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EDITORIAL

Dear Members

and all those who are always interested in **Thai Journal of Pharmacology**. This year is twentieth year of the Pharmacological and Therapeutic society of Thailand. We hope all of you will more enjoy and profit from reading the journal. The new approaches to **Thai Journal of Pharmacology** are developed in the coming years as we plan to publish the journal every 4 months. We are convinced that Thai Journal of Pharmacology will serve all pharmacologist and all researchers in the area of pharmacology or related fields.

As medical knowledge increases apace, the need researches for new drug development are still our duty. We are proudly present the two original articles obtained from our young pharmacologists, Dr. Pravit Akarasereenont, MD, PhD and Dr. Laddawal Phivthong-ngam, PhD. Both of them had an excellent opportunities to do those researches in international standard laboratories and devoted their original articles for our journal. In the first article, Dr Pravit presents data on the inducible isoform of cyclooxygenase (COX-2) and the roles of protein kinases in the induction of COX-2. In the second article Dr. Laddawal shows her experimental data on the cholesterol lowering action of lovastatin in high cholesterol rabbit. The case report in this issue is a long-termed study of Dr. Kobkarn and her colleges from the faculty of Dentistry, Chulalongkorn University. The application of fluocinolone in the treatment of oral lichen planus which failed to response the other drugs has been observed (if you remember her case report in our journal 2 years ago). The two reviews in this issue are NMDA receptor and nitric oxide (NO). NMDA receptor was presented by Dr. Panya in the meeting of the Pharmacological society and Therapeutic of Thailand held on 2 May 1997. The another review is about NO from Dr. Somchai from Chiangmai. This review is special for our members who are interested in NO, one of the most popular substances in this decade. In this review, NO is focused on its roles in biological function.

As you can see in this issue, the new drugs and pharmacological digest can be your entertainment and knowledge quick sources. Thanks to my young energetic staffs. They are very helpful and supportive. I myself appreciate their motivation for our journal and society. I want to express my sincerely thanks to all of sponsors who are continue to support the journal in spite of the IMF (I am fool- abbreviation for someone who are responsible for that) economic situation in Thailand. See you in the next issue which will be launched in August this year, I hope to. Please join us by sending your manuscripts or letters. We are waiting for any message from you.

ROLE OF PROTEIN KINASES IN THE INDUCTION OF INDUCIBLE ISOFORM OF CYCLOOXYGENASE-2 (COX-2) BY ENDOTOXIN-ACTIVATED ENDOTHELIAL CELLS

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ABSTRACT

Cyclooxygenase (COX) exists in at least two isoforms. COX-1 is present constitutively under physiological condition. COX-2 is induced in various cell types by mitogens and cytokines including endotoxin (lipopolysaccharides, LPS). Recently, we have shown that COX-2 can be induced by endotoxin in endothelial cells. The signal transduction mechanism of COX-2 induction is still unclear. Some cell membrane receptors have an intracellular protein kinase domain, activation of which results in the phosphorylation of proteins following ligand binding. In the present report, protein kinase inhibitors (erbstatin and genistein for tyrosine kinase inhibitors, staurosporine and calphostin C for protein kinase C inhibitors) were used as pharmacological tools to investigate the potential role of protein kinase in COX-2 induction in bovine aortic endothelial cells (BAEC) activated with endotoxin. The predominant COX metabolite, 6-oxo-prostaglandin (PG) $F_{1\alpha}$ was measured by radioimmunoassay under the following experimental conditions: (i) accumulation of COX metabolites of endogenous arachidonic acid was measured at 24 h after addition of LPS (1 μg/ml); (ii) determination of "COX activity" by measuring COX metabolites generated by LPS-activated BAEC after incubation with exogenous arachidonic acid (30 μM) for 15 min. Erbstatin (0.25 to 25 μM) or genistein (0.15 to 150 μM) caused a dose-dependent inhibition of the accumulation of COX metabolites in the supernatant of LPS-activated BAEC. Erbstatin or genistein also caused a dose-dependent inhibition of "COX activity" in BAEC. Western blot analysis with a specific antibody to COX-2 which determined the expression of COX-2 protein induced by LPS in cell extracts showed that erbstatin (25 μM) or genistein (150 μM) inhibited the expression of COX-2 protein in LPS-activated BAEC. In contrast to tyrosine kinase inhibitors, COX-2 induction in BAEC stimulated with LPS was not inhibited by protein kinase C (PKC) inhibitors, either staurosporine (0.0002-0.2 μM) or calphostin C (0.0015-1.5 μM). These results showed that tyrosine phosphorylation, not protein kinase C, was part of the signal transduction mechanism that mediated the induction of COX-2 elicited by LPS in BAEC.

Key words : lipopolysaccharide, prostaglandins, tyrosine kinase, protein kinase C

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INTRODUCTION

Prostaglandins (PGs) have numerous cardiovascular and inflammatory effects[1]. Cyclooxygenase (COX) is the first enzyme in the pathway in which arachidonic acid is converted to PGs[2-3]. COX exists in at least two isoforms. One is the constitutive enzyme, COX-1, producing regulatory prostanoids under physiological conditions[4], whereas the other, COX-2, is induced by mitogens[5-6], and proinflammatory cytokines[7] during pathological states such as inflammation. Recently, we have shown that COX-2 can be induced by endotoxin (lipopolysaccharides, LPS) in endothelial cells[8]. Little is known about the cellular mechanisms of these events as the signal transduction pathways involved in LPS induced alterations in cell function are not completely understood. Changes in the number of intracellular signal transduction systems have been observed after exposure of cells to LPS. Activation of the G-protein system has been im-plicated in intracellular signal transduction in cells exposed to LPS[9-12]. Also increased phosphorylation of certain proteins occurs after exposure of murine peritoneal macrophages to LPS, raising the possibility that protein kinases may be involved in the signal transduction process[13]. Activation of calcium and phospholipid-dependent protein kinase C has been observed after exposure of cells to either LPS or lipid A[14-15]. More recently, the phosphorylation of tyro-sine residues on cellular proteins leading to liberation of arachidonic acid metabolites has been observed in macrophages[16-17], neutrophils[18-21], smooth mus-cle cells[22], platelets[23], basophilic cells[24-25] and rat Kupffer cells[26] in response to agents such as endotoxin and IL-1. Therefore, the involvement of tyrosine kinase and of protein kinase C in the LPS-induced changes in COX in endo-thelial cells has been evaluated by using specific inhibitors such as erbstatin[27-29] or genistein[30] and calphostin C[31] or stauro-sporine[32-33].

METERIALS AND METHODS

Cell Culture

Bovine aortic endothelial cells (BAEC) were obtained from fresh bovine aortae as previously described[34] and cultured in 96-well plates with Dulbecco's Modified Eagle's Medium (DMEM; 200 µl/well) containing 10% foetal calf serum (Gibco) and 4 mM L-glutamine. The agents, dissolved in distilled water, were sterilized by filtration through a filter (pore size: 0.22 micron) before being added to the cells under sterile conditions. Cells were incubated at 37^0C in a humidified incubator.

Measurement of the release of COX metabolites

The main COX metabolites released after activation of cells with LPS (6-oxo-$PGF_{1\alpha}$for BAEC[8]) were measured by radioimmunoassay[35]. In experiments to measure the effects of tyrosine kinase inhibitors on the release of COX metabolites from endogenous arachidonic acid, cells were treated with LPS (1 µg/ml) together with the tyrosine kinase inhibitors erbstatin (0.25, 2.5 and 25 µM) or genistein (0.15, 1.5, 15 and 150 µM) or the protein kinase C inhibitors staurosporine (0.0002, 0.002, 0.02 and 0.2 µM) or calphostin C (0.0015, 0.015, 0.15 and 1.5 µM) for 24 h, and the medium was subsequently removed for radioimmuno-assay. In separate experiments designed to measure the effect of protein kinase inhibitors on "COX activity", cells were treated with LPS

(1 μg/ml) together with the respective protein kinase inhibitors (as above) for 12 h, after which time the cells were washed and fresh medium containing AA (30 μM) was added for 15 min at 37^0C. The formation of COX metabolites was then assessed by radioimmunoassay of the cell culture supernatant.

Immunoblot (Western blot) Analysis

BAEC which were untreated (control), treated with LPS alone (1 μg/ml), treated with LPS (1 μg/ml) plus erbstatin (25 μM) or with LPS plus genistein (150 μM) were cultured in 6-well culture plates (37^0C; for 24 h). After incubation, cells were extracted and analysed by immuno-blotting for COX-2 protein as previously described[8].

Measurement of cell viability

Cell respiration, an indicator of cell viability, was assessed by the mitochondrial dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan[36]. At the end of each experiment, cells in 96-well plates were incubated (37^0C; 1 h) with MTT (0.2 mg/ml) dissolved in culture medium, after which time, the medium was removed by aspiration and cells were solubilized in DMSO (200 μl). The extent of reduction of MTT to formazan within cells was quantitated by the measurement of optical density at 650 nm (OD_{650}) using a Molecular Devices microplate reader (Richmond, CA, USA).

Statistical analysis

The results are shown as mean ± SEM of triplicate determinations (wells) from 3 separate experimental days (n=9). Student's paired or unpaired *t*-tests, as appropriate, were used for the determination of significance of differences between means and a p -value of less than 0.05 was taken as statistically significant.

RESULTS

Effect of tyrosine kinase inhibitors on the release of COX metabolites from endogenous stores of arachidonic acids by BAEC activated by LPS

LPS enhanced the accumulation of 6-oxo $PGF_{1\alpha}$ in cultures of BAEC[8]. This accumulation was inhibited by erbstatin or genistein in a dose-dependent manner (Figure 1). A significant inhibition was achieved with 2.5 μM for erbstatin and 0.15 μM for genistein.

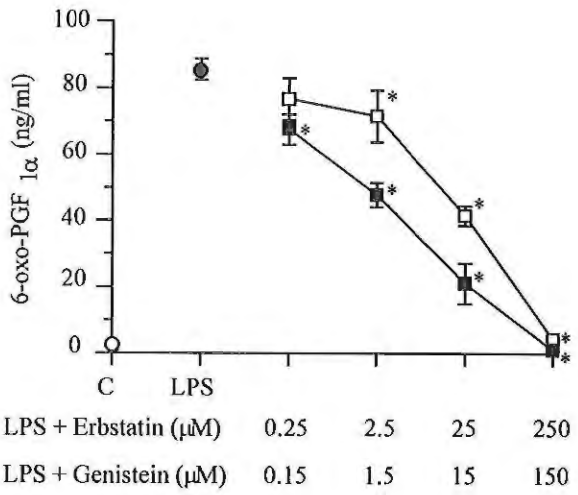

Figure 1 The effects of erbstatin (open squares) and genistein (closed squares) on the stimulation of COX in BAEC activated with LPS (1 μg/ml) measured at 24 h by the accumulation of 6-oxo-$PGF_{1\alpha}$ in culture medium. Data are expressed as mean ± SEM of triplicate determinations (wells) from 3 separate experimental days (n=9). * $p < 0.05$ when compared to LPS-treated cells at 24 h (LPS).

Neither of the tyrosine kinase inhibitors used (up to 250 μM for erbstatin and 150 μM for genistein) had any effect on cell viability, either alone or in combination with LPS (cell viability 84 ± 1 % of control untreated cells).

Effect of tyrosine kinase inhibitors on COX activity

In BAEC, the increase in COX activity caused by LPS, measured in the presence of exogenous arachidonic acid, was almost four-folds that of the control cells at 12 h (Figures 2 and 3). These figures also show the dose-dependent inhibition of COX activity by erbstatin or genistein to values almost as low as those of untreated cells at the highest concentrations of inhibition. The addition of either erbstatin (25 μM) or genistein (150 μM) to cell culture during the 15 min incubation with exogenous arachidonic acids did not affect the formation of 6-oxo $PGF_{1\alpha}$.

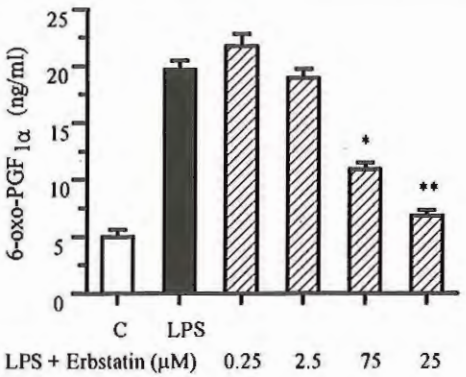

Figure 2 Dose-dependent inhibition of the increase in COX activity by erbstatin in LPS-activated BAEC measured at 12 h by the formation of the 6-oxo-$PGF_{1\alpha}$ in the presence of exogenous arachidonic acid (30 μM; 15 min). Data are expressed as mean ± SEM of 9 determinations from at least 3 separate experimental days. $^{*}p < 0.05$ when compared to LPS-treated cells at 12 h (LPS).

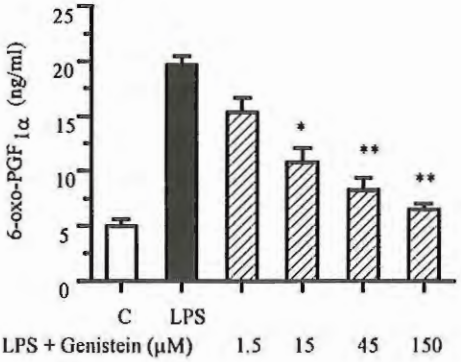

Figure 3 Dose-dependent inhibition of the increase in COX activity by genistein in LPS-activated BAEC measured at 12 h by the formation of 6-oxo-$PGF_{1\alpha}$ in the presence of exogenous arachidonic acid (30 μM; 15 min). Data are expressed as mean ± SEM of 9 determinations from at least 3 separate experimental days. $^{*}p < 0.05$ when compared to LPS-treated cells at 12 h (LPS).

The effect of tyrosine kinase inhibitors on COX-2 protein in LPS-treated BAEC

In these experiments, two control conditions untreated and LPS-treated were repeated to ensure reproducibility. Untreated BAEC contained no COX-2 protein (Figure 4; lane 1). In contrast, BAEC ac-tivated with LPS (1 μg/ml; 24 h) contained a protein of approximately 70 kDa, which was recognised by a specific antibody to COX-2 (Figure 4; lane 2). This induction of COX-2 protein by LPS in BAEC was prevented by erbstatin (25 μM; Figure 4; lane 3) or genistein (150 μM; Figure 4; lane 4).

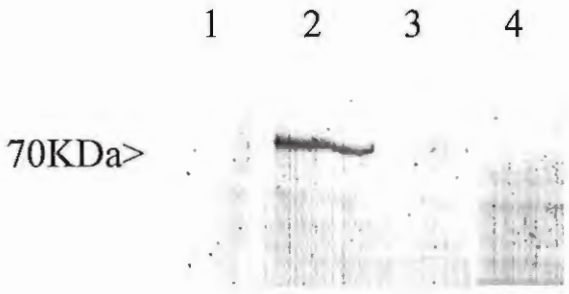

Figure 4 Western blots using polyclonal antibodies to COX-2 of cell extracts from LPS-treated and untreated BAEC. Equal amounts of protein were loaded in all lanes of BAEC (30 μg/lane). Control untreated BAEC (lane 1) contained no COX-2 protein. In contrast, LPS-activated (1 μg/ml for 24 h) BAEC contained COX-2 protein (lane 2). The induction of COX-2 protein by LPS in BAEC was abolished by erbstatin (25 μM; lane 3) and genistein (150 μM; lane 4). Similar results were obtained using cell extracts from 3 separate batches of cells.

Effect of protein kinase C inhibitors on the release of COX metabolites from endogenous stores of arachidonic acid of BAEC activated by LPS

Two protein kinase C inhibitors, calphostin C and stauro-sporine, were used. Either stauro-sporine (0.0002 to 0.2 μM) or calphostin C (0.0015 to 1.5 μM) did not significantly inhibit an increase in the accumulation of 6-oxo $PGF_{1\alpha}$ in BAEC activated with LPS (Figure 5).

Incubation of BAEC with either of the protein kinase C inhibitors (up to 0.2 μM for staurosporine and 1.5 μM for calphostin C) had no effect on cell viability, either alone or in combina-tion with LPS (cell viability 83 ±2 % of control untreated cells).

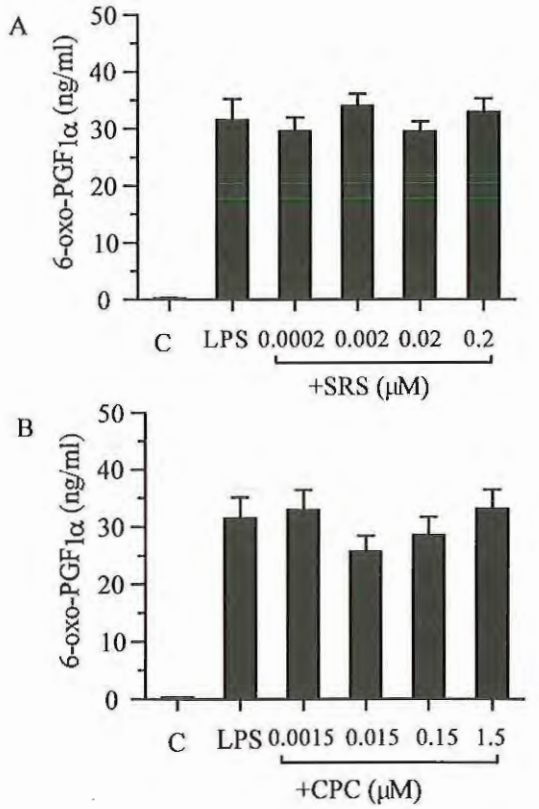

Figure 5 The effect of protein kinase C inhibitors, staurosporine (SRS; panel A) and calphostin C (CPC; panel B) on the accumulation of 6-oxo-$PGF_{1\alpha}$ in BAEC activated with LPS (1 μg/ml for 24 h). Data are expressed as mean ± SEM of 9 determinations from at least 3 separate experimen-tal days. $^{*}p < 0.05$ when compared to treated cells at 24 h (LPS).

Effect of protein kinase C inhibitors on COX activity

In BAEC, the increase in COX activity following LPS was inhibited by calphostin C only at the highest con-centration (1.5 μM; Figure 6), but not by staurosporine (up to 0.2 μM; Figure 6). The addition of either calphostin C (1.5 μM) or staurosporin (0.2 μM) to cells during the 15 min incubation with exogenous AA did not affect the formation of 6-oxo $PGF_{1\alpha}$.

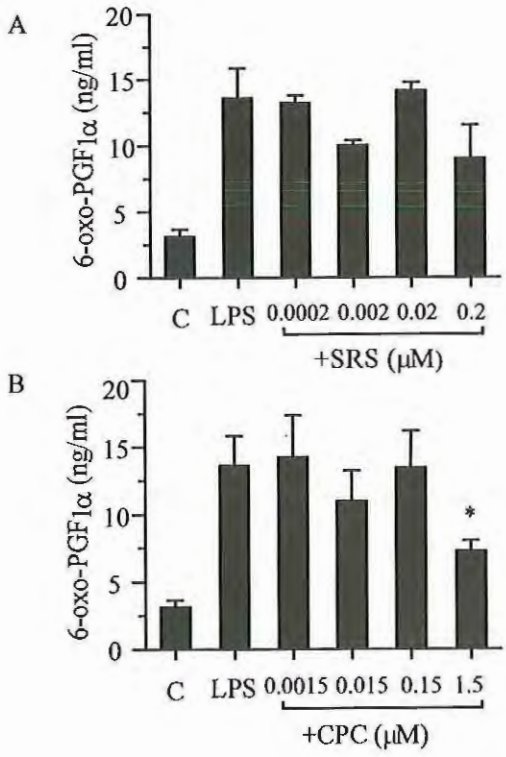

Figure 6 The effect of protein kinase C inhibitors, staurosporine (SRS; panel A) and calphostin C (CPC; panel B) on COX activity in BAEC activated with LPS (1 μg/ml for 24 h). Data are expressed as mean ± SEM of 9 determinations from at least 3 separate experimental days. $^{*}p < 0.05$ when compared to treated cells at 24 h (LPS).

DISCUSSION

The results show that the tyrosine kinase inhibitors, erbstatin or genistein, but not the protein kinase C inhibitors, calphostin C or staurosporine, concentration dependently in-hibit the expression by LPS of COX-2 activity and protein in endothelial cells. These findings clearly demonstrate that the signal transduction pathways leading to induction of COX-2 by LPS involve the phosphorylation of protein tyrosine kinase.

The experiments described in this study were designed to assess the role of two intracellular signalling pathways, protein tyrosine kinase and protein kinase C (PKC), in the transduction of the effects of LPS on COX-2 activity. These results demonstrate a more important role of tyrosine kinase than PKC in the signal transduction pathway leading to expression of COX-2. Neither set of inhibitors, however, interfered directly with the enzyme activity measured, i.e. they were not inhibitors of the COX-2 activity, but of the sequential steps leading to the expression of the respective proteins.

LPS is a major component of the outer membrane of gram negative bacteria. Although it interacts with many types of cells and is linked to numerous events associated with sepsis and endotoxin shock[37-38], the mechanisms underlying these actions are poorly understood. Two distinct pathways for LPS stimulation of cells have been identified, the direct stimulation by LPS and the indirect stimulation via cytokines release from LPS-stimulated cells[38-39]. The later step after LPS activation is the signal transduction mechanisms by which LPS induces alterations in cell function or the expression of proteins such as COX-2.

It is well-documented that LPS binds to a number of different constituents on the surface of cells or in the plasma/serum (i.e. LPS-binding protein or LBP[38-39]). Recently, two different cell surface molecules, CD14 and CD18, have been proposed as LPS receptors[40-42], but their contribution to the cellular activation afforded by LPS is unclear. LBP which is soluble[43-46], binds to the lipid A component of LPS[47], and these LPS-LBP complexes are recognized by receptors such as CD14 or CD18 molecules[38] present on monocytes[42]. In macrophages, CD14 is the im-portant cell binding protein[42], but not CD18 as with the scavenger receptors[38,41]. Although

endothelial cells do not have mCD14 (cell membrane CD14), LPS-LBP complexes react with another form of CD14, soluble CD14; sCD14, to form sCD14-LPS complexes on endothelial cells[48].

What are the intracellular signaling events that mediate specific LPS responses in endothelial cells? In some cell types such as lymphocytes, macrophages or mesangial cells, a pertussis toxin-sensitive guanine nucleotide binding protein (G-protein) has been identified which couples the binding of LPS to the cell surface to the intracellular signal transduction pathways leading to the response such as altered gene expression of some cytokines e.g. IL-1[9-12]. However, some of the actions of LPS in cells are unaffected by pertussis toxin treatment[10,49], suggesting that these responses elicited by LPS do not occur through a single class of a G-protein-dependent signalling mechanism. A Ca^{2+} signalling following activation of PAF receptor has recently been shown to be involved in the activation of guinea-pig macrophages and neutrophils by LPS[50].

An increased phosphorylation of certain proteins occurs after exposure of murine peritoneal macrophages to LPS, raising the possibility that protein kinases may be involved in the signal transduction process[13]. The two major types of protein kinase are serine-/threonine and tyrosine kinase. The phospholipid/Ca^{2+} dependent protein kinases (serine/threonine protein kinase, protein kinase C) could play a crucial role in the signal transduction and altered gene expression after LPS exposure[14-15,39]. In this study, protein kinase C inhibitors, however, did not inhibit the induction of COX-2 in endothelial cells after activation with LPS. In addition to protein kinase C, which is serine/threonine protein kinase, tyrosine kinase has also been suggested to play a role in the signal transduction mechanism. Binding of different ligands (i.e. EGF or PDGF) to cell surface receptors stimulates tyrosine phosphorylation and this event is thought to be part of the signal transduction mechanism that mediates later cellular responses[51]. The phosphorylation of tyrosine residues on cellular proteins, leading to formation of AA metabolites, has been observed in many cell types. Activation of tyrosine kinase may, therefore, be an integral part of the signal transduction pathways leading to expression of COX-2 in various cell types. In our experiments, roles of tyrosine kinases were implicated through the use of the inhibitors, erbstatin and genistein which were shown to be a selective inhibitor of tyrosine (vs serine/threonine) kinases in 1987[27,30] and have been used widely since then.

The demonstration that inhibitors of tyrosine kinase suppress the induction of COX-2 provides a possible explanation for the anti-inflammatory activities of tyrosine kinase inhibitors such as herbimycin A, leflunomide or tyrphostins in a variety of disease models, i.e rheumatoid synovitis in rat[52-55], and the prevention by a tyrphostin (AG126) of the mortality caused by endotoxaemia in mouse[56]. Furthermore, the finding that inhibitors of tyrosine kinase suppress the induction of COX-2, reinforces the hypothesis that agents which prevent the induction of COX-2 may well be useful in the prevention of local (inflammation) or systemic inflammatory conditions (e.g. systemic inflammatory response syndrome, arteriosclerosis, etc.).

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EFFECT OF LOVASTATIN ON PLAQUE FORMATION AND LDL OXIDATION IN CHOLESTEROL-FED RABBITS

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ABSTRACT

The present study investigated the effect of lovastatin, a HMG-CoA reductase inhibitor, on the regression or progression of atherosclerosis and on the susceptibility of LDL to oxidation in vitro in cholesterol-fed rabbits. New Zealand White Rabbits were fed 1% cholesterol for 4 weeks, and 0.5% cholesterol for additional 12 weeks. One subgroup of cholesterol-fed rabbits was treated with lovastatin (10 mg/day) from week 5-16. By the method of Cu^{2+}-mediated LDL oxidation, oxidative susceptibility of LDL was measured at 4 and 16 weeks of the experimental period. Cholesterol-feeding induced a marked increase in oxidative susceptibility of LDL. Treatment with lovastatin had no effect on lag time, maximal diene production, or diene production rate, even though it reduced plasma total cholesterol concentrations by about 50%. At the time of sacrifice, the extent of atherosclerotic lesions was measured by morphometric analysis of intimal plaque area in the common carotid arteries. Carotid intimal plaque area was 15.8±4.6% after 4 weeks of cholesterol-feeding and 44.0±5.2% after 16 weeks. Lovastatin suppressed the progression of plaque formation (to 27.0±5.1%) but did not induce regression. These data suggest that lovastatin can slow the progression of atherosclerotic plaque formation in rabbits induced by cholesterol-feeding without decreasing the oxidative susceptibility of LDL.

Key words: intimal thickening, oxidized LDL, atherosclerosis

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INTRODUCTION

Clinical and experimental studies have established that elevated concentrations of low-density lipoprotein (LDL) are a risk factor for atherosclerosis[1-4]. The lipids deposited in the atherosclerotic lesions are mostly derived from plasma LDL, which is modified by oxidative processes, resulting in an enhanced uptake by the scavenger receptor of macrophages[4-6], leading to foam cell formation. Lipid peroxidation plays a critical role in atherosclerosis[7,8]. Oxidized LDL has been found to be chemotactic for monocytes[9]; it induces endothelial cell damage[10,11] and stimulates cytokine and growth factor release from cells of the arterial wall[4,7]. The severity of atherosclerosis has been found to correlate with the susceptibility of LDL to oxidation and the concentration of lipid peroxides[12]. The method of promotion of LDL oxidation by Cu^{2+} has provided an assay allowing to study the oxidizability of LDL ex vivo[13,14].

3-Hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase inhibitors, like lovastatin, simvastatin, or pravastatin, are widely used to lower plasma LDL cholesterol concentrations. They are not only potent competitive inhibitors of the rate limiting enzyme in cholesterol biosynthesis[15,16], but also partly regulate LDL clearance by increasing the number of LDL receptors on the surface of liver cells[17,18]. There is increasing evidence that lovastatin can reduce atherosclerotic lesion development[19,20], as well as preserve endothelium-dependent relaxation both in hypercholesterolemic animal models and in humans[20-22]. In addition to reduced plasma cholesterol concentrations, the antiatherosclerotic activity of this class of compounds has been suggested to be due to reduced LDL oxidation[23-25]. However, this has not been supported by others[26].

To investigate the mechanism of antiatherosclerotic properties of HMG-CoA reductase inhibitors, the present experiment was designed to examine the effect of chronic treatment with lovastatin in cholesterol-fed rabbits with overt intimal lesions on the in vitro oxidation of LDL and the regression of atherosclerosis.

MATERIALS AND METHODS

Animals and study design

46 male New Zealand white rabbits weighing initially 1.5-2 kg were used in this study, which conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication. No 85-23, revised 1985) and had been approved by the Hannover supervisory committee for studies in animals. After a 2-week period of adaptation, the rabbits were exposed to dietary treatment for a period of 4 or 16 weeks. They were randomly divided into 4 groups. Rabbits in group I (n=12) were fed normal rabbit chow (Nohrlin 20 ZH5, Eggersmann, Germany) and plain tap water throughout the study and served as control group. The rabbits in group II (n=8) received 1% cholesterol diet (Altromin, Lage Germany) for 4 weeks (Cholesterol-4 group). The other two groups of eight rabbits were fed a diet containing 1% cholesterol for the first 4 weeks followed by 0.5% cholesterol (Cholesterol-16 group), or 0.5 % cholesterol supple-mented with 10 mg/day of lovastatin orally (MSD Sharp and Dohme, Germany; Lovastatin group) for the next 12 weeks. Animals were housed individually. Food and water were allowed ad libitum. Weight, food, and water consumption were measured in weekly intervals.

Blood samples were obtained from the central ear artery at baseline and at 4 and 16 weeks. Following

centrifugation (1500 x g, 10 min), plasma was removed and stored at -20°C prior to measuring plasma cholesterol. Another plasma sample was processed immidiately for the isolation and oxidation of LDL (see below).

At the end of 4 weeks, the rabbits in Cholesterol-4 group and 4 animals from the control group (Control-4 group) were sacrificed. The remaining rabbits were killed at 16 weeks. The carotid artery, thoracic and abdominal aortas were removed and cleaned of adherent fat and fascia.

Biochemical analyses

Plasma total cholesterol, LDL and HDL-cholesterol concentrations were quantified by commercially available enzymatic methods (Boehringer-Mannheim, Mannheim, Germany).

LDL isolation and oxidation

Fresh whole blood was collected into 1 mg EDTA/mL blood (Monovette-KE, Sarstedt, Germany) and centrifuged (1500 x g, 10 min) to generate plasma. LDL was isolated from plasma by high speed centrifugation through a discontinuous KBr gradient according to the method described by Esterbauer et al.[13]. Briefly, 5 mL of fresh plasma was adjusted to a density of 1.006 with KBr and centrifuged at 436,000 x g at 4°C for 2.5 hr using a Beckman TL-100 ultracentrifuge equipped with a Beckman TLA 100-2 Rotor (Beckman Instrument, U.S.A.). The VLDL fraction was collected, and the remaining solution was adjusted to a density of 1.063 with KBr. The LDL fraction was isolated by centrifugation at 436,000 x g for 2.5 hr at 4°C. EDTA (1 mg/mL) was present throughout all preparation steps. The EDTA-containing LDL stock solution could be stored overnight at 4°C in the dark in a nitrogen atmosphere. Before the susceptibility of LDL to oxidation was determined, the LDL stock solution was freed from EDTA by filtration through a disposable desalting column Econo-Pac 10 DG (Bio-Rad, Munich, Germany), using phosphate buffered saline solution (PBS) as eluent. Protein content was determined by the method of Bradford[27] using bovine serum albumin (BSA) as a standard.

LDL was adjusted with PBS to a concentration of 75 µg LDL protein/mL. Oxidation was triggered by adding 2.5 µM $CuSO_4$ to the LDL solution. Diene conjugation was monitored at 234 nm in 10 minute intervals using a Hitachi U-2000 spectrophotometer (Hitachi, Germany) for 6 hours. The concentration of conjugated dienes was calculated using the extinction coefficient of 2.8 x 10^4 $M^{-1}cm^{-1}$[13].

Using this method, LDL oxidation can be divided into three phases[13] : lag phase, propagation phase and decomposition phase which together describe the oxidizability of that particular LDL-preparation. The lag phase is defined as the period during which no oxidation occurs, i. e., the interval between the addition of $CuSO_4$ and the beginning of extensive lipid oxidation. The propagation phase occurs once LDL is depleted of its antioxidants and fatty acids are rapidly oxidized to form conjugated dienes, during which the rate of diene production can be calculated from the slope of the absorbance curve and expressed as micromoles of diene produced per minute per mg of LDL-protein. The levels of conjugated dienes reach a maximum and subsequently fall during the decomposition phase. The maximal diene production serves as one parameter, expressed as micromoles per mg of LDL-protein.

Morphometric and histological analyses

The proximal common carotid arteries were dissected free of adventitial tissue and cleared of remaining blood by infusion of 0.9% NaCl. Thereafter the arteries were opened longitudinally and placed on an even surface for photography of intimal lesions. Photographs were digitalized and measurements of total intimal area and plaque area were made by planimetry of the photographic images. The plaque areas were expressed as percentage of total intimal surface area.

Segments of the thoracic and abdominal aorta were excised, fixed in formalin, embedded in paraffin, and stained with hematoxylin/eosin for the morphometric measurement of intimal and medial cross-sectional areas by planimetry[28]. Four sections from each animal were analysed, and the values were averaged.

All histological measurements were performed by skilled observers who were blinded to the treatment groups.

Statistical analysis

All values are given as means ± standard error of the mean (S.E.M.). Statistical analysis was performed with the use of one way analysis of variance (ANOVA) followed by Fisher's protected least-significant difference test. Correlations were performed by linear regression analysis. A p value of less than 0.05 was considered significant.

Table 1 Body weight, plasma total cholesterol and lipoprotein cholesterol concentrations of the rabbits on various groups.

	Control-4	cholesterol-4	control-16	cholesterol-16	chol.+lovastatin
Body weight (kg)					
baseline	2.7±0.07	2.66±0.66	2.78±0.05	2.76±0.06	2.70±0.08
4 weeks	3.21±0.04	3.25±0.14	3.29±0.06	3.29±0.07	3.40±0.07
16 weeks	-	-	4.21±0.09	3.66±0.11*	3.73±0.12*
Total cholesterol (mmol/L)					
baseline	0.73±0.03	1.06±0.11	1.12±0.07	1.21±0.06	1.23±0.09
4 weeks	0.66±0.06	41.14±3.78*	0.92±0.11	49.44±4.66*	46.28±6.20*
16 weeks	-	-	0.49±0.07	37.10±3.06*	23.38±1.34*#
LDL- cholesterol (mmol/L)					
baseline	0.12±0.02	0.17±0.04	0.28±0.04	0.26±0.02	0.34±0.05
4 weeks	0.07±0.02	39.20±3.69*	0.18±0.06	44.74±4.26*	41.72±5.74*
16 weeks	-	-	0.05±0.01	33.18±3.09*	20.75±0.64*#
HDL- cholesterol (mmol/L)					
baseline	0.42±0.02	0.56±0.05	0.56±0.04	0.55±0.02	0.57±0.04
4 weeks	0.33±0.05	0.35±0.03	0.46±0.06	0.40±0.04	0.47±0.02
16 weeks	-	-	0.21±0.03	0.22±0.03	0.21±0.02

All values are mean + S.E.M.

* P<0.05 VS control group at the same period

# P<0.05 VS cholesterol group at the same period

RESULTS

Body weight

The changes in body weights during the study period in all groups of rabbits are summarized in Table 1. There was a progressive increase in body weight with no significant differences between all experimental groups during the first 4 weeks. Thereafter the rabbits in the control group grew faster than those rabbits fed a cholesterol-enriched diet, and showed significantly higher body weights than the cholesterol-fed rabbits at 16 weeks ($P<0.05$). No significant difference in body weights was found between the cholesterol-16 and lovastatin group.

Plasma lipids and lipoproteins

The effects of the cholesterol diets and treatment with lovastatin on plasma total cholesterol and lipoprotein concentrations are shown in Table 1. Total cholesterol was 1.15 ± 0.03 mmol/L (n = 36) at baseline with no significant differences between the groups. It remained unchanged during the whole experimental period in the control-4 and control-16 groups. Plasma total cholesterol concentrations increased to 46.82 ± 2.52 mmol/L (n = 16) after 4 weeks of 1% cholesterol feeding. After switching to 0.5% cholesterol feeding for further 12 weeks, total cholesterol concentrations slightly decreased and then remained on a high level (37,10 ± 3.06 mmol/L). It decreased by about 50% during 12 weeks of treatment with lovastatin, although the cholesterol-enriched diet was continued (46.28 ± 6.20 and 23.38 ± 1.34 mmol/L at 4 and 16 weeks, respectively; $P<0.05$), and was significantly lower than those in cholesterol-16 group.

The changes in LDL-cholesterol concentrations went parallel to the plasma total cholesterol levels in all groups. There were no significant differences in HDL-cholesterol concentrations at any time point between any of the groups.

Cu^{2+}-catalyzed oxidation of LDL ex vivo

Figure 1 shows the typical curves of in vitro LDL oxidation after the addition of $CuSO_4$ monitored as the formation of conjugated dienes of LDL. This figure clearly shows the effect of cholesterol-enriched diet on an increased susceptibility of LDL to oxidation, as demonstrated by short lag time, elevated total and rate of diene production.

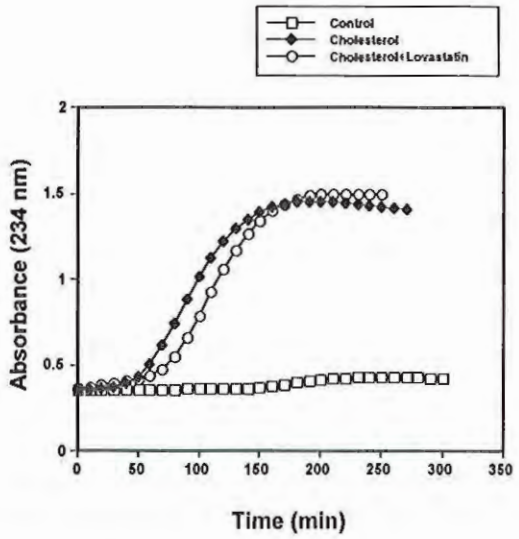

Figure.1 The example of the kinetic absorbance curves of LDL isolated from the one rabbits of the control-16, cholesterol-16, or lovastatin groups at 16 weeks. Effects of the cholesterol feeding on the maximal and rates of diene production are clearly shown.

The lag time of LDL oxidation obtained from rabbits fed a 1% cholesterol-containing diet for 4 weeks was significantly shorter as compared to that observed in the rabbits fed normal chow (85.8 ± 5.3 vs. 161.8 ± 5.0 min; $P<0.05$; Figure 2). After additional 12 weeks of a 0.5% cholesterol-enriched diet, the lag time was 118.9 ± 11.3 min, as compared to 183.0 ± 4.0 min in the control-16 group. Treatment with lovastatin did not result in a significantly different lag time

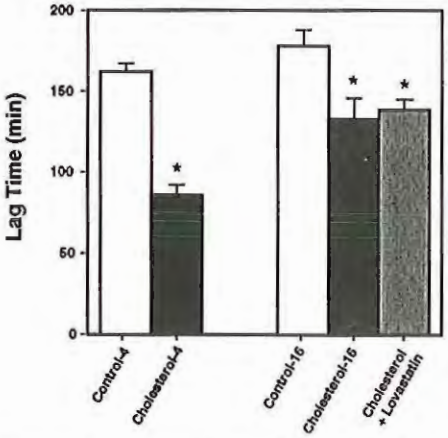

Figure 2 Susceptibility of rabbit-LDL to copper mediated oxidation before (at 4 weeks) and after (at 16 weeks) lovastatin treatment. The resistance of LDL to oxidation decreased markely after 4 or 16 weeks of cholesterol-enriched diet. No significant difference between the cholesterol-16 and lovastatin groups are observed. Data are mean ± S.E.M. * P<0.05 vs. Control

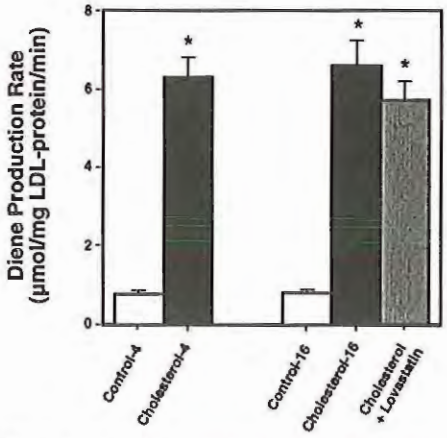

Figure 3 The maximal oxidation rate of LDL derived from rabbits in each groups, exposed to copper. The values were calculated from the slope of kinetic absorbance curve. After 4 or 16 weeks of cholesterol-enriched diet, the rates of diene production increased significantly, as compared to the control group. Lovastatin treatment did not induce significant difference from the cholesterol-16 group. Data are mean ± S.E.M. * P<0.05 vs. Control

as compared to rabbits fed the cholesterol-enriched diet alone (138.3 ± 5.4 min vs. 118.9 ± 11.3 min; P = n.s.).

Concomitantly, the maximal rate of diene production was significantly increased in cholesterol-fed rabbits (Figure 3). In the control group, the mean oxidation rates were 0.75 ± 0.08 and 0.77 ± 0.12 µmol/min/mg LDL protein for the time of 4 and 16 weeks, respectively. The cholesterol diet was associated with a nine-fold elevation in the maximal rate of diene production (6.28 ± 0.52 and 6.59 ± 0.66 µmol/min/mg LDL-protein in the cholesterol-4 and 16 groups, respectively; each P<0.05 vs control). Administration of lovastatin in the last 12 weeks did not significantly change the high rate of diene production as compared to the cholesterol-4 and -16 groups (5.72 ± 0.47 µmol/min/mg LDL protein).

Total diene production was 208.81 ± 6.26 and 261.25 ± 18.74 µmol/mg LDL-protein in the control-4 and -16 groups (Figure 4). Cholesterol

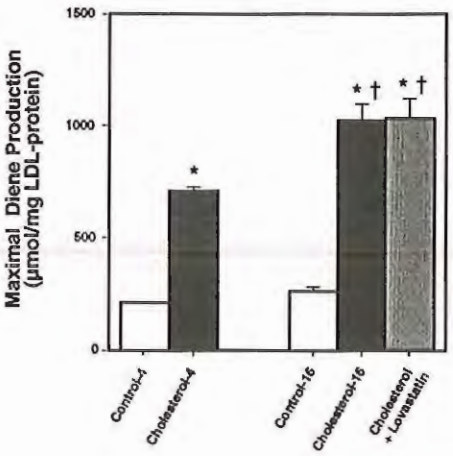

Figure 4 The maximal production of conjugated dienes during the LDL oxidation induced by copper ex vivo. The concentration of conjugated dienes was calculated using the extinction coefficient of 2.8 $x10^4$ $M^{-1}cm^{-1}$. The maximal diend production significantly increased after 4 weeks of a 1% cholesterol-enriched diet and progressively increased after the further 12 weeks of 0.5% cholesterol-enriched diet. There is no significant difference in conjugated diene production between the cholesterol-16 and lovastatin groups. Data are mean ± S.E.M. * P < 0.05 vs. Control, † P<0.05 vs. cholesterol-4 group

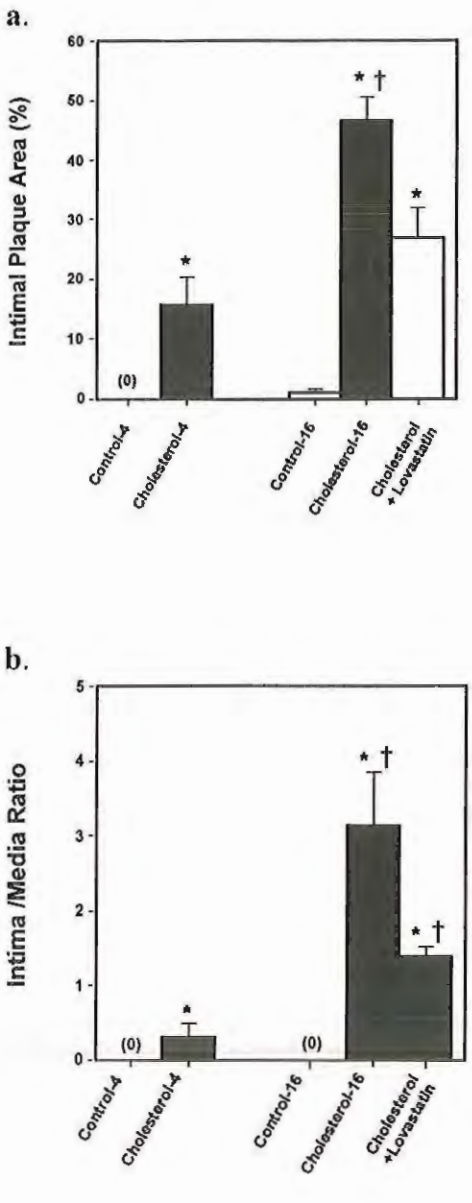

Figure 5 The effect of lovastatin treatment on the carotid arterial intimal plaque area (a) and aortic intimal thickening (b). At the end of treatment period in each groups, the rabbits were sacrified. The intimal lesion of the carotid arteries were assessed by planimetry of digitized photographys and aortic intima/media ratios were assessed by micromorphometry of semithin sections of the thoracic aortas stained with hematoxylineosin. The plague formation increased significantly in the groups of rabbits fed cholesterol-enriched diet. Treatment with lovastatin can slow the progression of the plaque formation and shows significantly difference from those in the cholesterol-16 group. Data are mean ± S.E.M. * P<0.05 vs. Control † P<0.05 vs. cholesterol-4 group

feeding for 4 weeks induced a three-fold increase in the production of diene (703.51 ± 22.3 μmol/mg LDL-protein), which was significantly different from the control-4 group (P<0.05). After further 12 weeks of 0.5% cholesterol feeding, the diene production increased further to 1021.14 ± 91.48 μmol/mg LDL-protein (P<0.05 vs control-4 and cholesterol-4 groups). Treatment with lovastatin had no effect on the total production of diene (1032.78 ± 91.48 μ mol/mg LDL-protein) as compared to the cholesterol-16 group (P = n.s.).

Morphometric and histological analyses

The percentage of intimal plaques in the carotid arteries and aortic intima/media ratios are shown in Figure 5a and 5b. No plaques were found in common carotid arteries in the control-4 group. After 4 weeks of cholesterol feeding, plaques covered 15.8 ± 4.6 % of the total intimal area. Plaque formation progressed to 46.6 ± 4.1 % after additional 12 weeks of 0.5% cholesterol feeding (cholesterol-16 group), but remained low in the control-16 group (0.9 ± 0.6%; P<0.05). Lovastatin treatment nearly abolished the progression of intimal plaque formation (27.0 ± 5.1 %; P<0.05 vs. cholesterol-16 group). It was slightly, but not significantly higher than in the cholesterol-4 group. The percentage of carotid arterial intimal plaque areas were significantly correlated with plasma total cholesterol (r = 0.597, P<0.02; Figure 6) but not with the measures of LDL-oxidation ex vivo.

In both control groups, no intimal thickening of thoracic aortic cross sections was detectable. Cholesterol feeding significantly and progressively increased intima/media ratios to 0.3±0.2 (4 weeks) and 3.1±0.7 (16 weeks). Lovastatin treatment significantly reduced the the intima/media ratios (1.4±0.1; P<0.05 vs. cholesterol 16 group).

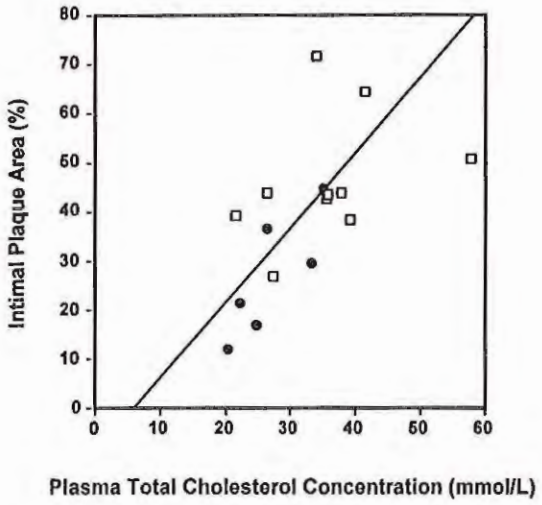

Figure 6 Correlation between plasma total cholesterol concentrations and the carotid arterial intimal plaque area in cholesterol-fed rabbits for 16 weeks treated (●) or untreated (□) with lovastatin. Each value represents a single animals. a) $r = 0.597$, $P<0.02$

DISCUSSION

We have demonstrated that lovastatin treatment suppresses the progression of plaque formation in the carotid arteries in rabbits fed a high cholesterol-diet, while it did not improve oxidative susceptibility of LDL. In the present study, lovastatin was administered for 12 weeks to rabbits after they had already been fed a 1% cholesterol diet for 4 weeks.

Due to inability to compensate for increased input of cholesterol by raising the excretion of cholesterol into the bile, the rabbit is well known among experimental animal species for its tendency to develop severe hypercholesterolemia when dietary cholesterol is increased[29], and to produce a strong relationship between the levels of plasma cholesterol and the degree of aortic lesions with a marked thickening of the intima[2]. In the present study, plasma total cholesterol concentration markedly increased by 41-fold in rabbits receiving 1% cholesterol for 4 weeks, and was maintained on this high level throughout the experimental period, even though the cholesterol content in diet was reduced to 0.5% during the last 12 weeks. This increase was mainly due to an increase in LDL-cholesterol, whereas no changes in the HDL-cholesterol were observed. Treatment with 10 mg/day of lovastatin in hypercholesterolemic rabbits reduced plasma total cholesterol and LDL-cholesterol by about 50%. These data are consistent with previous studies[22,30].

Hypercholesterolemia is one risk factor of atherosclerosis, in which oxidative modification of LDL plays a crucial role. Oxidatively modified LDL may impair the function of signal transduction pathways in endothelial cells, and stimulate many growth factors[9-11]. Monocyte-derived macrophages take up oxLDL by scavenger receptors more rapidly than native LDL[5-7]. There has been much interest in the modulation of oxidative susceptibility of LDL by antiatherosclerotic agents. It is assumed that the lower the resistance of plasma LDL to oxidation, the greater the chance of developing atherosclerotic disease. While the method to detect in vivo LDL oxidation remains unclear, LDL can be oxidized by traces of transition metal ions in a cell-free system ex vivo, particularly effective are Cu^{2+} ions. LDL extracted from human and rabbit lesions was shown to resemble LDL oxidized in vitro by Cu^{2+}[31,32]. In the current investigation we used the method of ex vivo oxidation of LDL by Cu^{2+} ions, which was proved by O'Leary and co-workers[14] to be a reliable index to study the effects of drugs on LDL oxidation. In the present study, neither lag time, maximal diene production nor diene production rate was changed by lovastatin treatment. This finding is in accordance with the result of Yoshida et al.[26] who found no effect of low dose

simvastatin (5 mg/day) on oxidative susceptibility of LDL in hypercholesterolemic patients. However, antioxidant effects of HMG-CoA reductase inhibitors have been previously reported both in vitro and in vivo[23-25]. Kleinveld et al.[25] showed that simvastatin and pravastatin altered the composition of LDL derived from plasma of hypercholesterolemic patients and resulted in its reduced oxidizability by decreasing total and maximal rate of diene production with unchanged lag time. In contrast, a study by Aviram et al.[23] in hypercholesterolemic patients demonstrated antioxidant properties of lovastatin both in vitro and in vivo without any change in LDL composition, and suggested that lovastatin might bind to the LDL surface and alter polyunsaturated fatty acid availability to in vitro oxidation. This discussion is still controversial. In addition, these studies were performed in patients with mild hypercholesterolemia without atherosclerosis. There is no previous report on antioxidant properties of lovastatin in animals. In the present experiment, we studied the effects of lovastatin in rabbits with severe hypercholesterolemia (total cholesterol concentration of 46.82 ± 2.52 mmol/L) and pre-established atherosclerotic lesions (carotid intimal plaque area of 15.8 ± 4.6% and aortic intima/media ratio of 0.3±0.2). Moreover, various compounds within the group of HMG-CoA reductase inhibitors have been shown to possess varying degrees of hepatoselectivity and plasma cholesterol lowering[30]. Therefore, a compound specific effect may be one cause for the different results.

A study of Gilligan et al.[33] showed that there is a strong negative correlation of the lag time of LDL oxidation with the levels of plasma cholesterol in hypercholesterolemic patients. A similar result was found in the prsent study. Lipoproteins derived from hypercholesterolemic patients contain increased concentrations of cholesterol, resulting in enhanced in vitro susceptibility of LDL for oxidation[13,34]. The lack of effect of lovastatin on LDL oxidation in our present study suggests that other components of the LDL particle beyond cholesterol influence the susceptibility of LDL to oxidation, which are not influenced by lovastatin treatment. There are evidences that oleate-enriched diets reduce the susceptibility of LDL to oxidation, whereas linoleate-enriched diets increase the susceptibility[35]. In addition, smaller, more dense LDLs display enhanced susceptibility to Cu^{2+}-induced oxidation when compared to larger, more buoyant lipoprotein particles[36].

The earliest histologically recognizable atherosclerotic lesion is the fatty streak. It is clearly shown that a decrease of plasma lipid levels can reduce atherosclerotic lesion formation[2,20,21]. In the current investigation, after 4 weeks of cholesterol feeding, intimal plaque in the carotid arteries and intimal thickening in aorta significantly increased and aggressively progressed when the cholesterol-feeding was further continued for 12 weeks. Dietary supplementation with lovastatin decreased these progression despite continued intake of the high-cholesterol diet. Intimal plaque formation significantly correlated with plasma total cholesterol concentrations. The beneficial effect seen with lovastatin therefore seemed to be due to its cholesterol-lowering effect. A reduction in lesion size of atherosclerotic arteries in high cholesterol-fed cynomolgus monkeys was seen after the cholesterol level was decreased by returning to a normal diet[37]. Extent and type of atherosclerotic lesions induced in rabbits has been shown to be dependent upon the overall plasma cholesterol exposure, VLDL- and LDL-cholesterol content[2]. Soma et al.[38]

reported that lovastatin administered at 20 mg/kg inhibited neointimal formation by 33% in normocholesterolemic rabbits when lesions were induced by surrounding the carotid artery with a flexible collar. In other studies[20] in which lovastatin therapy was initiated simultaneously with the cholesterol diet and plasma cholesterol levels were maintained relatively low, lovastatin was found to prevent atherosclerotic lesion formation. Similar results have been reported by Bocan et al.[2] who found a development of minimal atherosclerosis in the thoracic aorta of rabbits when cholesterol levels were maintained below 700 mg/dL. A regression of aortic atherosclerosis in cholesterol-fed rabbits was found by the study of Zhu et al.[39] who used a high dose of lovastatin (6 mg/kg/day) combined with a normal diet, inducing a reduction of plasma cholesterol levels by 95%.

In addition, there is evidence that mevalonate and other intermediates (isoprenoids) of cholesterol synthesis are essential for DNA synthesis and cell proliferation[40]. Thus, inhibition of HMG-CoA reductase may prevent an increase in DNA synthesis induced by platelet-derived growth factor, and subsequent cell growth. It is therefore possible that the beneficial antiatherosclerotic effect of lovastatin is due in part to reduced intimal hyperplasia by inhibiting smooth muscle cell proliferation. Previous observations demonstrated that treatment of cells with lovastatin and compactin resulted in growth arrest unrelated to the presence of cholesterol[41]. Subsequently, lovastatin, fluvastatin, and simvastatin have been shown to dose-dependently decrease smooth muscle cell migration and proliferation in cell culture[42]. On the other hand, one study showed that administration of lovastatin to hypercholesterolemic rabbits markedly attenuated both the reduced basal NO production and the increased adhesiveness of the endothelium[43]. More recently, lovastatin has been shown to reduce the expression of scavenger receptor CD36 in human monocytic cells[44].

In conclusion, the results show that lovastatin can slow the progression of intimal plaque lesions in the carotid arteries even in the presence of preestablished lesions. This effect is not associated with a decreased susceptibility of LDL to Cu^{2+}-mediated LDL oxidation ex vivo.

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EFFICACY OF FLUOCINOLONE ACETONIDE IN THE TREATMENT OF ORAL LICHEN PLANUS : A 5 - YEAR FOLLOW UP STUDY

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ABSTRACT

Thirteen patients with atrophic or erosive oral lichen planus (OLP) failed to respond to other medications were selected for the treatment with fluocinolone acetonide 0.1% in orabase (FAO). The drug was applied to the lesions four times daily in the first month, after which the application frequency was gradually reduced. Examinations of the oral mucosa were carried out at 2 weeks, 1 month, 6 months, 1 year , 3 years and 5 years after starting the treatment. At one month, the lesions were satisfactorily in remission in 11 of the 13 patients, but at 5 years, only one patient was completely in remission. Acute pseudomembranous candidiasis was frequently complicated during the FAO treatment , but was effectively treated with topical antifungal agent in every instances. This study shows that FAC is a useful drug for the short term treatment of refractory OLP but is ineffective for the long term treatment, although serious long term side-effect is negligible.

Key words : fluocinolone acetonide, oral lichen planus

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INTRODUCTION

Lichen planus is a common chronic inflammatory disease of skin and oral mucosa with unknown etiology[1]. In non-erosive oral lichen planus (OLP), patients often complain of roughness of the oral mucosa or burning sensation with hot or spicy food. In erosive type, OLP may interfere with speaking, eating and swallowing which makes the patients suffer most. Various treatments for the disease have been tried, but complete cure is rather rare. Vitamin A analogues can eliminate the white lesions of OLP but rapid recurrence is noted after discontinuation of the drug[2]. Human interferon-β has been reported to have high therapeutic efficacy but needs advanced genetic engineering for its synthesis[3]. Antifungal agent and cryosurgery have also been tried in the treatment of OLP[4-5]. Cyclosporin has recently been reported to be beneficial in the treatment of several mucocutaneous disorders but it does not seem to be reliably effective in severe OLP[6-7]. Furthermore, it is rather expensive and has some serious side effects[8]. Therefore, both systemic and topical corticosteroids have been widely used to alleviate pain and inflammation in OLP. In this study, a new preparation of topical steroid, fluocinolone acetonide 0.1% in orabase (FAO), was assessed for its efficacy and safety in the treatment of refractory cases of OLP .

PATIENTS AND METHODS

Thirteen patients with atrophic or erosive OLP who had failed to respond to other medications and had no serious systemic diseases were selected. The number of female patients was greater than males (10 vs 3), and the age range was 30-79 years with a mean of 48 years. The duration of disease varied from 364 months. Lesions were found most in buccal mucosa followed by mucobuccal fold, lip, palate, gingiva and tongue, respectively (Table 1). The diagnosis was confirmed by tissue biopsy. In addition, candida staining was performed in all cases before and during treatment. Patients were asked to stop any medications for the treatment of OLP at least 2 weeks before the commencement of FAO.

The lesions were scored 0-5 according to the following criteria.

5 = white striae with erosive area larger than 1 x1 cm^2 with severe symptoms
4 = white striae with erosive area smaller than 1x1 cm^2 with moderate symptoms
3 = white striae with erythematous area larger than 1x1 cm^2 with mild symptoms
2 = white striae with erythematous area smaller than 1x1cm^2 with mild symptoms
1 = very mild white striae without symptoms
0 = normal mucosa without symptoms

The criteria for cure or remission were the absences of erythematous or inflammatory area, white striae and symptoms, i.e. a score of 0 or 1. Score 2 represented partial remission and scores 3-5 represented no response to treatment. FAO 0.1% was prepared in the Pharmacological Department. Patients were advised to apply the drug to the lesions four times daily during the first month. When the lesions had responded to the treatment (score 2), the application was reduced to three times daily and gradually to twice (score 1) and once (score 0) daily, and finally stopped. One year after beginning the treatment,

the patients were advised to apply either FAO or fluocinolone acetonide 0.1 % in solution (FAS) topically to the lesions when they recurred as the above criteria. Evaluations by oral examinations together with photographs were performed at 2 weeks, 1 month, 6 months, 1 year, 3 years and 5 years after starting treatment respectively.

RESULTS

After the first 2 weeks, 9 out of 13 cases (69.23%) remitted while after 1 month, 11 cases (84.61%) re-mitted. After 5 years, however, only one case (7.69%) was completely in re-mission, whose oral mucosa had be-come normal without any symptoms (Table 2). The lesions before and after treatment were illustrated for clinical comparison as shown in Figure 1-6. Acute pseudomembranous candidiasis was frequently found 5 out of 13 cases (38.46%) during the first 2-6 months after beginning treatment but was completely cured by miconazole gel in all cases.

DISCUSSION

Both systemic and topical corticosteroids have been widely used in the treatment of OLP for alleviating pain and inflammation[9-11]. Steroids used topically or by local injection are often more effective and safer than systemic steroids[12-13]. It was shown in previous study that fluocinolone acetonide cream did not result in significant differences in blood pressure and synacthen test before and after treatment[14]. Moreover, our recent study indicated no permanent adrenal suppression after treatment with FAO for 6 months[15]. This present study showed that only one case of OLP out of 13 cases was still in complete remission after 5 years follow-up. It is not known whether the remission of lesions in this case occurred as a consequence of the drug or did it occur spontaneously. The lesions in this case had persisted for longer than 34 months before treatment with FAO without remission. Even though the FAO may not help curing the disease, it may help reducing pain. Nine patients dropped out during the follow up period. It was ascertained through telephone communication that most of them had had only partial remission with mild symptoms. Only two cases had shown no response to the treatment while most patients responded very well after one month. Acute pseudomembranous candidiasis occurred as a complication of the treatment was common but could readily be cured with topical miconazole in all cases.

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Table 1 Sex, age, site and duration of disease in OLP patients.

No	Sex	Age(years)	Sites of the lesions*	Duration (months)
1	M	79	B, M	56
2	F	53	P	35
3	F	50	B, T	34
4	F	32	B, L	36
5	F	56	B, L	34
6	F	38	B, M, G	42
7	F	52	G	6
8	M	67	B, L	24
9	F	39	B	6
10	F	30	P	4
11	F	33	L	64
12	M	36	B, M	3
13	F	57	B, M	60
Total = 13	F:M = 10:3	Mean± SD = 47.85±14.84	B = 9 P = 2 M = 4 G = 2 L = 4 T = 1	range = 3-64 months

* B = buccal mucosa, G = gingiva, L = lip,
M = mucobuccal fold, P = palate, T = tongue

Table 2 Result of the FAO treatment at 2 weeks, 1 month, 6 months, 1 year, 3 years and 5 years in 13 OLP patients.

Duration of treatment	Number of patients			
	remission	partial remission	no response	dropped out
2 weeks	9	2	-	2
1 month	11	2	-	-
6 months	9	2	-	2
1 year	2	3	-	8
3 years	2	2	-	9
5 years	1	1	2	9

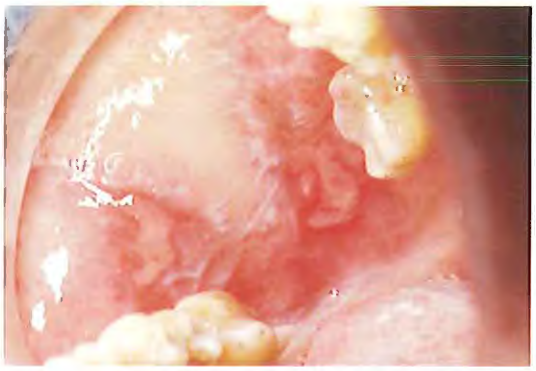

Figure 1 Case 1 patient with erosive lichen planus on right buccal mucosa before treatment with FAO.

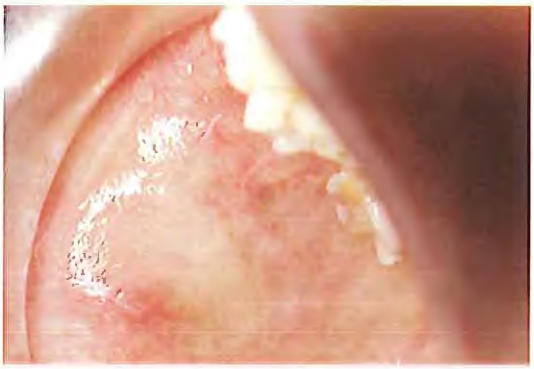

Figure 2 Case 1, one month after treatment with FAO.

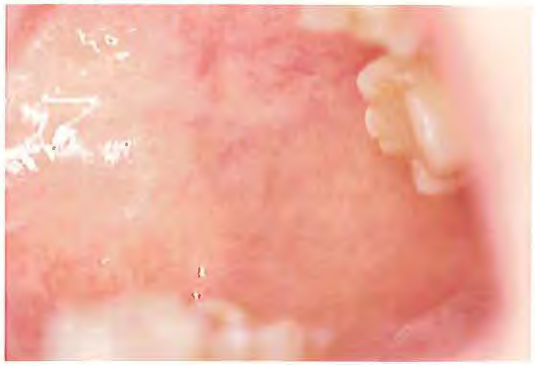

Figure 3 Case 1, five years after the start of treatment, there was neither white striae nor erythematous area.

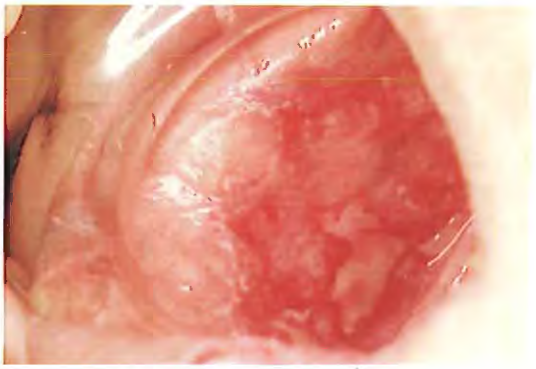

Figure 4 Case 2 patient with erosive lichen planus on right buccal mucosa before treatment with FAO.

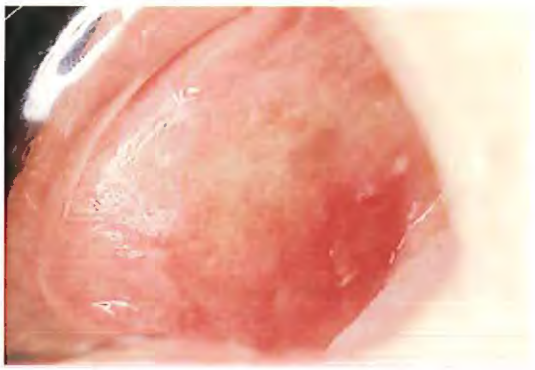

Figure 5 One month after treatment with FAO in case 2, the buccal mucosa showed good response to the treatment.

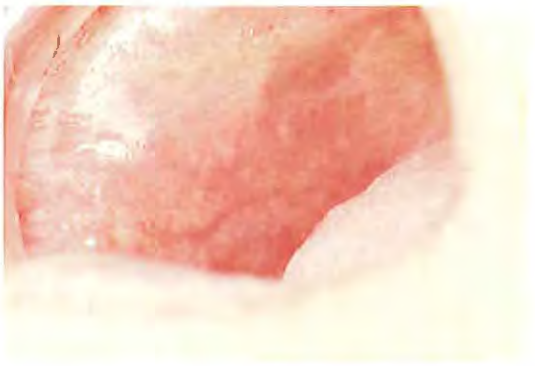

Figure 6 Case 2, one year after treatment with FAO. The mucosa appeared to be normal.

"โสม" มีหลายชนิดแต่ชนิดที่สำคัญและนำมาใช้เป็นยากันมากที่สุด คือ โสมเกาหลีหรือโสมคน ซึ่งมีชื่อทางวิทยาศาสตร์ว่า Panax ginseng C.A. Meyer "โสม" มีสารไกลโคไซด์ ชื่อจินเซโนไซด์ (ginsenosides) ซึ่งเป็นสารสำคัญที่แสดงฤทธิ์ทางยา

"จี 115" เป็นโสมสกัดมาตรฐาน มีจินเซโนไซด์ 8 ชนิด ในสัดส่วนคงที่ และปรับปริมาณให้มีความเข้มข้น 4% (พอเหมาะในการแสดงฤทธิ์ทางเภสัชวิทยา)

โสม กับผู้สูงอายุ

รศ. ดร. ภญ. นงลักษณ์ สุขวาณิชย์ศิลป์

ฤทธิ์ต้านความแก่

เมื่ออายุมากขึ้นเข้าสู่วัยชรา นอกจากสังขารภายนอกจะเสื่อมลงแล้ว การทำงานของระบบต่าง ๆ ภายในร่างกายก็เสื่อมลงตามอายุที่เพิ่มขึ้นด้วย ประสิทธิภาพในการทำงาน ความจำและการเรียนรู้ลดลง มีการกล่าวถึงการนำโสมมาใช้เพื่อต้านความแก่ โดยจะพบว่ามีการนำโสมมาใช้ในตำรับยาจีนหลายอย่าง [1,2] ในสหรัฐอเมริกาใส่โสมลงในเครื่องสำอาง เช่นสบู่และน้ำหอม [3] มีผู้สังเกตพบว่าในผู้สูงอายุที่ใช้ผลิตภัณฑ์โสมจะมีผิวหนังชุ่มชื่นขึ้น จุดด่างดำและรอยเหี่ยวย่นลดลง [4]

จากการที่พบว่า โสมมีฤทธิ์ป้องกันเซลล์ไม่ให้ได้รับอันตรายจากรังสี โสมจึงอาจมีบทบาทในการช่วยยืดอายุเซลล์ให้เสื่อมช้าลง [4,5] นอกจากนี้ผู้ทำการศึกษาในสัตว์ทดลอง พบว่าโสมสกัดช่วยทำให้การเรียนรู้และความจำที่ลดลงในหนูแก่กลับดีขึ้น [6] ในการศึกษาโดยใช้ DX-9386 (เป็นสูตรยาจีนซึ่งประกอบด้วยโสมและมีสารอื่น ๆ ผสมอยู่ด้วย) ให้กับหนูถีบจักรพันธุ์แก่เร็ว พบว่ายาตำรับนี้ช่วยให้กระบวนการออกซิเดชันของไขมันเกิดช้าลง (กระบวนการออกซิเดชันของไขมันทำให้เกิดสารที่เป็นต้นเหตุของการเกิดเม็ดสีในผู้สูงอายุ และยังเป็นต้นเหตุของการเกิดโรคบางชนิดในผู้สูงอายุ) ช่วยยืดอายุหนู ช่วยป้องกันการมีน้ำหนักลดลงเมื่อหนูอายุมากขึ้น ช่วยชะลอความจำที่เสื่อมลงตามอายุ และช่วยให้อาการอื่น ๆ ที่เกิดเนื่องจากการสูงอายุนั้นลดลง [4,7,8]

โสมกับสมรรถภาพผู้สูงอายุ

โสมสกัดช่วยให้การทำหน้าที่เกี่ยวกับระบบภูมิคุ้มกันของเซลล์น้ำเหลืองที่ลดลงในคนสูงอายุกลับเพิ่มขึ้น [9] ผู้สูงอายุที่ได้รับโสมสกัดมาตรฐานเป็นเวลา 12 สัปดาห์ เมื่อทำการทดสอบค่าต่าง ๆ เกี่ยวกับปฏิกริยาการตอบโต้ของร่างกาย และประสิทธิภาพในการทำงานตลอดจนวัดความเปลี่ยนแปลงทางด้านความจำ อารมณ์ ความตั้งใจในการทำงาน การนอนหลับ ฯลฯ พบว่ากลุ่มที่ได้รับโสมสกัดมีการเปลี่ยนแปลงในสิ่งเหล่านี้ไปในทางที่ดีขึ้นกว่ากลุ่มควบคุม [10]

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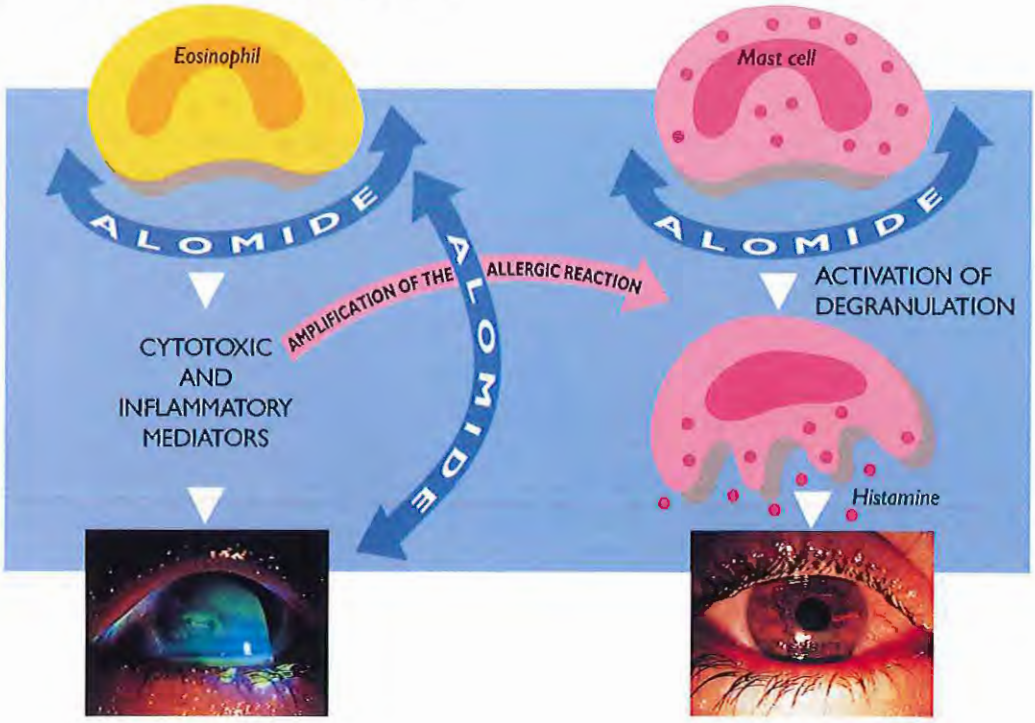

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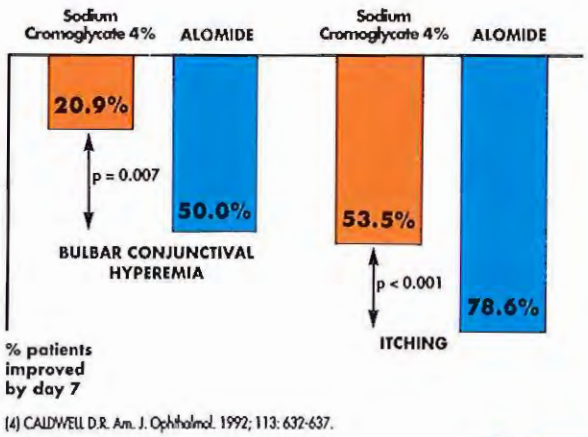

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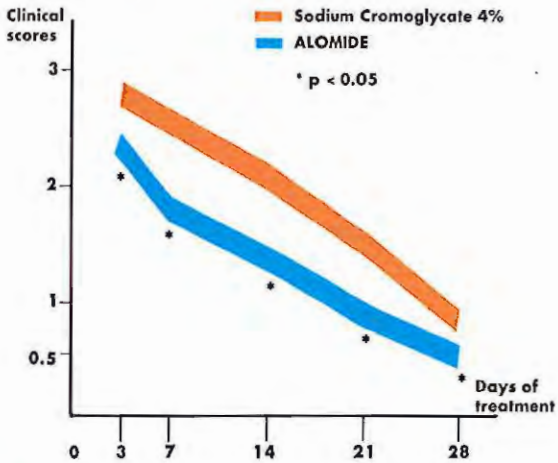

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NMDA RECEPTOR AND THE THERAPEUTIC POTENTIAL OF ITS LIGANDS

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ABSTRACT

NMDA is the excitatory amino acid receptor subtype that is most thoroughly studied. It is an ionotropic receptor with Mg^{2+} blockage in a voltage-dependent manner. Functional NMDA receptor consists of a heterodimer of NMDAR1 and NMDAR2 subunits. Alternative splicing of the products of exons 5, 21 and 22 results in 7 naturally occuring isoforms. Impotant allosteric binding sites on the NMDA receptors are Zn^{2+}, glycine and polyamine binding sites. Since the competitive NMDA antagonists and the noncompetitive antagonists are mostly associated with psychotomimetic effects, attention has recently been paid toward the development of ligands that bind to the glycine and polyamine binding sites. The most promising compounds that have potentials as anticonvulsants are partial and competitive antagonists of the glycine binding site and competitive antagonists of the polyamine bindings sites.

Key words : NMDA receptor, therapeutic potential

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INTRODUCTION

Excitatory amino acids that function as neurotransmitters are chiefly glutamate and aspartate. There are currently 4 subtypes of excitatory amino acid receptors named after the relatively specific agonists[1].

1. NMDA (N-methyl-D-aspartate) receptor
2. AMPA (a-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid) receptor
3. Kainate receptor
4. Metabotropic receptor

The first 3 subtypes are iono-tropic receptors by having integral cation-specific channel in the receptor molecule. The last subtype couple with G-protein to control the synthesis of intracellular messengers which involves the metabolism of phosphatidylinositol 4,5-bisphosphate.

NMDA receptor contains a single cation channel which is blocked by Mg^{2+} in a voltage-dependent manner. Partial depolarization of the membrane displaces Mg^{2+} from the channel lumen and allow cation entry (Ca^{2+} is preferred over Na^{+}) to depolarize the neuron. Phencyclidine (PCP), ketamine and dizocilpine (MK-801) are noncom-petitive antagonists which block open channel close to the magnesium binding site.

This review will focus on the subunit constitution of NMDA receptor, its important binding sites and the compounds that show some promises in therapeutic uses.

SUBUNIT CONSTITUTION OF NMDA RECEPTOR

Functional NMDA receptor must be at least a heterodimer having 2 subunits, NMDAR1 and NMDAR2[2]. NMDAR1 subunit is the product of a single gene. There are 7 isoforms from alternative splicing of the products of exons 5, 21 and 22[3] . Exon 5 encodes 21 amino acids at the N-terminal while exons 21 and 22 encodes 37 and 38 amino acids, respectively, at the C-terminal[4]. Therefore, there are 8 (2^3) possible splice variants of these exon products. So far, 7 isoforms have been identified (NMDAR1A to NMDAR1G) which contain 938, 959, 901, 922, 885, 922 and 906 amino acid residues, respectively. The splice variant that contains the products of exons 5 and 21 but not the product of exon 22 has not been found in the cDNA library. The characteristics of NMDAR1 subunit are[4]:

1. having a large extracellular N-ter-minal, up to half of the subunit's molecular weight.
2. having three transmembrane dom-ains (TM1, TM3 and TM4)
3. TM2 is bent in the membrane and line the cation channel (pore-forming region or P-region)[5]
4. having S1 and S2 regions that bind glutamate and glycine, respectively.
5. having 4 or 5 residues at the C-terminal that can be phosphorylated by protein kinase C.

NMDAR2 subunit has 4 subtypes from 4 different genes[1,6] (NMDAR2A to NMDAR2D) that contain 1464, 1482, 1250 and 1323 amino acid residues, respectively. Its structure in relation to the cell membrane seems to be similar to NMDAR1 with a large intracellular C-terminal extension from TM4. The

distribution of each subtype in the rat brain is heterogenous. From autoradiographic studies, each subtype is present with high densities in the following brain regions[1]:

NMDAR2A	-	hippocampus and cerebral cortex
NMDAR2B	-	forebrain
NMDAR2C	-	cerebellum
NMDAR2D	-	diencephalon and lower brainstem

ALLOSTERIC SITES ON THE NMDA RECEPTOR

A. Zn^{2+} binding site

Zn^{2+} is a potent noncompetitive NMDA receptor antagonist that blocks the open channel of NMDA receptor with little sensitivity to changes in glycine concentration or membrane voltage[7] . At low concentrations, Zn^{2+} block the response to NMDA in this manner[8,9]. At high concentrations, Zn^{2+} block the open channel in a voltage-dependent manner at the Mg^{2+} binding site. The latter type of blockade is not important because Zn^{2+} concentration has to be very high.

B. Glycine binding site

NMDA receptor is a new type of receptor that requires two different agonists to bind simultaneously to activate the ion channel. Endogenous agonists in this case are glutamate and glycine. Glycine is absolutely required for NMDA receptor activation[10]; without glycine, the receptor could not be activated. Glycine therefore acts as coagonist at this site of NMDA receptor which is strychnine-insensitive. Binding of glycine in the presence of NMDA agonist increases the frequency of cation channel opening with little effect on the mean open time. Extracellular concentration of glycine in most parts of the brain (> 1 mm) is more than enough to saturate the glycine binding sites[11]. In other words, glycine is present in the concentration that is high enough to activate the NMDA receptor together with the NMDA agonist. Although glutamate mediates synaptic transmission, changes in the extracellular glycine levels modulate the NMDA receptor's contribution to the transmission.

Partial agonists for the glycine binding site are HA-966[12] (3-amino-1-hydroxypyrrolidone) and D-cycloserine[13] . HA-966 noncompetitively inhibits glutamate binding and enhances the binding of competitive NMDA antagonists. Competitive antagonists of glycine's action are kynurenic acid derivatives e.g. 7-chlorokynurenic acid[14] and 5,7-dichlorokynurenic acid (MDL 29,814). Kynurenic acid is the product of kynurenine pathway of tryptophan metabolism. It is a nonselective antagonist that binds to the NMDA site, AMPA receptor and kainate receptor. The concentration in human brain tissue is about 1 mm. In the experimental animals it has anticonvulsant effect and protects the animals from global cerebral ischemia. 7-Chlorokynurenic acid antagonizes the action of glycine and HA-966.

In experimental animals R(+)-HA-966 and 5,7-dichlorokynurenic acid have anxiolytic effect[15] and blocks mesolimbic activation and associated hyperactivity by amphetamine[16]. Anxiolysis from these compounds is better than those from NMDA antagonists because there is less muscle relaxation at equivalent anxiolytic doses in the model that measures ultrasonic vocalization of rat pups following the separation from their mothers. 1-Amino-1-carboxycyclopropane and R(+)-cis-4-methyl-HA-966 (L 687414) are partial

agonists with potent anticonvulsant action. The latter compound does not have psychotomimetic effect or behavioral stimulation[17]. D-cycloserine has been shown to produce cognitive enhancing effect in experimental animals[18].

C. Polyamine binding site

Polyamines are highly charged cationic compounds that have their positive charges separated by their chain lengths. They have roles in all enzymatic processes that involve RNA or DNA in cellular growth and development[19]. Spermine and spermidine are polyamines that potentiate the response of NMDA receptor stimulation[20]. NMDA receptor activation is necessary to the potentiating effect of these polyamines. Ifenprodil and its derivative, eliprodil (SL 82.0715) are antagonists at the polyamine binding site[21]. These compounds have the following actions:

1. anticonvulsant action in the maximal electroshock models and in the NMDA-induced[22] and audiogenic seizures[23].
2. cytoprotective effect in focal cerebral ischemia model[24]. Besides the action on NMDA receptor, they have inverse steal action from constriction of pial vessels.
3. neuroprotective effect from the toxicity of NMDA[25]
4. eliprodil blocks voltage-sensitive calcium channel[26] and is a high affinity ligand to the s-receptor with unknown function.

NONCOMPETITIVE NMDA ANTAGONISTS

Although dizocilpine (MK-801) show promising results in many animal models of epilepsy and cerebral ischemia, trials of this compound and related antagonists in patients with epilepsy and stroke are disappointing because of associated psychotomimetic effects. The most promising compound in this group is memantine which is related to the antiviral amantadine. It produces good results in rat ischemic models and is clinically well-tolerated[27].

COMPETITIVE NMDA ANTAGONISTS

D-CPP-ene (SDZ EAA-494), a competitive antagonists at the NMDA binding site on the NMDA receptor, has no anticonvulsant action and many serious adverse effects in human complex partial seizures[28] although promising results have been reported in many animal models of epilepsy[29]. Selfotel (CGS 19755) has been shown to reduce intracranial pressure in patients with severe head injury[30]. Its use in stroke patients has been limited by hallucinations at doses similar to those used in head injury.

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Drug Tale

Muscarinic antagonist compounds are some of the oldest drugs employed in medicine. They are found in a variety of natural substances, mainly in plants belonging to the deadly nightshade family (*Solanaceous* plants) and in a few poisonous species of the *Amanita* genus of mushroom (eg, *A. muscaria*, or fly agaric, and *A. phalloides*, or death cup). Two chemically similar drugs, atropine and scopolamine, are found in various members of the above-mentioned plant and fungus groups. The active substances found in these sources are alkaloids known as L-hyoscyamine and L-hyoscine (scopolamine). In DL-hyoscyamine or atropine, a racemic mixture occurs during the extraction process. Such a mixture is a 50:50 combination of two stereoisomers, or optical isomers-a D-isomer and an L-isomer. L-Hyoscyamine is approximately 20 times more potent than D-hyoscyamine. Atropine and scopolamine are called **belladonna alkaloids** because they are found in the deadly nightshade plant (*Atropa belladonna*). Jimsonweed, also known as locoweed, thorn apple, or stinkweed (*Datura stramonium*), contains L-hyoscyamine; black henbane (Hyoscyamus niger) is a source of L-hyoscine. Over several centuries, in widely scattered countries, women have added extracts of belladonna to their eyes to produce expansive pupils. Ophthalmologists still do it today. The extremely large, dark eyes that result were seen as a sign of beauty (not of the inability to focus); hence the name *belladonna*, which means "beautiful woman."

Anonymous. Belladonna alkaloids and beauty. In: Cottrell GP, Surkin SB, eds. Pharmacology for respiratory care practitioners. 1st.ed.Philadelphia:F.A.Davis company, 1995:115.

AN INTERCELLULAR MESSENGER : NITRIC OXIDE

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ABSTRACT

Nitric oxide (NO), a potentially toxic molecule, plays a role as an important second messenger. It has been implicated in a wide range of biological functions, such as blood pressure regulation, blood clotting, and neurotransmission. The regulation, signal transduction and cytotoxicity of NO is strictly dependent upon its chemical reactivity with oxygen and metals rather than specific structural interactions with physiological targets. The properties, biosynthesis, physiological roles, involvement in pathophysiology and therapeutic potential of NO are reviewed.

Key words: Nitric oxide, NO, neurotransmitter, second messenger

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INTRODUCTION

Recently, the intense interest of nitric oxide (NO) research has occurred, because of the significant therapeutic potential. NO plays a role as an important biological second messenger in human physiological processes, which is involved in neurotransmission, blood clotting and blood pressure control. In addition, NO has been shown to serve as part of the immune system against cancer cells and intracellular parasites and microbes. Due to the molecule's reactivity, very small size and diffusibility, the actions of NO depend on its chemical reactivity with oxygen and metals rather than its molecular shape or specific structure interactions with physiological targets as any other biological messengers.

PROPERTIES AND BIOSYNTHESIS OF NO

NO is an inorganic gas produced by many mammalian cells. It is an uncharged molecule containing one unpaired electron and thus is both a paramagnetic compound and a free radical. Nitric oxide diffuses freely in a surprisingly large distance, in all directions from its site of origin[1] and acts as a dissolved nonelectrolyte in all its biological activities, with the exception of the lung in the presence of gaseous phase[2]. It is much more soluble in apolar solvents such as *n*-hexane and dissolve selectively in the membrane and lipid phase of cells.

The chemical reactions of NO with oxygen and metal in biological system are characterized as stabilization of the unpaired electron. The reaction of NO with oxygen species, such as O_2, superoxide anion ($O_2^{\cdot-}$) or peroxy radicals, results in the formation of a stabilized diamagnetic species. The most important reaction of NO with metal is its reaction with oxyferrohemoglobin. The reaction is a transfer of oxygen and electron to NO, forming nitrate anion and oxidized methemoglobin[2].

The reaction of NO with O_2

$$2\,NO + O_2 \longrightarrow 2\,NO_2 \text{ (nitrogen dioxide)}$$
$$NO_2 + NO_2 \longrightarrow N_2O_4 \longrightarrow NO^+$$
$$NO_2 + NO \longrightarrow N_2O_3 \longrightarrow NO^+$$

The transferation of nitrosonium ion : Transnitrosation

$$[NO^+] + OH^- \longrightarrow HNO_2 + H^+ + NO_2 \text{ (nitrite)}$$
$$[NO^+][NO_2^-] + H_2O \longrightarrow 2\,NO_2^- + 2\,H^+$$
$$[NO^+][NO_3^-] + H_2O \longrightarrow NO_2^- + NO_3^- + 2\,H^+ \text{ (nitrate)}$$

Nitrosation of biological nucleophilic targets

$$[NO^+] + R_2NH \longrightarrow R_2NNO + H^+ \text{ (nitrosamine)}$$
$$[NO^+] + RSH \longrightarrow RSNO + H^+ \text{ (s-nitrosothiol)}$$

Nitrosamine : potent mutagen and carcinogen
s-Nitrosothiol : source of NO

The reaction of NO with superoxide $O_2^{\cdot-}$

$$NO + O_2^{\cdot-} \longrightarrow \underset{\text{(peroxynitrite)}}{ONOO^-} \xrightarrow{H^+} ONOOH \longrightarrow NO_3^- + H^+$$

The reaction of NO with oxyferrohemoglobin

$$Hb(Fe^{2+})O_2 + NO \longrightarrow Hb(Fe^{3+}) + NO_3^-$$

Nitric oxide is produced from the substrates arginine, O_2 and reduced nicotinamide adenine dinucleotide phosphate (NADPH) by a Ca^{2+}-dependent mixed function oxidase, nitric oxide synthase (NOS). NOS is a homodimeric protein of 125 to 160 KD subunits. Each NOS subunits contains one flavin adenine dinucleotide (FAD), one flavin mononucleotide (FMN), one tetrahydrobiopterin (H_4B) and one Fe(III)-heme (iron protoporphyrin IX) as its prosthetic groups (Figure 1 and 2) or cofactors.

The synthesis of NO (Figure 3) is a two-step mechanism, the oxidation of arginine (Arg) to L-hydroxyarginine (NOHArg) at the heme site of NOS and the conversion of NOHArg to NO. The N in nitric oxide comes from the guanidino group of arginine, the electron from NADPH and the oxygens in both NO and citrulline from molecular O_2[3].

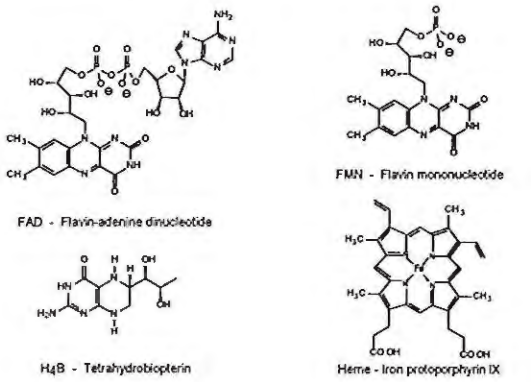

Figure 1 Nitric oxide synthase prosthetic groups.

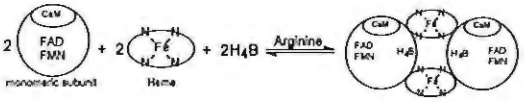

Figure 2 A model shows NOS associate with its prosthetic groups and Ca-calmodulin (CaM) to form an active form of NOS in catalysing the synthesis of nitric oxide from arginine.

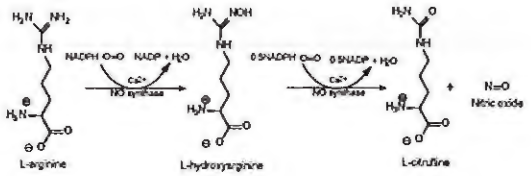

Figure 3 Biosynthesis of Nitric oxide (NO).

THE PHYSIOLOGICAL ROLE OF NO

The effects of NO have been many and varied due to its interplay between different possible reaction pathways. Several researches including the areas of cardiovascular pharmacology, immunology and neurobiology had been done to study the actions of NO in the biological system.

NO IN BLOOD PRESSURE REGULATION

The vascular endothelial cell is now regarded as an endocrine gland that releases several substances including NO[2] which is produced by the activation of NOS in vascular endothelial cells, eNOS. The activation of eNOS occurs through its interaction with Ca^{2+}-calmodulin complex which formed after a rise in intracellular Ca^{2+} causes by influx of Ca^{2+} to the cell. The activated eNOS then catalyses the synthesis of NO which rapidly diffuses from the endothelial cell to the adjacent vascular smooth muscle cells and interacts with its physiological target, the soluble guanylate cyclase (sGC)[4]. Soluble guanylate cyclase (sGC) is a cytosolic enzyme containing a heme as well as a copper ion[2]. The sGC catalyses the production of guanosine 3',5'-cyclic monophosphate (cGMP), an intracellular second messenger, that mediates smooth muscle relaxation through its stimulation of cGMP-dependent protein kinase or protein kinase G to phosphorylate the myosin light chain[1]. NO activates sGC by binding with very high affinity to iron in the heme of sGC displacing the heme iron from the plane of porphyrin ring, eliciting a conformational change and this change enhances the enzyme's catalytic activity[4]. Various nitrates have been used as vasodilators and antianginal agents. The relaxing properties of these agents were once thought to depend on the formation or the release of NO and this idea is now shown to be true. Some of these agents spontaneously decompose into NO and some of them, such as nitroglycerine and amyl nitrite, interact with the thiol groups on protein or enzymes and form an intermediate S-nitrosothiol (RS-NO) before releasing NO[5] (Figure 4).

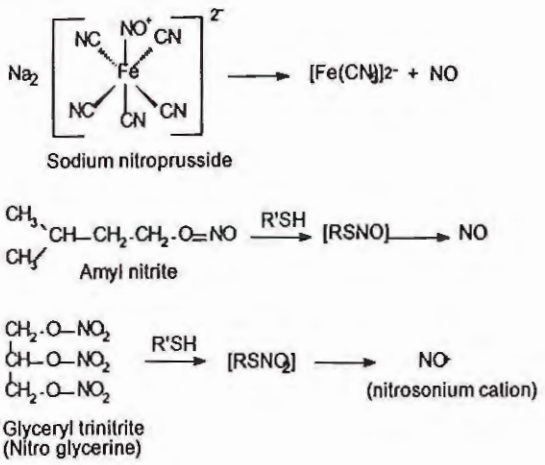

Figure 4 Examples of nitric oxide releasing agents

In addition to the effects on vascular smooth muscle, NO also inhibits platelet aggregation via sGC and cGMP dependent pathway. Together with prostacyclin, NO increases the platelet affinity for both the vascular endothelial surface (platelet adhesion) and for each other (platelet aggregation)[2].

NO AS NEUROTRANSMITTER

The second messenger role for NO in the central nervous system (CNS) and peripheral nervous system (PNS) followed studies in the cardiovascular and immune system. In 1988, John Garthwaite and colleages showed the relationship of NO and N-methyl-D-aspartate (NMDA) receptor, important receptor in the transmission of nerve impulse from cell to cell. They found that the slices from cerebellar portion of rat brain release a labile substance upon the activation of NMDA receptor.(6) This substance is called the endothelial relaxing factor (EDRF) which has been identified as NO or a close derivative that release NO[4].

The exact effector. pathways of NO (Figure 5) is activation of its physiological target, the enzyme soluble guanylate cyclase (sGC). Binding of NO to sGC caused a 100-fold increase in the production of cGMP which leads to the ultimate cellular response. NO does not fit the concept of classical neurotransmitter or neuromodulator. It is synthesized in the neurons when glutamate released from a stimulated neuron binds to and activates NMDA receptor on the adjacent neuron, causing an ion channel in the receptor to open, admitting influx of Ca^{2+} to the neuron. In the neuron, Ca^{2+} binds to calmodulin to form Ca^{2+}/calmodulin complex that associates with NOS and upon the presence of oxygen and nicotinamide-adenine dinucleotide phosphate (NADPH), it converts L-arginine to L-citrulline and NO. NO is released from the neuron through diffusion not the exocytosis[1]. Since it is synthesized on demand, there are no storage or formal uptake mechanism for NO and because of its highly reactivity, NO is rapidly inactivated *in situ*.

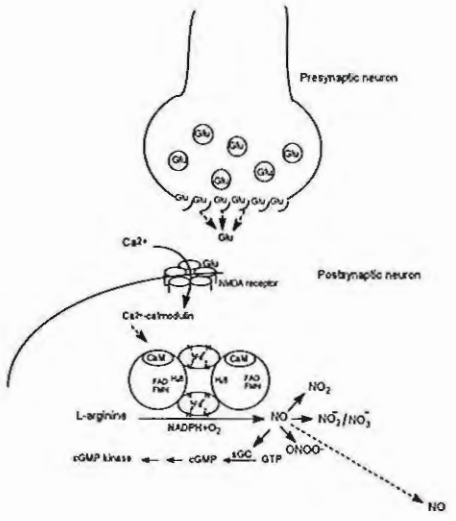

Figure 5 Overview of the biochemical pathway for nitric oxide synthesis and suggested targets for nitric oxide

NO potentiates transmitter release via direct stimulation of synaptic vesicle release. There are evidences that NO acts as a retrograde messenger,[7] that is, it diffuses back to the presynaptic neuron and binds to the iron of the heme cofactor of sGC leading to the increase of synthesis of cGMP which affects the amount of transmitter released. It has been found that because of the action of NO as retrograde messenger, a neuron or group of neurons in the hippocampus and the Purkinje cells of the cerebellum is repeatedly stimulated which leads to the occurrence of long term potentiation (LTP) and long term depression (LTD)[7,8]. LTP and LTD are identified as two processes that link with memory formation.

There are also several lines of evidence that suggest a role for NO in neurotoxicity of the brain[2,3]. During a stroke, delivery of oxygen and nutrients to affect regions of the brain is compromised and neurons in those areas become unable to exclude Ca^{2+}. When blood flow is restored to these calcium rich neurons, overstimulation of NMDA receptor occurs which in turn would activate the synthesis of large amounts of NO. The elevated level of NO has been linked to the inflammation associated tissue damage and the formation of NO active species such as peroxynitrite, a strong oxidant, capable of oxidizing thiols and DNA bases[9]. Mechanisms proposed for NO neurotoxicity are the same as its action in the immune system.

NO IN THE IMMUNE SYSTEM

The role of NO in the immune system is quite different from its function in either neurons or blood vessels. The activation of macrophages and neutrophils by cytokines and/or endotoxins, results in the synthesis of NOS, called inducible NOS[10] or iNOS. In contrast to NOS found in brain (bNOS) and vascular endothelial cells (eNOS), iNOS is not Ca^{2+}-dependent and always contains tightly bound calmodulin that allows the enzyme to be fully active at basal level of Ca^{2+}. The activity of iNOS lasts for many hours following the stimulation of its synthesis. The activated macrophages and neutrophils produce much more large quantities of NO reactive species, such as the potent oxidant peroxynitrite ($OONO^-$), than vascular endothelial cells or neurons do[3].

The reactive species of NO diffuse to tumor cells nearby and interfere with several cellular processes by interact with the iron-sulfur center of several important macromolecules including *cis*-acotinase, an enzyme involved in tricylic AMP cycle and the complex I and complex II in the mitochondrial electron transport chain by forming complexes of general formula $(RS)_2Fe(NO_2)^2$. These interaction diminish the cell's ability to produce and use NAPDH, leading to a decrease in ATP synthesis. NO is produced by the activated macrophages in enough amount to inhibit ribonucleotide reductase, an enzyme catalyses the forming of deoxynucleotides, which is a precursor in DNA synthesis. The interaction of NO to the active sites on this enzyme, a tyrosyl radical, a nonheme iron and thiol groups, causes depletion of deoxyribonucleotides and ultimately leads to inhibition of cell growth[3].

NO has also been reported for its neurotoxicity as well as tumoricidal and bactericidal action by inactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) through S-nitrosylation of the active site cysteine[2]. NO also enhances ADP-ribosylation of poly (ADP-ribose) synthase, (PARS), a nuclear enzymes-activated by DNA strand breaks.

The increase of ribosylation causes cell death through depletion of ATP and the source of ADP-ribose, β–nicotinamide adenine dinucleotide[11].

NO IN NANC TRANSMISSION

NO-sensitive neurons that do not response to the neurotransmitter acetylcholine or norepinephrine can be found in several peripheral tissues including the cardiovascular, urogenital, respiratory and digestive system. Therefore NO has also been implicated as one mediator of nonadrenergic noncholinergic (NANC) neurotransmission.

NOS can be found in the myenteric plexus of neurons in all regions of the gastrointestinal (GI) tract[12]. These neurons mediate the physiologic relaxation of the part of GI tract that participates in the normal peristaltic activities of digestion. The gastric mucosa can become more susceptible to lesion and ulceration, when NO synthesis in GI system is impaired[2]. These evidences suggest the involvement of NO in the muscle relaxation and the cytoprotection of the gastric lining. The abnormality of enlarged stomach was found in mutant mice lacking a form of NO-synthesizing enzyme called bNOS which can be found in brain and peripheral nerve cells. Lacking of bNOS leads to decrease production of NO which controls the sphincter that must open to allow food to pass from stomuch to intestine[2]. NO was also found to mediate penile erection by relaxing smooth muscle in the corpus cavernorsum, the major erectile tissue of this organ[13]. Relaxation of the tissue allows increased blood flow into the penis, causing erection.

FUTURE DIRECTIONS

As several studies have been done on NO as an important compound play roles in many different physiological processes, an understanding of the involvement of NO in pathophysiology is evolving. The physiology and biochemistry of NO-mediated processes have been translated into therapeutic benefits. There are several researches on NO leading to a potential drugs, either research on NO-releasing agents or the supplement to the old drugs with agents that will make them more effective to be used in vascular disorders, pulmonary hypertension[9] or erectile dysfunction or impotence and the apoptotic cell death induced by treatment with tumor necrosis factor α (TNFα) plus actinomycin D[14]. These NO donors may also play a beneficial role in the tissue transplant or autoimmune disease such as rheumatoid arthritis. Selective inhibitors of individual forms of NOS which offered the therapeutic benefit with lower toxicity can be used in cerebral ischemia or postischemic damage in stroke to prevent the neurotoxicity from NO. The inhibition of NO synthesis was also found to have a significant beneficient effect on disease states such as schizophrenia, migraine headache, Alzeimer's diseases, developement of colitis[15]. NOS inhibitors were also found to prevent tolerance to morphine and the destruction photoreceptors in the retina[9,15].

Although several studies have been done in NO research and results from these trials are promising, much remains to be accomplished and efforts will undoubtedly increase to use this knowledge to discover new NO-related therapies.

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Dietary Antioxidants

A diet rich in fruits, nuts, grains, and vegetables seem protective against cardiovascular disease and several forms of cancer. Some or all of this effect may be due to the antioxidants present, although there are many other protectives agents also.

Vitamin C

Water-soluble vitamin that has multiple metabolic roles. Antioxidatant action is only one of this effects. It is thought an important role in the respiratory tract in protecting against oxidizing air pollutants.

Vitamin E

Fat-soluble vitamin. Essential antioxidant in humans. Low intake increase risk of cardiovascular disease; severe deficiency leads to neurodegeneration.

Flavonoids, other plant phenolics, wine phenolics

Many plant phenols inhibit lipid peroxidation *in vivo*. It has been speculated that flavonoids in red wine could partly explain the so-called French paradox (the low incidence of death from heart disease despite a high-fat diet, high prevalence of smoking, and other risk factors). More data are needed on absorption and bioavailability of plant phenolics, but there is some evidence that wine and tea phenolics are absorbed, and can make blood lipids more resistant to peroxidation.

Beta-carotene, other carotenoids, related plant pigments

There is increasing epidemiological evidence that high body levels of these pigments are associated with diminished risk of cancer and cardiovascular disease, particular in smokers. Many carotenoids have been claimed to exert antioxidant events *in vivo* (although sometimes questionable assays have been used).

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NEW DRUGS

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This issue new drugs provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications.

Selective COX-2 inhibitors

The widely used nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the cyclooxygenase (COX) which converts arachidonic acid into prostaglandins (PGs). COX exists in at least two isoforms. COX-1 (constitutive isoform) is present constitutively under physiological condition. COX-2 (inducible isoform) is induced in various cell types by mitogens and cytokines including endotoxin. The marketed NSAIDs are differential effects on COX-1 and COX-2. PGs produced by COX-1 have important role in normal platelet, gastric and renal function. As a consequence, inhibition of COX-1 results in unwanted side effects. The developing selective COX-2 inhibitors may offer an advantage over the existing non-selective NSAIDs by reducing the associated renal and gastric toxicity.

Some of these new drugs are:

1. A novel series of 1,2-diarylcyclobutenes has evaluated as potential selective COX-2 inhibitors. 4,4-dimethyl-2-phenyl-3-[4-(methylsulphonyl)phenyl]cyclobutenone (figure 1) was shown to be particularly selective for COX-2 and orally active in the rat paw edema model (ED_{50} = 2.4 mg/kg). (Friesen and coworkers. *Bioorg Med Chem Lett* 1996; **6:** 2677-2682).

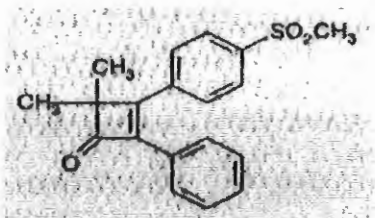

figure 1

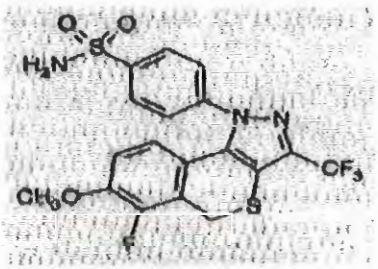

figure 2

2. The agents based on the synthesis and evaluation of series of 1,5-diarylpyrazones containing either a sulphone or sulphonamide moiety as COX-1 and COX-2 inhibitors have been described. The series of benzothiopyrano-pyrazoles, exemplified by figure 2, were found to be both selective COX-2 inhibitors *in vitro* and antiinflammatory agents *in vitro* in the air-pouch model of inflammation. (Bertenshaw and coworkers. *Bioorg Med Chem Lett* 1996; **6:** 2827-2830).

3. DuPont Merck (Wilmington, DE, USA) has described the new class of COX-2 inhibitors, the terphe-nyls, which they discovered while seeking to improve the in vitro selectivity of their selective COX-2 inhibitor, diarylthio-phen (figure 3 DuP697). The terphenyl compound (figure 4) was identified as a potential lead compound having good COX-2 selectivity and a better pharma-cokinetic profile than DuP697. (Pinto and coworkers. *Bioorg Med Chem Lett* 1996; **6:** 2907-2912).

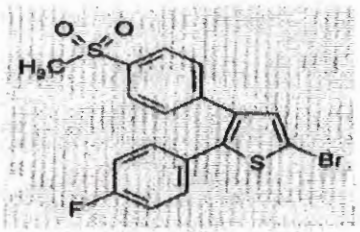

figure 3

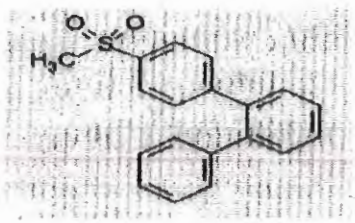

figure 4

4. The agents based on the synthesis and evaluation of a series of 3,4-diarylpyrazoles as potential COX-2 inhibitors have been described. A number of these compounds, exemplified by figure 5, were found to be potent selective inhibitors of COX-2 and shown to have oral antiinflammatory activity in a rat carrageenan-induced foot pad edema assay. (Pinning and coworkers. *Bioorg Med Chem Lett* 1996; **7:** 2122-2124).

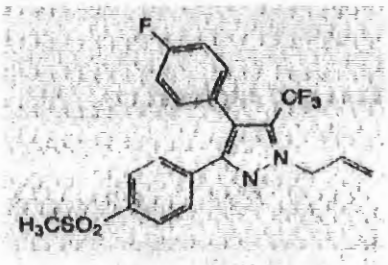

figure 5

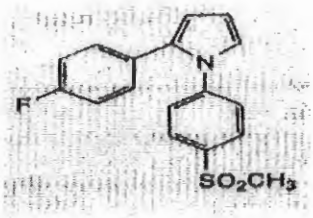

figure 6

5. A group from Searle Research and Development (Skokie, IL, USA) have described the identification of 1,2-diarylpyrroles as potent, selective inhi-bitors of COX-2 (IC_{50} = 15-100 nM). In vivo testing of these compounds in the rat carrageenan induced paw edema model established that the compounds were orally active anti-inflammatory compounds with the most potent inhibitor of edema (figure 6) having an ED_{50} of 4.7 mg/kg and a 200-fold selectivity for COX-2 over COX-1. (Khanne and coworkers. *J Med Chem* 1997; **40:** 1619-1633).

6. The agents based on the synthesis and evaluation of 1,2-diaryl-imidazoles as COX-2 inhibitors have been described. These compounds were also found to be potent and highly selective inhibitors of the human COX-2 enzyme. Several of these com-pounds, exemplified by figure 7, were found to exhibit excellent inhibition in the adjuvant-induced arthritis model (ED_{50} = 0.02 mg/kg). The 1,2-diarylimidazoles were also shown to inhibit carrageenan-induced rat paw edema and hyperalgesia, with several orally active compounds showing no gastrointestinal toxicity in either the rat or mouse at up to 200 mg/kg, suggesting that these compounds offer potential as anti-inflammatory agents with reduced side effects. (Khanne and coworkers. *J Med Chem* 1997; **40:** 1634-1647).

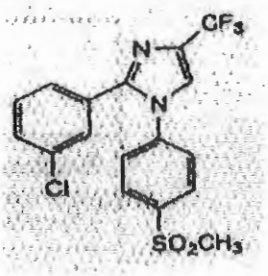

figure 7

PHARMACOLOGICAL DIGEST

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Use of Clostridial Collagenase in Clinical Practice

Mammalian collagenases belong to the family of metalloproteinases. They specifically cleave collagen and thus play an important role in the metabolism of collagen in mammalian tissues. The localisation of the enzyme in burn wounds - within cells as well as within the wound site - suggests that the enzyme directly takes part in the healing mechanisms. In healthy subjects, where wounds normally heal by primary intention, the amount and activity of endogenous collagenases are sufficient for the removal of dead tissue from the wound. In patients presenting with chronic nonhealing wounds such as pressure sores, venous leg ulcers and diabetic ulcers etc. may cause an impairment of endogenous collagenase production and activity, and thus lead to insufficient removal of dead tissue. Although the mechanism of action of collagenases during the wound-healing process is not yet fully understood, pharmacological and clinical data show the beneficial effect that can be achieved by the supplementation of clostridial collagenase to necrotising wounds.For this reason it seems appropriate to consider the application of clostridial collagenase to such wounds in order to reinforce the body's own cleaning and healing mechanisms.

[Journal of Clinical Drug Investigation 1998; 15(3): 245-252.]

Use of Famotidine in Infants and Children : Despite the Lack of FDA Approval

The histamine type-2 receptor antagonists (H2RAs) have made a significant impact on the prevention and management of gastroesophageal reflux and ulcers. The University of Virginia Pharmacy and Therapeutics Committee has recently approved a therapeutic interchange program for this class, with famotidine as the agent of choice. It offers the advantages of having relatively few adverse effects and no significant drug interactions. Famotidine is currently approved by the Food and Drug Administration for both treatment and maintenance therapy of duodenal ulcers, gastric ulcers, pathological hyper-secretory conditions, and gastro-esophageal reflux disease in adults, but not for use in children. Despite the lack of FDA approval, the H2RAs have been widely used in the pediatric population. There are nearly a dozen publications describing famotidine use in infants and children. The majority of these studies have focused on the use of famotidine in the prevention of stress erosions. The ideal dosing regimen is still debated. However, additional clinical trials, particularly in infants and young children, are needed to better establish the safety and efficacy of current dosing strategies.

[Pediatric Pharmacotherapy 1998; 4(2)]

Is Enalapril Safer Than Nisoldipine in Hypertensive Diabetics?

Cardiovascular disease is the leading cause of death in people with non-insulin-dependent diabetes mellitus (NIDDM). One means of reducing cardiovascular risk in this group is to treat hypertension. ACE inhibitors, such as enalapril, and calcium-channel blockers, such as nisoldipine, are commonly used antihypertensives. As part of the Appropriate Blood Pressure Control in Diabetes (ABCD) Trial, this study compared the incidence of fatal and nonfatal myocardial infarction (MI) over a 5-year period between 235 NIDDM patients receiving nisoldipine and 235 NIDDM patients receiving enalapril. The results appear to indicate that "an ACE inhibitor is the preferred antihypertensive agent, rather than a dihydropyridine calcium-channel blocker, for the prevention of cardiovascular complications, specifically myocardial infarction, in patients with NIDDM."

[The New England Journal of Medicine. 1998; 338: 645-652.]

New Research on Cancer Genes

The human gene most frequently linked to oncogenesis -- p53 -- was the subject of much research. The p53 gene, in normal cells, suppresses tumor activity. Mutations in the gene allow unrestricted cellular growth and tumor formation. The Schering-Plough Gene Therapy Study Group used an adenovirus to deliver the normal p53 gene into tumors in patients with various types of tumors. Phase I trials showed that the gene was successfully delivered into various solid tumors: colon cancer metastatic to the liver, ovarian cancer, melanoma, head and neck cancer, and nonsmall-cell lung cancer. In a study of children with neuroblastoma, researchers found abnormal levels of the unmutated p53 protein in tumor cells in cytoplasm, which suggest that, in neuroblastoma, the p53 is not mutated but is somehow inactivated. E1A is another suppression gene, one that has suppressed metastasis, induced apoptosis (programmed cell death), and countered the overexpression of the breast cancer gene HER-2/neu. In a 12-patient study, researchers were able to transfer E1A into tumor cells using liposomes, and E1A expression led to HER-2/neu suppression in breast and ovarian cancer cells. Several studies looked at p73, a gene thought to be produce a p53-like protein. Discovery and cloning of p51, another possible p53 family member, was reported at the annual meeting of the American Association for Cancer Research (AACR) by Japanese researchers.

[Pharmacotherapy News Network : Apr. 1, 1998: Cancer Update: News from the annual meeting of the American Association for Cancer Research.]

Memantine: An Investigational Drug for the Alleviation of Neuropathic Pain

Memantine is an orally available N-methyl-D-aspartate (NMDA) antagonist, which has been shown to inhibit prophylactically or therapeutically enhanced feelings of pain caused by nerve damage. It currently is undergoing clinical trials in patients with neuropathic pain. In a recent phase II clinical trial, memantine subjects with painful diabetic neuropathy experienced significant reductions in pain compared with placebo-treated subjects. In contrast, it was not effective in controlling pain in subjects with postherpetic neuralgia.

Published reports indicate that memantine also may be effective in the treatment of Parkinson's disease and acquired pendular nystagmus in patients with multiple sclerosis.
[From issue No. 246 (March, 1998) of Medical Sciences Bulletin]

First Oral Drug for Erectile Dysfunction Approved

Sildenafil citrate, the first pill to treat erectile dysfunction was approved last month by the Food and Drug Administration. It acts by enhancing the by enhancing the smooth-muscle relaxant effects of nitric oxide, a chemical normally released in response to sexual stimulation. This smooth muscle relaxation allows increased blood flow into certain areas of the penis, leading to an erection. The pill, taken about an hour before sexual intercourse, is effective in about 70% of patients. The most common side effects include headache and indigestion. Some patients on sildenafil citrate (about 3%) also reported changes in vision, principally altered color perception.The drug should not be used with organic nitrates such as nitroglycerin patches or sublingual tablets because the combination may lower blood pressure. Because new drug has not been studied in combination with other treatments for impotence, the FDA does not recommend the use of such combinations.
[Medical Tribune: Family Physician Edition 1998: 39(8)]

Treatment of LRTIs: Two Antimicrobials Compared

Lower respiratory tract infections (LRTIs) commonly require antimicrobial therapy. Historically, ampicillin has been used as a primary empiric agent. However, antimicrobial resistance among these organisms continues to increase. This has led to combining ampicillin with a beta-lactamase inhibitor, such as sulbactam, which protects ampicillin from enzymatic degradation. The efficacy and safety of intravenous ampicillin/sulbactam and cefoxitin, a second-generation cephalosporin, were compared in 75 inpatients with bacterial infections of the lower respiratory tract. Results demonstrated that ampicillin/sulbactam and cefoxitin have similar efficacy and safety profiles in the treatment of lower respiratory tract infections. Ampicillin/sulbactam is a cost-effective alternative in the treatment of these infections.
[Infect Med 1998: 15(4):256,259-263]

Montelukast Approved for Asthma in Adults and Children

Montelukast was recently approved by the FDA for the prevention and chronic treatment of asthma in adults and children aged 6 years and older. It is the third antileukotriene agent (the first two being zafirlukast and zileuton) to be approved for treatment of asthma and the only antileukotriene approved for use in children. Montelukast is a potent and specific antagonist of the cysteinyl leukotriene receptor, known as the CysLT1 receptor, and thus inhibits the physiologic action of leukotriene D4 at this receptor. After oral administration, montelukast is rapidly absorbed, with mean oral bioavailability of 64%. Maximum plasma concentrations are reached 3 to 4 hours after administration of a 10-mg film-coated tablet and 2 to 2.5 hours after administration of a 5-mg chewable tablet. It is eliminated predominantly by metabolism followed by biliary excretion. The mean plasma half-life in young adults ranges from 2.7 to

to 5.5 hours. Clinical trial results suggest that it can be administered as controller therapy for patients with mild, persistent asthma whose symptoms are not controlled with as-needed beta agonist.
[issue No. 246 of Medical Sciences Bulletin 1998]

Endocannabinoids: A New Class of Vasoactive Substance

Endogenous cannabinoids (endocannabinoids) have recently been identified in the CNS and attention has now turned to their cardiovascular actions. The prototypic endocannabinoid, anandamide, dirived from arachidonic acid, has been shown to be a vsorelaxant, particularly in the resistance vasculature. This vasorelaxation has been shown to be both endothelium-independent and -dependent, depending on the vascular bed. It has been proposed that an endocannabinoid may mediate the nitric oxide - and prostanoid-independent component of endothelium-dependent relaxations, as these responses are sensitive to a cannabinoid receptor antagonist and show similarities to anandamide-induced relaxations. In addition, it has recently been shown that anandamide is produced by endothelial cells. Clearly, much work is required to adequately define the physiological significance of endocannabinoids in the cardiovascular system.
[TiPs 1998; 19: 55-58]

Nerve Growth Factor May Heal Corneal Ulcers

Neurotrophic corneal ulcers occur when the sensory innervation of the cornea is disrupted. Such ulcers lead to progressive corneal scarring and visual loss. There has been no effective known treatment for such ulcers, and management has consisted of protecting the remaining cornea to prevent extension of scarring and superimposed infection by surgically closing the eye or by the patient wearing a patch or protective contact lenses.A preliminary report by Italian researchers, Alessandro Lambiase and colleagues, showa that Topical application of eye drops containing nerve growth factor can heal corneal ulcers and restore damaged vision. It is the first to show a promising medical treatment for the disorder. If the treatment proves beneficial in the long term it may obviate the need for many corneal transplants.
[BMJ 1998;316:1333]

Thai Journal of Pharmacology

Instruction for Authors

The Thai Journal of Pharmacology serves as the official journal of the Pharmacological and Therapeutic Society of Thailand. The journal is designed to contribute to the publication of researches and information exchanges in the field of pharmacology and related fields. The manuscripts should not have been published before. Original full length scientific research papers, reviews, short communication, case report, new drugs development and pharmacological digest will be included in this journal .

Manuscripts

Three copies of manuscript, diskette(s) and illustration(s) are required. Manuscript should be written in English, the others can be English or Thai. The preparation of the manuscript should be in the form of Microsoft Word (front: Times New Roman with size 12). Pages should be numbered consecutively, including the title page.

Table and Illustration should be numbered with arabic figures consecutively in the order of first citation in the text and supply a brief title for each. Explain in footnotes all non-standard abbreviation that are used. Illustrations should be professionally drawn and photographed or produced on a laser printer.

Nomenclature should follow the recommendations of the International Union for Pure and Applied Chemistry (IUPAC), and the International Union for Biochemistry (IUB). All measurements must be in System International (SI) units.

Research articles

The research papers should contain a) title, b) abstract, c) keywords, d) introduction, e) materials and methods, f) results, g) discussion, h) references.

***The title page*:** Should contain the title of the article, author(s) name and affiliation (s) laboratory or institute of origin and address. Name and complete address of author responsible for correspondence about the manuscript should be also placed at the foot of the title page. *An abstract* limited to approximately 250 words should be carried in this page. It should be informative and state concisely what was done, results obtained and conclusion. Three to ten *key words* or short phrases appropriate for subject indexing should be typed at the bottom of abstract.

***Introduction*:** State clearly the purpose of article, the rationale for the study or observation. Relevant previous study should be cited and do not review the subject extensively .

***Materials and Methods*:** Describe the sufficient detail of the method, experimental subjects (patients or experimental animals, including controls) clearly. Identify the method, apparatus (manufacturer's name and address in parenthesis). Give references to established method, study design and statistical method .

***Results*:** Present your results logical sequence in the text, tables, and illustrations. Only important observations should be summarized and emphasized. Do not repeat in the text all the data in the table or illustrations.

Discussion: Comment on the results and integrate them with the existing knowledge and point out the field. Recommendation may be also included.
Acknowledgment: Persons, financial or technical helps which have contributed to the paper can be acknowledged in a paragraph.
References: Place the number references consecutively in the order in which they are first mention in the text. Use the style of the examples below:

Examples
Articles in journals
(1) Standard journal article (List all authors, but if the number exceeds three give three followed by et al)

You CH, Lee KY, Chen RY, Menguy R. Electrogastrographic study of patients with unexplained nausea, bloting and vomitting . *Gastroenterology* 1980; 79:311-314.

(2) Organisation as author

The Royal Marsden Hospital Bone-marrow Transplantation Team. Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. *Lancet* 1977;2:742-724.

(3) No author given

Coffeee drinking and cancer of the pancrease (editorial) BMJ 1981;283-628.

(4) Volume with supplement

Magni F, Borghi S, Berti F. BN-52021 protects guinea-pig from heart anaphylaxis. *Pharmacol Res Commun* 1988;20 suppl 5:75-8.

(5) Books and other monographs

5.1 Personal author(s)
Colson JH, Armour WJ. *Sports injuries and their treatment*. 2nd rev ed. London: S Paul, 1986
5.2 Editor(s), compiler as author
Diener HC, Wilkinson M, editors. *Drug-induced headache*. New York Springer-Verlag, 1988.
5.3 Chapter in a book
Jaffe JH, Martin WR. Opioid analgesics and antagonists. In: Gilman AG, Goodman LS, Gilman A, editors. *The Pharmacological basic of therapeutics*. 6th ed. New York: MacMillan Publishing, 1980:494-543.
5.4 Conference proceedings
Vivian VL, editor. Child abuse and neglect: a medical community response. *Proceeding of the first AMA National Conference on Child Abuse and Neglect;* 1984 Mar 30-31; Chicago. Chicago: American Medical Association, 1985.

(6) Dissertation

Youseff NM. *School adjustment of children with conjenital heart disease* (dissertation). Pittsburg (PA): Univ of Pittsburg, 1988.

(7) In press

Lillywhite HB, Donald JA. Pulmonary blood flow regulation in an aquatic snake. *Science.* In press.

Reviews

All Reviews are usually peer-reviewed. If the manuscript is written in Thai, English title and abstract are also required.

Short Communications

Short communication should contain new and unpublished results in a short form. It should not exceed 2 print pages and may contain one table and one illustration.

Manuscript submission

All manuscripts are to be submitted to editor or associate editors, Thai Journal of Pharmacology, Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Chulalongkorn Hospital, Rama IV Road, Bangkok 10330, Thailand. All paper are critically reviewed by the invited referees. Reviewers' comments are usually returned to the authors.The editorial board will decide upon the time of publication and retain the right to modify the style of contribution. However, major changes will be agreed with the authors. Authors will receive 25 reprints free.

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เรียน ผู้จัดการวารสารเภสัชวิทยา

ข้าพเจ้า.....
ที่อยู่.....
.....

มีความประสงค์จะรับวารสารเภสัชวิทยา ปีที่.....ฉบับที่.....เป็นต้นไป รวม.....ฉบับ พร้อมกันนี้ได้แนบเช็คไปรษณีย์หรือธนาณัติในนาม " ผู้จัดการวารสารเภสัชวิทยา" สั่งจ่าย ณ.ปณ.ปทุมวัน เป็นจำนวนเงิน.....บาทมาเป็นค่าสนับสนุนการจัดทำวารสารด้วยแล้ว

ลงชื่อ.....
( )

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ใบสมัครเข้าเป็นสมาชิก

เขียนที่.....

วันที่.....เดือน.....พ.ศ.....

นาย

ข้าพเจ้า นาง.....ชื่อสกุล.....

นางสาว

อาชีพ.....ขอสมัครเข้าเป็นสมาชิกสมาคมเภสัชวิทยาแห่งประเทศไทย และขอรับรองว่าจะปฏิบัติตามระเบียบข้อบังคับของสมาคมฯ ทุกประการ

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ทะเบียนประวัติ

นาย

1. ชื่อ นาง.. .....ชื่อสกุล.....

นางสาว

ชื่อภาษาอังกฤษ (ตัวพิมพ์ใหญ่).....

2. เกิดเมื่อวันที่.....เดือน.....พ.ศ.....

3. ตำแหน่งหน้าที่หรือตำแหน่งทางวิชาการในปัจจุบัน

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4. สถานที่ทำงาน.....

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.....โทรศัพท์.....

5. ที่อยู่ปัจจุบัน.....

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.....โทรศัพท์.....

6. ที่อยู่ถาวร.....

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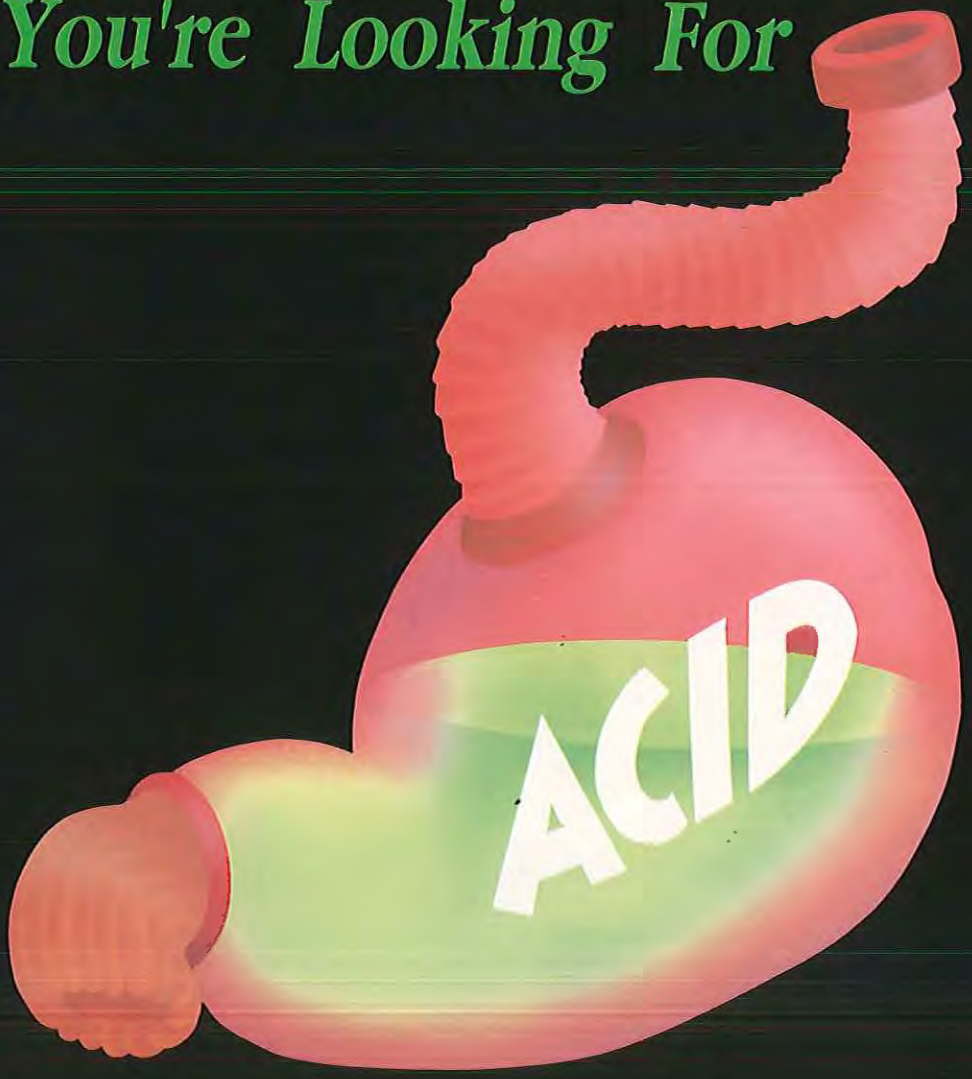

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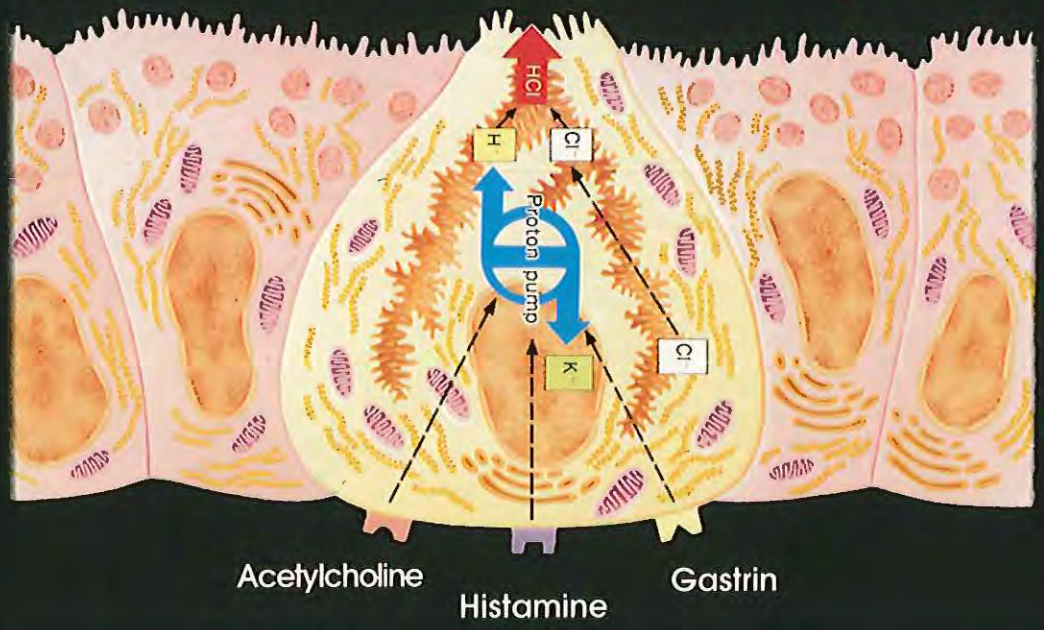

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Presentation white hard capsule containing 30 mg Lansoprazole as enteric coated granules. **Indication** Duodenal ulcer,gastric ulcer, stomal ulcer, reflux esophagitis and Zollinger-Ellison syndrome. **Dosage and Administration** Usually for adults, administer one capsule (30 mg) orally once daily. Duodenal ulcer: 30 mg once daily for 4 weeks. Gastric ulcer, reflux esophagitis & stomal ulcer: 30 mg once daily for 4-8 weeks. Zollinger-Ellison syndrome: The dosage should be adjusted according to the patients signs and symptoms. **Precautions** Its use should be avoided in pregnant women and nursing mothers unless the potential benefits outweigh the possible hazards. For nursing mothers, breast feeding should be discontinued if the use of lansoprazole is considered essential. **Side effects** Generally trasient and self-limiting, including gastrointestinal symptoms, headache, dizziness, rash and increases in liver function tests. Hematological changes have been reported rarely. **Storage** Store at room temperature (below 30° c). **Expiration** 3 years after manufacture. Package Box of 3 X 10 capsules.

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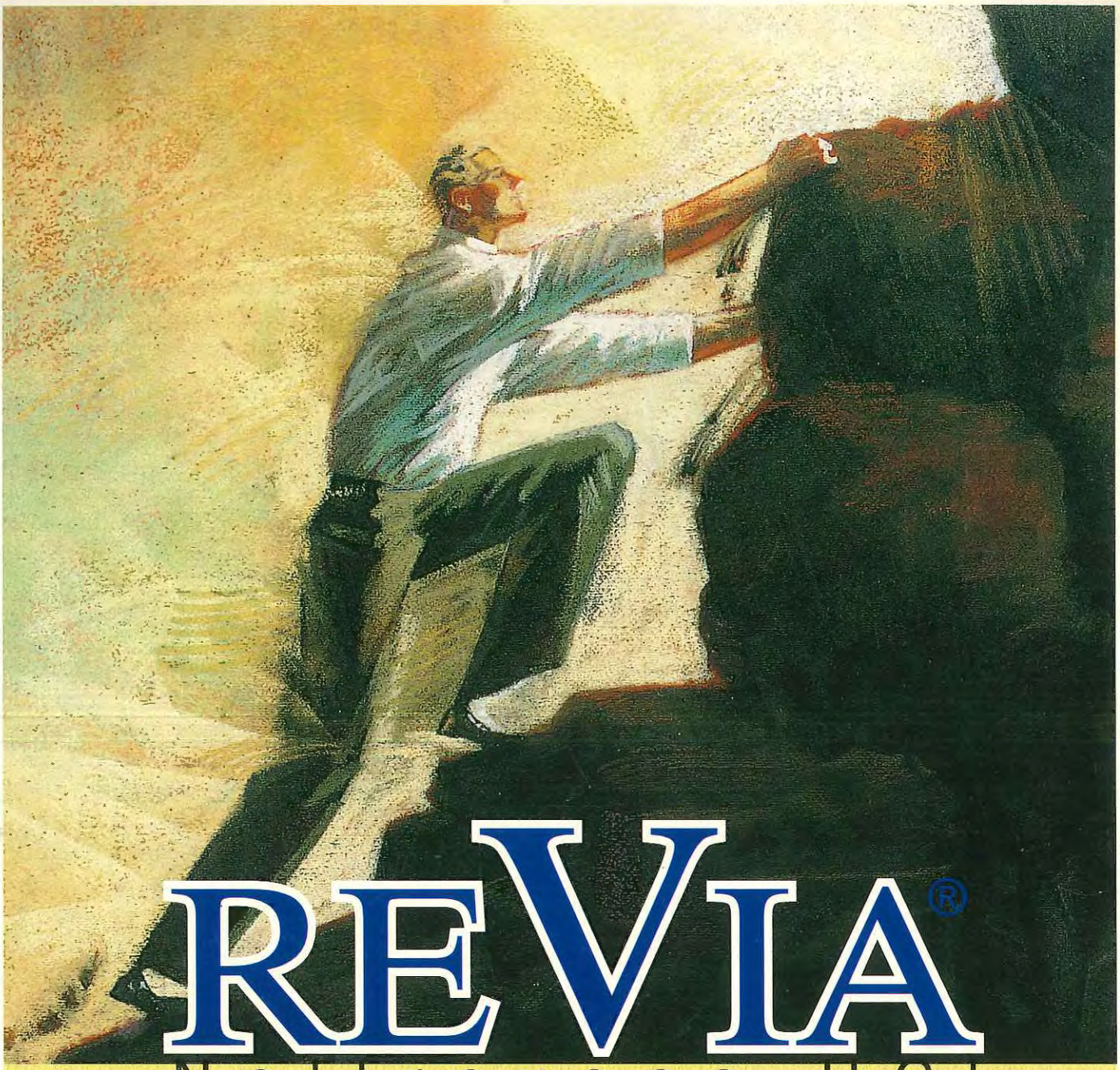

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References:

1. Product Monograph, Data on Files/DuPont Pharma U.S.A. 2. Clinical Information, Data on File/DuPont Pharma U.S.A. 3. Opioid Addiction, Medical Progress, April 1997: p.39-44. 4.Addiction Medicine/Contempo 1997. JAMA SEA Nov. 1997: p.11-17. 5.Experts Debate Merits of 1-Day Opiate Detoxification Under Anesthesia. JAMA SEA July 1997; p.11-14.

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