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ORIGINAL ARTICLE

INHIBITION OF HEPATIC DRUG METABOLISM BY ANTICANCER AGENTS

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SUMMARY

Pretreatment of the rats with methotrexate, cyclophosphamide and 5-fluorouracil could cause inhibition of hepatic drug metabolism of zoxazolamine, aminopyrine and aniline, as evidenced by a prolongation of zoxazolamine paralysis time and a decrease in the elimination of the latter two amines from the plasma. However, the rate of absorption of both aminopyrine and aniline were unaffected. *In vitro* investigations were consistent with the view that the most likely cause of this inhibited hepatic drug metabolism produced by these anticancer agents was the reduction in one or more components of the drug-metabolizing enzyme system, including the cytochrome P-450 content.

Chemotherapy plays a very important role in the treatment of neoplastic diseases. Single drug or several drugs in combination have been successfully employed either alone or in combination with surgery or radiotherapy. The therapy is mostly palliative but not curative (1). However, the majority of drugs used are highly toxic and also affect rapidly growing cells other than those of the neoplastic process. As a consequence, their therapeutic index is low.

Many studies have reported that anticancer drugs are cytotoxic to liver cells. Hepatitis, cirrhosis, fibrosis and fatty change in liver cells have been found in liver biopsy samples from psoriatic patients treated chronically with methotrexate (2). In the study of

Custer et al. (3) it was found that rats given more than 125 μg/kg methotrexate intraperitoneally five times per week for 24 months developed serious liver damage-namely, varying degrees of fatty metamorphosis, necrosis, atrophy of hepatic cord and fibrosis. Liver toxicity caused by cyclophosphamide is also well documented but 5-fluorouracil-induced hepatotoxicity is rare.

Relatively little is known of the effects of anticancer drugs on the action, metabolism and toxicity of other drugs used concomitantly in cancer patients. Decreased activities of hepatic microsomal drug-metabolizing enzymes have been reported in rats treated with 5-fluorouracil, cyclophosphamide, methotrexate, mechlorethamine, 6-mercaptopurine, or daunorubicin (4-7). The basis for these decreased enzymatic activities has not been fully elucidated.

The objective of this study is to determine the effects of subacute treatment of methotrexate, cyclophosphamide and 5-fluorouracil on hepatic drug-metabolizing enzymes.

MATERIALS AND METHODS

Experimental Animals and Chemicals

Adult male Fisher rats of about 60 days of age and weighing 170-200 g were used in the study. The animals were supplied by the Animal Center of the Faculty of Science, Mahidol University, Bangkok. Four rats were kept in one cage suspended over Absorb-Dri. All animals were allowed access of food (Purina Laboratory Chow, Zuellig Pte, Ltd., Singapore) and tap water ad libitum until 16 hours before sacrifice, during which time they were allowed access to water only.

Chemical agents used in this experiment were obtained commercially in the highest grade available without further purification.

Treatment of Experimantal Animals

The experimental animals were fasted overnight prior to treatment with the anticancer agents, which were freshly prepared just before use as a suspension in normal saline. All drugs were given to the animals in the morning around 8.00 to 9.00 A.M. After having been given the drugs, the rats were again fasted for about 16 hours before sacrifice.

1. Subacute experiments

The animals were intubated once daily for 5 consecutive days with methotrexate (1, 2, 3 and 5 mg/kg) and cyclophosphamide (20, 40 and 60 mg/kg) and the others were injected intraperitoneally with 5-fluorouracil (10, 20 and 30 mg/kg). Control animals received an identical treatment with normal saline solution. The animals were weighed and sacrificed 48 hours after last dose the drug.

2. Recovery experiments

The animals were treated orally with 2 mg/kg methotrexate and 40 mg/kg cyclophosphamide and intraperineally with 30 mg/kg 5-fluorouracil, once daily for 5 days. They were killed at 2, 4, 6, 10, 15 and 20 days after the last dose of the 5-day pretreatment schedule.

3. Collection of blood samples

Blood samples were collected from rats by heart puncture under light ether anesthesia. Heparin sodium solution (200 units per ml blood) was preintroduced into the syringe to prevent blood coagulation. The blood was then centrifuged at 3,000 rpm for 20 min in the International Centrifuge Size 2 Model K and the plasma was removed with a pasteur pipette. The plasma was kept under deep freeze at -20° C for further quantitative determination of drugs.

Analytical Assays

In the assay of aminopyrine N-demethylase or aniline hydroxy-

lase, the hepatic postmitochondrial fraction was used as enzyme source. In both cases, however, enzymatic activity was expressed per mg microsomal protein. Both the enzyme source and the microsomes were prepared by the method described by Unchern and Thithapandha (8).

The activities of aminopyrine N-demethylase and aniline hydroxylase were determined by the methods described by Mazel (9), except that 20 μmole of aminopyrine or 10 μmole of aniline and 1 μmole of NADP were used.

Microsomal protein was determined by the method of Lowry et al (10) with bovine serum albumin as the standard. Cytochrome P-450 in the microsomal pellet was estimated by the method of Omura and Sato(11).

The amount of aminopyrine in the plasma was determined by the method described by Brodie et al (12). Palsma aniline concentration was measured by the method of Kupfer and Bruggeman (13).

Zoxazolamine Paralysis Time

Zoxazolamine paralysis time was determined by measuring the time interval between the onset of paralysis and the regain of the movement after an intraperitoneal administration of 60 mg/kg zoxazolamine. Zoxazolamine solution was freshly prepared at the concentration of 20 mg/ml, according to the method described by Conney et al (14).

Pharmacokinetic Analysis and Statistical Analyses

All pharmacokinetic parameters obtained were computed according to the one-compartment open model (15).

The results are reported as the mean value ± S.E. The statistical significance was analyzed by multiple comparison using the method of Newman and Keul's as described by Grimm (16) with the level of significance $p < 0.05$. The method of least square was employed to draw all regression lines for pharmacokinetic data.

RESULTS

As shown in Table 1, pretreatment of rats with methotrexate, cyclophosphamide and 5-fluorouracil at the doses indicated produced a certain degree of toxicities to the animals, as evidenced by the formation of blood stool and a significant reduction in body weight. Many of them died a few days later.

After the animals had been pretreated with these drugs, even at the reduced doses, zoxazolamine paralysis time were markedly prolonged when compared with the control (Table 2). These results thus suggested that the anticancer drugs might inhibit the hepatic microsomal metabolism of the muscle relaxant. When the effects of each drug were investigated further, it was found that methotrexate depressed the enzymatic activities of both aminopyrine N-demethylase and aniline hydroxylase in a somewhat dose-dependent manner (Table 3). It also caused a dose-dependent reduction in hepatic cytochrome P-450 content,

Table 1. The effect of anticancer agents on rat body weight

	Body Weight (g)			
	Control	Metho-trexate	Cyclophos-phamide	5-Fluoro-uracil
Before treatment	166 ± 3.2	179 ± 7.2	184 ± 3.9	178 ± 4.7
After treatment	175 ± 2.8*	164 ± 5.5*	155 ± 5.3*	148 ± 8.5*

Six rats in each group were treated with the drug once daily for 5 days as follows : methotrexate (5 mg/kg, PO) ; cyclophosphamide (60 mg/kg, PO) ; and 5-fluororacil (30 mg/kg, IP).
Control animals received an equal volume of saline. The asterisk indicates a significant difference between before and after treatment ($P < 0.005$).

Table 2. The effect of methotrexate, cyclophosphamide and 5-fluorouracil pretreatment on zoxazolamine paralysis time

Pretreatment	Paralysis Time (min)
Control	45 ± 3
Methotrexate	83 ± 6*
Cyclophosphamide	119 ± 5*
5-Fluorouracil	141 ± 10*

Male rats weighing 170-200 g were pretreated once daily for 5 days with methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO) and 5-fluorouracil (30 mg/kg, IP). Control animals received an equal volume of saline. Zoxazolamine (60 mg/kg) was injected (i.p.) about 50 hours after the last dose of the pretreatment; the paralysis time was then recorded. Results are expressed as mean ± S.E. from 7 rats. * $P < 0.01$ (from control; Newman-Keule' test)

while the liver weight and the microsomal protein level were unaffected (Table 4). The effects of cyclophosphamide on cytochrome P-450 content and the activities of these two drug-metabolizing enzymes qualitatively similar to those of methotrexate (Table 4), except that this alkylating agent also decreased microsomal protein content even at a low dose. 5-Fluorouracil at low doses had no effect on all of the above parameters though physically the animals appeared to be sick (Table 5). However, at a higher dose such as 30 mg/kg the drug could decrease all of these parameters except the liver weight.

The effects of methotrexate, cyclophosphamide and 5-fluorouracil seemed to be long lasting inasmuch as it took more than 20 days after the drug's last dose for aniline hydroxylase activity to return to normal (Fig. 1). Many of the treated rats died during this course of enzymatic recovery. When they died their drug-metabolizing activity

Table 3. Effects of methotrexate on various parameters of drug-metabolizing enzyme systems

Methotrexate (PO, Once daily for 5 days) (mg/kg)	Liver Weight (% of Body Weight)	Microsomal Protein (mg/g liver)	Aminopyrine N-Demethylase Activity[a]	Aniline Hydroxylase Activity[b]	Cytochrome P-450 (nmole/mg protein)
0	3.00 ± 0.05	31.80 ± 0.31	137.50 ± 1.79	14.15 ± 0.30	0.56 ± 0.01
1	3.02 ± 0.09	31.06 ± 0.50	121.00 ±10.70	11.98 ± 1.61	0.57 ± 0.02
2	3.97 ± 0.23	28.46 ± 1.06	68.54 ±12.57*	4.52 ± 0.75*	0.38 ± 0.03*
3	3.94 ± 0.17	29.14 ± 0.46	58.55 ± 9.75*	4.03 ± 0.87*	0.32 ± 0.03*
5	3.58 ± 0.09	31.09 ± 1.08	21.43 ± 2.15*	2.25 ± 0.20*	0.24 ± 0.03*

Values are expressed as mean ± SE from 6 determinations
a = nmole formaldehyde formed / mg protein / 30 min.
b = nmole p-aminophenol formed / mg protein / 20 min.
Asterisks indicate significant difference from control ($P < 0.05$; Newman-Keuls' test)

Table 4. Effects of cyclophosphamide on various parameters of drug-metabolizing enzyme systems

Cyclophosphamide (PO, Once daily for 5 days) (mg/kg)	Liver Weight (% of Body Weight)	Microsomal Protein (mg/g liver)	Aminopyrine N-Demethylase Activity[a]	Aniline Hydroxylase Activity[b]	Cytochrome P-450 (nmole/mg protein)
0	2.90 ± 0.08	38.98 ± 1.22	144.07 ± 6.61	13.77 ± 0.69	0.58 ± 0.02
20	2.87 ± 0.03	33.94 ± 0.85*	102.48 ± 4.27*	9.41 ± 0.68*	0.42 ± 0.06*
40	3.64 ± 0.18	31.57 ± 1.81*	83.73 ± 5.79*	5.16 ± 0.38*	0.28 ± 0.02*
60	4.02 ± 0.14	28.97 ± 1.23*	60.84 ± 7.46*	3.46 ± 0.30*	0.21 ± 0.01*

Values are expressed as mean ± SE from 6 determinations
a = nmole formadehyde formed/mg protein/30 min.
b = nmole p-aminophenol formed/mg protein/20 min.
Asterisks indicate significant difference from control ($P < 0.05$; Newman-Keuls' test)

Table 5. Effects of 5-fluorouracil on various parameters of drug-metabolizing enzyme systems

5-FU (IP, Once daily for 5 days) (mg/kg)	Liver Weight (% of Body Weight)	Microsomal Protein (mg/g liver)	Aminopyrine N-Demethylase Activity[a]	Aniline Hydroxylase Activity[b]	Cytochrome P-450 (nmole/mg protein)
0	3.08 ± 0.06	27.23 ± 1.02	127.22 ± 9.53	13.36 ± 1.03	0.52 ± 0.03
10	3.15 ± 0.06	27.93 ± 1.93	118.36 ± 8.04	10.76 ± 0.90	0.48 ± 0.03
20	2.97 ± 0.04	27.70 ± 1.59	120.53 ± 4.85	13.45 ± 0.54	0.55 ± 0.02
30	3.48 ± 0.10	22.50 ± 0.88*	59.77 ± 5.45*	7.36 ± 1.11*	0.32 ± 0.04*

Values are expressed as mean ± SE from 6 determinations.
a = nmole formaldehyde formed/mg protein/30 min.
b = nmole p-aminophenol formed/mg protein/20 min.
Asterisks indicate significant difference from control ($P < 0.05$; Newman-Keuls' test)

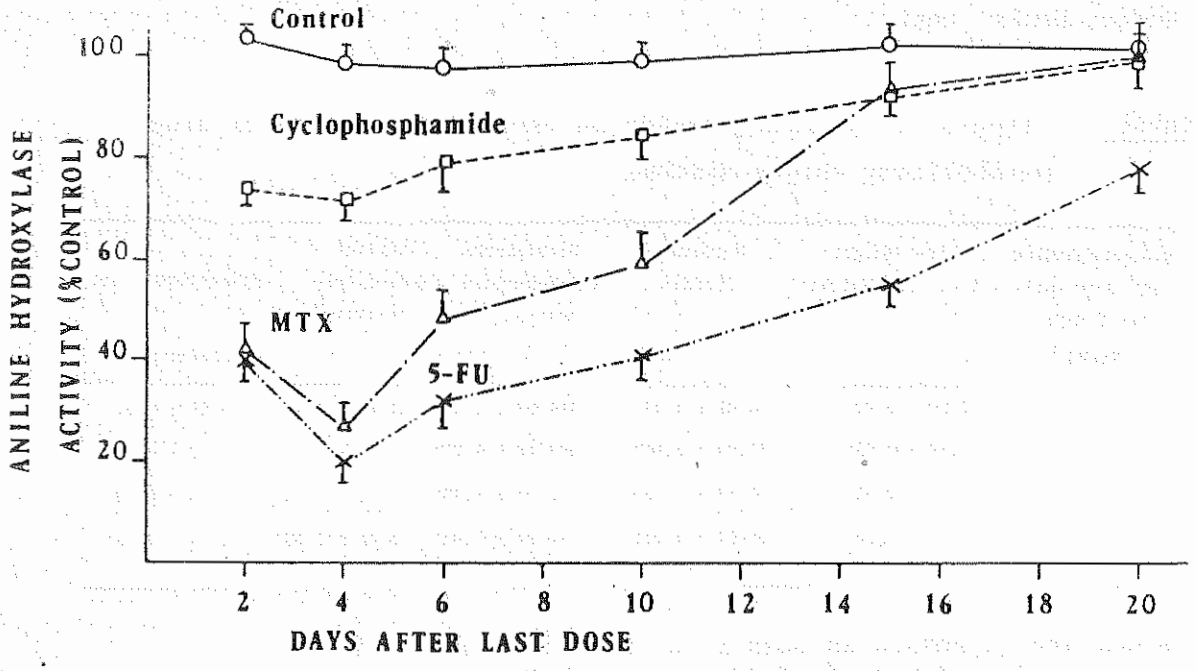

Figure 1. Time-course of the effects of anticancer agents on aniline hydroxylase activity. Methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO), and 5-fluorouracil (30 mg/kg, IP) were given once daily for 5 days as described previously. Each point represents mean ± S.E. from 6 separate determinations.

(aniline hydroxylase) was still low, approximately 20-40 % of control. Similar results were also obtained with aminopyrine N-demethylase (Fig. 2).

The plasma concentration-time curves of aminopyrine and aniline in control and drug-treated animals are shown in Figs. 3 and 4; their pharmacokinetic parameters are tabulated in Tables 6 and 7 . All of the three anticancer drugs caused an increase in both the plasma half-life and volume of distribution of the two tested amines. The drugs did not seem to cause any change in the initial rates of absorption of aminopyrine though they did produce somewhat lower peak levels of aniline in the treated groups (Figs. 3 and 4).

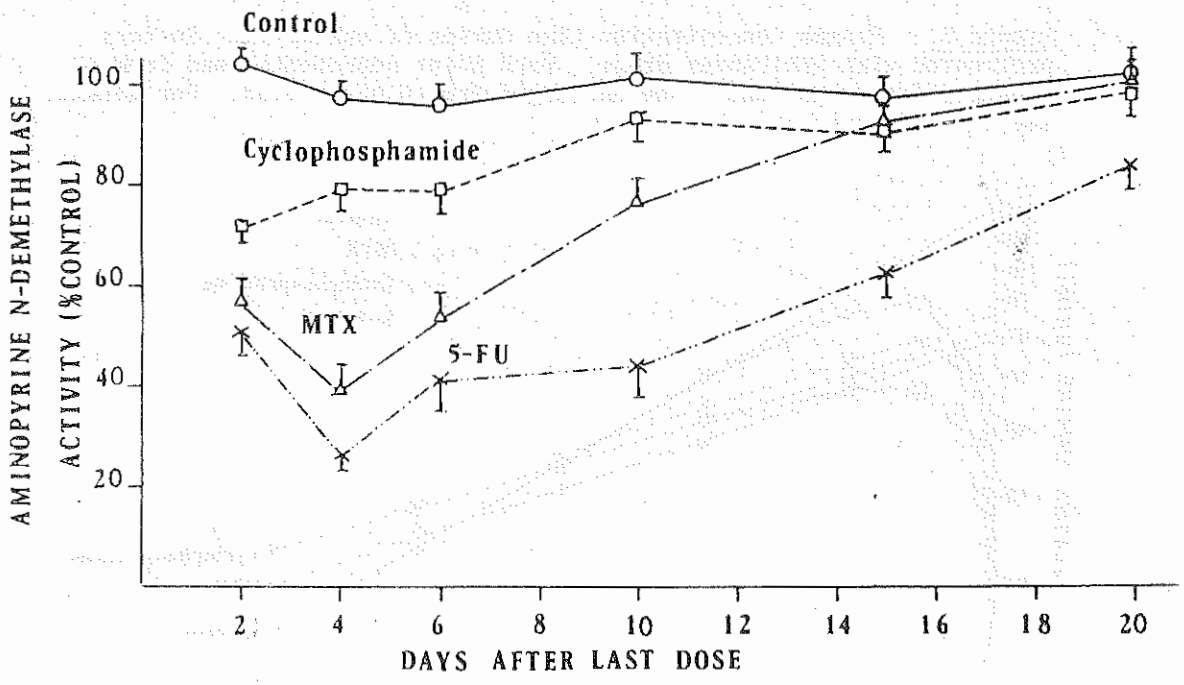

Figure 2. Time-course of the anticancer agents on aminopyrine N-demethylase activity. Methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO) and 5-fluorouracil (30 mg/kg, IP) were given once daily for 5 days as described previously. Each point represents mean ± S.E. from 6 separate determinations.

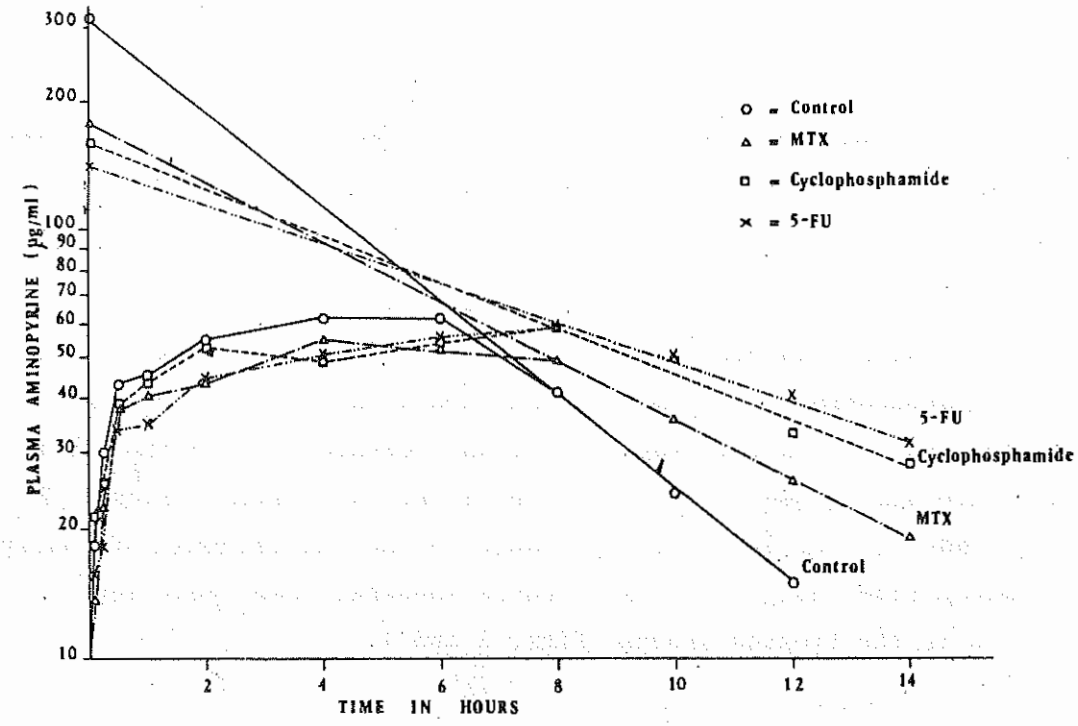

Figure 3. Plasma concentration-time curves of aminopyrine in rats pretreated with anticancer drugs. Each point represents mean from 4 separate determinations. The SE. ranged from 0.63 to 8.33. For detail, see Table 6.

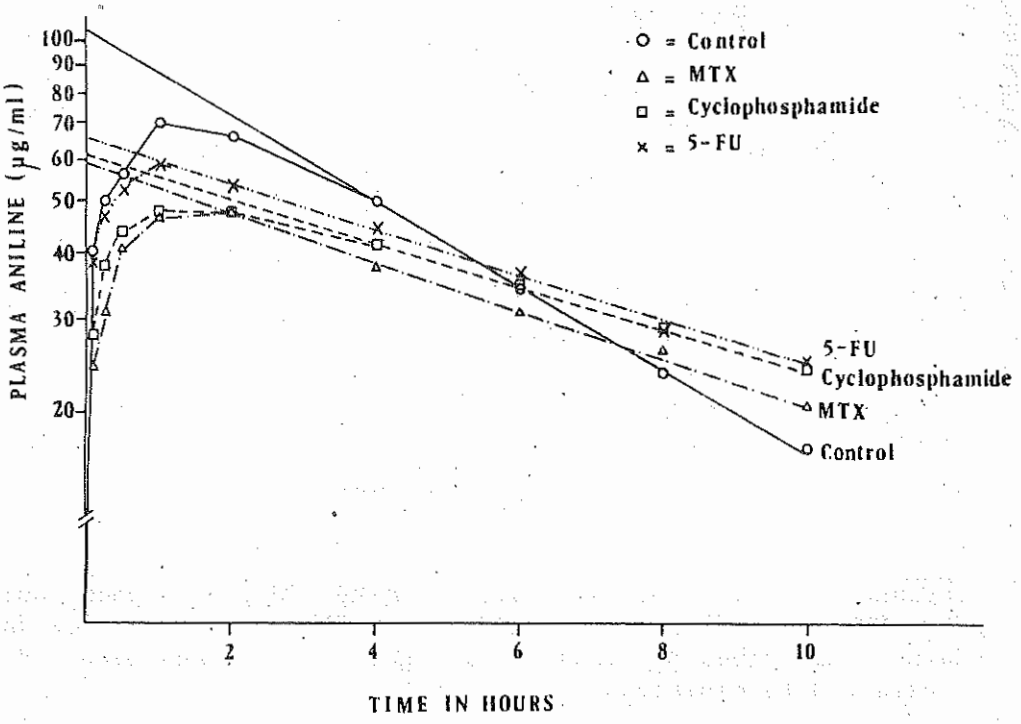

Figure 4. Plasma concentration-time curves of aniline in rats pretreated with anticancer drugs, Each point represents mean from 4 separate determinations. The SE. ranged from 0.56 to 4.78. For detail, see Table 7.

Table 6. The pharmacokinetics of aminopyrine in animals pretreated with anticancer agents

Pretreatment	Half-life (hrs)	Plasma Clearance (L/kg/hr)	Vd (L/kg)
Control	2.84 ± 0.14	0.16 ± 0.008	0.65 ± 0.01
Methotrexate	4.42 ± 0.34*	0.18 ± 0.015	1.19 ± 0.15*
Cyclophosphamide	5.56 ± 0.08†	0.15 ± 0.017	1.27 ± 0.31†
5-Fluorouracil	7.60 ± 1.20†	0.14 ± 0.008	1.49 ± 0.28†

Male rats weighing 170-200 g were pretreated once daily for 5 days with methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO), and 5-fluorouracil (30 mg/kg, IP). Control animals received an equal volume of normal saline. Aminopyrine (200 mg/kg) was given orally 48 hours after the last dose of pretreatment. The animals were then sacrified at various time intervals by heart puncture under light ether anesthesia. The plasma level of aminopyrine and the pharmacokinetic constants were determined as described for once-compartment open model, with the assumption that aminopyrine was completely absorbed in all groups. The results are expressed as mean ± SE from 4 separate determinations. * $P < 0.05$ (from control) ; † $P < 0.01$ (from control)

DISCUSSION

It is not uncommon to give drugs to cancer patients who have already been treated with cancer chemotherapeutic agents with little regard to the possible hepatotoxic actions of these compounds. The results obtained in the present study that pretreatment of rats with methotrexate, cyclophosphamide and 5-fluorouracil could prolong zoxazolamine paralysis time and increase the plasma half-life of both aminopyrine and aniline (Table 2, 6 and 7; Figs 3 and 4) provide a good evidence that anticancer agents at appropriate dose can have a profound effect on hepatic drug metabolism. One might regard that the experi-

Table 7. The pharmacokinetics of aniline in animals pretreated with anticancer agents

Pretreatment	Half-life (hrs)	Plasma Clearance (L/kg/hr)	Vd (L/kg)
Control	3.83 ± 0.29	0.17 ± 0.003	0.95 ± 0.06
Methotrexate	7.13 ± 1.22*	0.17 ± 0.011	1.68 ± 0.19*
Cyclophosphamide	7.54 ± 0.80*	0.15 ± 0.007	1.63 ± 0.11*
5-Fluorouracil	6.10 ± 0.22*	0.15 ± 0.004	1.46 ± 0.60*

Male rats weighing 170-200 g were treated once daily for 5 days with methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO) and 5-fluorouracil (30 mg/kg. IP). Control animals received an equal volume of normal saline. Aniline hydrochloride (100 mg/kg) was given orally 48 hours after the last dose of the drug treatment. The animals were then sacrified at various time intervals by heart puncture under light ether anestesia. The plasma levels of aniline were measured as described in Materials & Methods. The pharmacokinetic constants were obtained as described for the one-compartment open model, with the assumption that aniline was completely absorbed in all groups. The results are expressed as mean ± SE from 4 separate determinations.
* $P < 0.05$ (from control)

ments with anticancer agents are comparable with those associated with various forms of liver disease. In liver disease the prolongation of drug action or increase in the drug's plasma half-life is generally ascribed to either a reduction in the ability of hepatic cells to eliminate the drug, to a reduced functional cell mass or to a reduced perfusion of each cell with the drug. The *in vitro* results on the effects of methotrexate, cyclophosphamide and 5-fluorouracil on various hepatic parameters (Tables 3-5) were complementary to those obtained with aminopyrine or aniline pharmacokinetics (Tables 6 and 7) and sug-

gested that decrease in hepatic drug metabolism was a key factor in the observed impaired plasma clearance of the above two amines. Delayed absorption of either aminopyrine or aniline from the gut was not likely to be the cause since the plasma concentration-time profiles of either amine during the absorption phase in control and drug-pretreated groups were very similar (Figs. 3 and 4).

However, these three anticancer agents at the doses employed in the present study did not cause any change in SGPT and SGOT levels (data not shown). Thus, there was no correlation between the transaminase activity and the abillity of the liver to metabolize drugs.

The results obtained from this study are similar to those of Capel et al. (17), who found that pretreatment of rats with 5 anticancer drugs retarded and decreased absorption from the gastrointestinal tract and the elimination of antipyrine from the plasma; the latter effect was believed to be due to a reduction in hepatic cytochrome P-450 content. In the present study, however, the effect of anticancer drugs on the absorption profile of either aminopyrine or aniline was not as clear cut as it was found with antipyrine by the above authors. The discrepancy has yet to be settled.

The effect of anticancer drugs on hepatic drug metabolism may have clinical relevance. Drug dosage should be modified on cancer patients receiving chemotherapy. Capel et al. (17) have suggested that antipyrine be used in patients before and after treatment with anticancer drugs to obtain a direct indication of the metabolic status of the liver as a result of the therapy. This method seems reliable since each patient can serve his or her own control. Moreover, in view of the fact that anticancer drugs may be given to patients in combination their effects on liver function could be more serious. Without dosage adjustment several high-risk drugs may produce a fatal outcome in these patients.

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ขอเชิญเข้าร่วมประชุมวิชาการ และเสนอผลงานวิจัย

ใน

การประชุมสามัญและวิชาการประจำปี

ของ

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SHORT COMMUNICATION

DECREASE IN PITUITARY GROWTH HORMONE LEVEL IN RAT AFTER PROLONG ADMINISTRATION OF BOVINE THYROTROPIN

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SUMMARY

Female Fisher rats aged 35 days were injected intraperitoneally with 50 µg and 100 µg per day of bovine thyrotropin(TSH) for 44 days. GH content and concentration in the TSH treated groups were significantly lower than that of the control group. Wet weight of anterior pituitary and thyroid gland were not altered by TSH treatment, while the doses of 100 µg TSH significantly lowered adrenal, ovarian and uterine weights. The formation of antibodies to bovine TSH after chronic administration, which can cross react to rat endogenous TSH, may lead to thyroid hypofunction which in turn lower pituitary GH synthesis. The bovine TSH antiserum may also cross react with rat FSH and LH and lead to adrenal and ovarian hypofunction.

The biological role of TSH in stimulating thyroid function has been well documented. Acute effects of TSH include the stimulation of adenylate cyclase and cyclic-AMP formation (1,2), increasing the height of thyroid follicular cells (3,4) and the stimulation of thyroid hormone secretion (5,6). Thyrotropin may also cause subsequent enzyme induction leading to T_3 and T_4 synthesis (7). However, chronic administration of TSH has been reported to cause a suppression of metabolic rate and T_3 and T_4 levels (8,9) The serum of animals that had been treated chronically with TSH, when given to other animals for a prolong period, caused

a drop in the basal metabolic rate. Werner et al (10) suggested that the antibodies to bovine TSH in the rabbit can neutralize the thyrotropic effect of the hormone. Passcasio and Selenkow (11) reported that antisera to bovine TSH was capable in neutralizing the biologic effects of endogenous guinea pig and rat TSH. Hays et al (12) also showed that highly purified heterologous TSH was an antegenic substance in man. Thus, it is interesting to examine the chronic effects of bovine TSH on rat pituitary GH content as presented in this study.

MATERIALS AND METHODS

Female Fisher rats aged 35 days were used in this study. They were kept in room temperature of 25-30° c with 12 hours of daily light. Zuelling diet (Gold Coin Mills) and tap water were available *ad libitum*. The animals were injected intraperitoneally and daily with 50 µg or 100 µg of bovine TSH for 44 days. The control animals were injected with equivalent volume of sterile normal saline solution. They were sacrified one day after the last injection and the pituitary GH was assayed by the quantitative compliment fixation immunoassay (see 13 for detail description). Body length (from nose to tip of tail) and wet weight of the ovary, uterus, thyroid and adrenal glands were also measured. A student t-test was used to determine for the difference between several mean values.

RESULTS

The final body weight and length and pituitary wet weight of animals treated with bovine TSH were not different from control animals. Significant decreases in pituitary GH content and concentration were produced by prolong administration of either 50 µg or 100 µg of bovine TSH (Table 1). The absolute and relative weights of thyroid gland, absolute adrenal weight, and ovarian weight of rats treated with 50 µg of TSH

Table 1 Effects of prolong treatments with bovine thyrotropin (TSH) in female rats.

	Control	TSH injected 50 µg/day	TSH injected 100 µg/day
Final Body Weight (g)	150.0± 1.9	154.0± 3.6	161.0± 4.0
Final Body Length (cm)	35.4± 0.43	34.8± 3.16	34.3± 0.31
Anterior Pituitary Wet Weight			
Absolute (mg)	6.75± 0.41	6.78± 0.38	6.30± 0.33
Relative (mg/100 g BW)	4.41± 0.24	4.26± 0.23	3.90± 0.16
Anterior Pituitary GH			
Content (µg/gland)	285.0± 17.6	151.0± 9.9**	172.0± 17.1**
Level (µg/mg tissue)	39.7± 3.94	23.5± 1.64**	27.4± 2.56**
Thyroid Gland Weight			
Absolute (mg)	9.94± 0.76	9.98± 0.60	7.16± 0.29**
Relative (mg/100 g BW)	6.64± 0.56	6.63± 0.37	4.45± 0.17**
Adrenal Gland Weight			
Absolute (mg)	46.24± 2.47	42.18± 2.46	40.04± 2.31
Relative (mg/100 g BW)	30.68± 1.38	28.45± 1.56	24.68± 0.96
Ovary Weight			
Absolute (mg)	87.58± 5.40	81.52± 5.10	70.97± 3.16*
Relative (mg/100 g BW)	58.48± 3.97	52.59± 2.45	43.60± 2.78**
Uterus Weight			
Absolute (mg)	331.1± 16.6	250.0± 18.3**	224.6± 27.2**
Relative (mg/100 g BW)	218.7± 11.7	173.4± 10.1**	137.9± 15.1**

* and ** represent significant differences from control group with $p < 0.05$ and $p < 0.01$ respectively. All values are mean ± S.E. of 10 rats in each experimental group.

were not significantly changed ; but those of the 100 μg TSH treated group were significantly lower than the corresponding values in the saline-injected controls. The uterine weight in both TSH treated groups were significantly lower than the corresponding control values.

DISCUSSION

It is shown in the present experiments that pituitary GH content and concentration in rats decreased significantly following chronic administrations of bovine TSH. The reduction in pituitary GH in this study may be due to the formation of antibodies to bovine TSH and neutralization of the thyrotropic effect of endogenous rat TSH leading to thyroid hypofunction. Cross reactivity of bovine TSH antiserum against endogenous rat TSH has been suggested by investigators (see 8, 11, 12). The decreases in pituitary GH and growth rate due to low serum thyroid hormone have been well documented (see 13-18).

The formation of antibodies to bovine TSH may also responsible for the suppression of ovarian and uterine weight in rats found in this study, as cross reactivities between TSH antiserum and the endogenous LH or FSH may occur since LH, FSH and TSH molecules posses similar α - chain. Koneff (19) and Wong (18) had demonstrated decreases in pituitary LH and FSH following the decrease in serum thyroid hormone level. Additionally, the decrease in serum thyroid hormone level may lead to lowering of cell metabolism in general, including the gonadal cells and the FSH and LH cells. Further investigations are necessary to elaborate the mechanisms responsible for the reduction of pituitary GH and ovarian and uterine weight observed in the present study.

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REVIEW ARTICLE

ENDOGENOUS OPIATE : CHARACTERIZATION AND PHYSIOLOGICAL ROLE IN PAIN MODULATION

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The hypothesis that opiates exert their pharmacological actions by interacting with the specific receptor sites located on the surface or inside the nerve cells in the brain and possibly other tissues has been proposed since the last decade. The reason for postulating receptors for morphine and the related natural and synthetic narcotics is the finding that all these opiate agonists, or analgesically active compounds, show basic similarities in their molecular structures. A slight modification of this basic structure could result in an increase or a decrease in potency, or could transform the compound to the antagonist. In addition, a high degree of steric and structural specificity inherent in many of the actions of the opiates observed,i.e. for a large number of morphine-like analgesics studied, it is always the levorotatory (-) isomer that is active, whereas the dextrorotatory (+) isomer has little or no analgesic or any other actions associated with opiates. Moreover, all pharmacological effects of an opiate could be obtained with a very small dose of the drug, in turn suggesting an interaction of the drug with the highly selective receptor sites.

The discovery of opiate receptors in the brain had led to a search for an endogenous morphine-like substance that would act as a natural opiate receptor ligand. There is evidence for at least five opioid peptides in mammalian nervous tissues: methionine- and leucine-enkephalins, β-endorphin, dynorphin and most recently α-neo-endorphin.

This article describes the experiments carried out by a number of laboratories to characterize the endogenous opiates, and to implicate their physiological role in the modulation of pain perception.

Enkephalins

Hughes (1) observed an opiate-like activity of the extract of a low molecular weight substance from the brain of rabbits, guinea-pig, rats and pigs. The opiate-like substance had been isolated and identified (2,3) and found to consist of two similar pentapeptides with sequences of H-Tyr-Gly-Gly-Phe-Met-OH (Methionine-enkephalin) and H-Tyr-Gly-Gly-Phe-Leu-OH (Leucine-enkephalin). The existence of these two opiate peptides has been confirmed by Pasternak et al (4) and Simantov and Snyder (5). It has been found that bovine brain contains 4 times as much leu-enkephalin as met-enkephalin whereas in pig brain this ratio is reversed. The competition for opiate receptor binding by leu-enkephalin suggests that leu-enkephalin may be a "pure" agonist than met-enkephalin. The structure of met-enkephalin is contained within the sequence (residues 61 to 65) of 91 amino acids of β-lipotropin, a peptide isolated from the pituitary gland of several vertebrate species (6,7).

In the studies of Hughes and coworkers (1,2), it was found that both met- and leu-enkephalins had potent agonistic action at opiate receptor sites in that they produced a dose-related inhibition of electrically evoked contraction of the mouse vas deferens and the guinea-pig ileum preparations. These inhibitory effects could be completely antagonized by naloxone. Met-enkephalin is about twenty times more active than normorphine in the mouse vas deferens and equipotent to normorphine in the guinea-pig ileum. With the modified purification procedure, enkephalins were found unevenly distributed in the brain (pig, rat, rabbit) the highest concentrations occurring in the striatum, midbrain, pons and medulla. No enkephalins were detected in cerebellum, liver and lung (1).

The regional distribution of enkephalins in the monkey brain has been examined by the inhibition of ^{3}H-naloxone binding to rat brain membranes (8). It has been found that the regional distribution of enkephalin activity in the monkey brain resembles in general with the regional distribution of opiate receptor binding. However, the most marked discrepancies between the distribution of enkephalins and the opiate receptors involve the amygdala which contains the highest density of opiate receptor binding in the monkey brain but only moderate to low levels of enkephalins. The periaqueductal gray, one of the areas most enriched in opiate receptor binding, also contains only moderate levels of enkephalins. The medial thalamus, implicated in the mediation of affective components of pain, is highly enriched in opiate receptor binding and contains about three times as much opiate receptor binding as the lateral thalamus. Enkephalin activity in the medial thalamus is fairly low, but is more than twice of the levels in the lateral thalamus (8). However, the correlation of the regional distribution of enkephalin activity and that of opiate receptor binding in the brain has been reported in rabbits (1), calves (4,9) and rats (4,9), which in turn suggests the possible roles of enkephalins in the modulation of pain perception.

β-Endorphin

Simultaneously with the discovery of enkephalins, a 31-amino acid peptide with opiate-like properties, β-endorphin has been isolated and identified from the pituitaries of bovine (10), porcine (10,11), camel (12), and human (13,14). The amino acid sequence of β-endorphin is identical to the sequence of the carboxy terminal 31 amino acids of human β-lipotropin (i.e. β-LPH61-91). Immunohistochemical studies show a dense staining for β-endorphin-containing cells in pars intermedia, with a sparser distribution of immunoreactive cells in the adenohypophysis, the neurohypophysis appears devoid of such activity (15).

In the rat brain, the β-endorphin-like activity has been found to be about 3-4 times lower than in the pituitary gland (16). High β-endorphin-like immunoreactivity is found in the medial hypothalamus, where it is threefold that in the next higher brain areas (preoptic region and mesencephalon) and nearly fivefold the value in the lateral hypothalamus. Moderate β-endorphin-like immunoreactivities are present in the amygdala, septum and nucleus accumbens. Despite the high enkephalin level in the striatum, only low β-endorphin-like immunoreactivity is found in this area, especially in the globus pallidus. The thalamus, the cerebral cortex as well as the hippocampus also contain low β-endorphin levels (16).

It has been reported that β-endorphin, adrenocorticotropin (ACTH), β-MSH and α-MSH are all formed from a common precursor of 28-41K Daltons which has been termed pro-opiocortin (17-20). Furthermore, ACTH and β-endorphin are released concomitantly after stressful stimuli in rats such as footshock (21) or leg break (22). The association between β-endorphin and ACTH might well reflect the proposed common origin and biogenesis of these two peptides. Footshock stress in rats can also elicit an analgesic response which is partially reversed by naloxone (23-25). It is possible that β-endorphin has an unknown peripheral physiological role, perhaps related to changes in adrenocortical function or intermediary metabolism during stress. The presence of opiate receptors in the pituitary (26) also reflects the possible intrapituitary function of β-endorphin which could involve in the modulation of hormone synthesis or release. Alternatively, β-endorphin may be released into the hypothalamic portal system (27) and be retrogradely transported so that it could act on hypothalamic or other brain centers(28).

Dynorphin and α-neo-endorphin

Goldstein et al (29) have partially sequenced a peptide from commercial pituitary powder that contains the leu-enkephalin sequence

with a basic carboxy terminal extension. This peptide, named dynorphin has its first 13 residues as Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys. It appears to be remarkably potent in the guinea-pig ileum assay, being some 730 times that of morphine and 54 times that of β-endorphin. Its effects on this tissue are blocked completely by naloxone, but the apparent affinity of naloxone is 1/13th that for blockade of leu-enkephalin or normorphine. In the mouse vas deferens, dynorphin (1-13) is three times more potent than leu-enkephalin. In the pituitary (porcine, bovine and rat), immunoreactive dynorphin is found predominantly in pars nervosa. In the central nervous system, it is distributed widely, with high concentrations in the hypothalamus, the medulla-pons, the midbrain and the spinal cord (30). In rabbits and rats, the concentration of immunoreactive dynorphin is high in the dorsal aspect of the spinal cord ,but is low in the root ganglia of both species (31)

Millan et al (32) observed the distribution of immunoreactive -dynophin (ir-dyn) in the pituitary, discrete regions of brain and the spinal cord, and observed the influence of a 5 min footshock stress upon levels of ir-dyn in these structures in rats. It was found that footshock produced a significant fall in the anterior pituitary lobe content of ir-dyn but no significant change in its neurointermediate lobe. In the hypothalamus, a significant elevation in level of ir-dyn was observed. With the exception of the frontal cortex, in which a decrease in level of ir-dyn was found, in all other brain regions examined no significant changes emerged. A significant diminution in concentrations of ir-dyn in both the lumbosacral and thoracic sections of the spinal cord was also detected. These authors suggested that, due to a particularly high potency of dynorphin (29), the changes observed in their experiments might be of considerable functional significance in the response to stress (32).

Wuster et al (33) employed the technique of chronic opioid infusion to produce tolerance attributable to specific receptor population in the mouse vas deferens. They observed that the mouse vas

deferens preparations taken from mice previously simultaneously infused with D-Ala^{2}D-Leu^{5}enkephalin (a δ-agonist) and sufentanyl (a μ-agonist) for a period of six days, developed tolerance to δ-agonist and μ-agonist action. However, it was observed that such tolerance did not develop to the activity of dynorphin (1-13). These authors therefore suggested the existence of highly selective dynorphin receptors in the mouse vas deferens, which might function independently of the μ- and δ-receptors in this tissue. Friedman et al (34) demonstrated the significant effect of dynorphin(1-13) on morphine and β-endorphin-induced analgesia after the intracerebroventricular injection in mice (the tail-flick test). They observed that dynorphin(1-13) itself did not produce any significant analgesia. This peptide attenuated the analgesia produced by all doses of morphine used. The peptide attenuated the analgesic effect of high doses of β-endorphin whereas it potentiated that of the low doses, suggesting some common sites might exist between β-endorphin and dynorphin(1-13).

Pierce et al (35) observed an analgesic activity of dynorphin (1-13) by the tail-flick test but not by the hot plate test and only after the intraspinal administration, not either after the intracranial or the intraperitoneal administration. In the same experiment, ethylketocyclazocine was found to be most potent by the intraspinal route irrespective of the analgesic tests. The finding that dynorphin relied solely on the spinal sites for its analgesic activity and ethylketocyclazocine, a κ-receptor agonist also showed a preference on the spinal cord activity had led the authors (35) to suggest that dynorphin(1-13) might be an endogenous ligand for the κ-receptor. This hypothesis has been further supported by Chavkin et al (36) who demonstrated that dynorphin(1-13) and ethylketocyclazocine had equally poor sensitivity to naloxone antagonism on the guinea-pig ileum preparation. Moreover, in the binding assays with the membranes from guinea-pig brains, both compounds were more potent in displacing ^{3}H-ethylketocyclazocine than in displacing μ- or δ-opiate receptor ligands

Kangawa and co-workers (37,38) have recently isolated and identified a new opiate-like decapeptide, α-neo-endorphin from porcine hypothalamus. The sequence of this peptide has been determined to be Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys. This peptide showed a very potent opiate activity in the guinea-pig ileum assay, 6.7 times as high as met-enkephalin and 5 times as high as β-endorphin.

It seems, from the studies of β-endorphin, dynorphin and α-neo-endorphin, that, chemically speaking, there are at least two families of opiate peptide based on met-enkephalin and leu-enkephalin respectively. It also appears that carboxy-terminal extension can greatly enhance potency of both enkaphalins. In all three cases, β-endorphin, dynorphin and α-neo-endorphin have a number of strongly basic side chains. Carboxy-terminal extension employing neutral amino acids does not give this enhanced potency (40).

Specific receptor sites for endogenous opiates

The relation of the endogenous opiates, enkephalins and β-endorphin, to the opiate receptors has been established by the multiple parallel assays in the laboratory of Lord et al (41). Two of the assays were pharmacological in which the depression of the electrically induced contractions in the guinea-pig ileum and in the mouse vas deferens were observed. The other two were based on the inhibition of the binding of ^{3}H-leu-enkephalin and opiate antagonist ^{3}H-naloxone, in homogenates of guinea-pig brain. It was found that β-endorphin is equipotent in the two pharmacological models and also in the two binding assays. Leu-enkephalin behaves differently in that it is much more potent in the mouse vas deferens than in the guinea-pig ileum and also more effective in inhibiting ^{3}H-leu-enkephalin binding than in inhibiting ^{3}H-nalaxone binding. The activity pattern of met-enkephalin is intermediate between those of β-endorphin and leu-enkephalin. In contrast, morphine is more potent in the guinea-pig ileum than in the mouse vas deferens and

is also a better inhibitor of ^{3}H-naloxone binding than of ^{3}H-leu-enkephalin binding. It was concluded that the receptors preponderant in the mouse vas deferens (δ-receptors) corresponded to the leu-enkephalin binding sites in the brain and that those preponderant in the guinea-pig ileum (μ-receptors) were correlated to the naloxone binding sites (41).

In conclusion, a number of studies (e.g.41-43) have clearly defined at least three receptor types of which none of the ligands (natural and synthetic) is absolutely specific for one receptor type but preferences are clearly discernable in various bindings or bioassays. At the present time, the μ-receptor is defined as having a preference towards morphine, dihydromorphine and β-endorphin as agonists and naloxone as an antagonist. The δ-receptor has a greater affinity for the enkephalins as well as β-endorphin but a poor affinity for naloxone. The κ-receptor is less well defined but apparently prefers compounds of the ketocyclazocine series and only has a weak affinity for naloxone. Specific receptor blocking agents are not available for probing these different receptor types, i.e. naloxone does has a high affinity for the μ-receptor but it is not specific in that it will also interact with the δ-and κ-receptors.

Possible physiological role of endogenous opiates in the modulation of pain perception

The physiological role of endogenous opiates in the modulation of pain perception has been proposed by a number of investigators who employed various experimental techniques, such as naloxone antagonism, microinjection of the compounds into various brain areas, electrical stimulation of the brain, acupuncture, stress-induced analgesia, etc.

Jacob et al (44) provided the first evidence in experimental animals (mice and rats) that naloxone produced hyperalgesia and hypothesized that this was due to the interference of the natural occurring substances that are physiological regulators of pain sensitivity and

reaction to pain. These observations have been confirmed by some laboratories, (e.g.45-47), but not by others (e.g.48-50) possibly because of slight differences in methodology. Experiments with naloxone injection into humans have also been investigated. El-Sobky et al (51) found no reduction in pain thresholds in a situation where pain was induced acutely by electrical stimulation of the skin in the healthy volunteers. Naloxone was administered on a double blind basis, and the reaction to ischemic arm pain measured. No effect on pain thresholds was observed whereas the subjects reported slight changes in mood and feeling, and an increase in anxiety, hostility and depression, which could in fact have due to a blockade of endogenous opiate activity. Morphine is known to affect mainly the reaction to pain tolerance rather than pain thresholds, and the blockade of endogenous opiates would be expected to affect this psychosomatic component.

It has been well documented that the mesencephalic periaqueductal periventricular gray (PAG) regions in the midbrain appear to be the major sites for opiate analgesic activity in the CNS. Electrical stimulation of these regions or the nearby regions could result in an analgesic response in animals and human (52-56). Such stimulation produced analgesia are partially or completely reversed by naloxone (53-56). Furthermore, it has been demonstrated that the stimulation produced analgesia outlasts the brain stimulation for a few minutes to several hours, depending on the duration and intensity of the stimulation (52,57,58). This observation may suggest that the electrical stimulation of the medial brainstem appears to produce analgesia by activation of an endogenous pain inhibitory system, and the endogenous opiate system appears to be the most potential candidate for this phenomenon. This hypothesis has been supported by the observation of the opiate agonistic effects, particularly analgesia and catatonia, in animals which have been microinjected with enkephalins (leu-and met-enkephalins) or β-endorphin into the periaqueductal gray regions (e.g.59-61).

Mayer et al (62) observed that the pain threshold to electrical stimulation of the tooth was significantly increased by acupuncture needling of Hoku points between the thumb and index finger. This increase was completely reversed by naloxone 5 min after injection. The reversal was incomplete 10 min after the injection and disappeared within 15 min. In contrast, these authors found no effect of saline injection on analgesia produced by acupuncture and no effect of naloxone on the base-line pain threshold. The reversal effect of naloxone on acupuncture analgesia in human has been reported by many investigators (e.g.63,64). These results suggest the possible involvement of endogenous opiates in acupuncture analgesia.

It has been accepted that animals which are submitted to a stressful procedure exhibit antinociceptive (analgesic) response. The antinociception induced by stressful events was linked to the brain endogenous opiates because acute exposure to stress induced changes in levels of brain opiate peptides (23,24) and changes in brain opiate receptor binding characteristics (65,66). It was also noted that β-endorphin and ACTH were found to be concomitantly released from the pituitary gland following an acute stress in rats (21,22). Rossier et al (67) found a decrease in leu-enkephalin levels in hypothalamus of rats following footshock. No changes were observed in other parts of the brain. These data provided evidence in support of the involvement of endogenous opioids in stress-induced antinociception. On the other hand, there is evidence which casts doubt on this hypothesis. For example, the evidence provided by Rossier et al (21) who reported an increase in β-endorphin level in the blood but not in the brain following footshock in rats. Furthermore, Fratta et al (68) found no change in the met-enkephalin content of the whole brain of rats after footshock. A lesion placed in the dorsal part of the lateral funiculus of rat spinal cord, which reduced or abolished morphine and electrical brain stimulation-induced analgesia (69) , had no effect upon analgesia induced by electrical footshock (70)

Similar observations have arisen from a number of studies which used morphine and its antagonist naloxone, as the experimental tools to implicate endogenous opiates in stress-induced antinociception. Stress-induced antinociception was found to be fully or partially reversed by naloxone in some studies (e.g.23,25,47,50,71-75),but not in other (25,75-77). The cross tolerance between morphine and stress-induced antinociception has been reported by some observers (50, 74,75,78,79), but again not by others (80,81).

Tolerance to and physical dependence on endogenous opiates

Simultaneously with the observation in the experimental animals of the analgesic activity of an intracerebroventricular dose of enkephalins and β-endorphin (e.g.59-61) an attempt has been made to investigate whether repeated administration of the endogenous opiates would lead to tolerance and physical dependence in the same manner as observed in exogenous opiates (82-87).

The antinociceptive effect of human β-endorphin after an intracerebroventricular injection observed in rats was found to be 21 times more potent than morphine (85). This peptide also produced morphine-like catatonia and hypothermia. The responses were blocked by naloxone. Repeated injection of the peptide (94 μg twice daily for 2 days) induced tolerance to analgesic response, catatonia and hypothermia. Furthermore,a cross-tolerance to morphine was also observed. The similar results were also reported by Van Ree et al (82).

Loh et al (83) investigated an analgesic activity of β-endorphin after an intracerebroventricular injection, which was found to be 18 to 33 times more potent than morphine and its action was blocked by naloxone. The authors found that met-enkephalin produced a weak analgesic effect of short duration (less than 5-10 min) after an intracerebroventricular injection. After 70 h of infusion of β-endorphin into the rat brains, all the animals demonstrated a naloxone-precipitated

morphine-like withdrawal syndrome. Such results were also observed with met-enkephalin (84)

In the experiment of Huidobro-Toro and Leong Way (86) a β-endorphin injected intracerebroventricularly in mice produced a rapid onset, dose-dependent antinociceptive effect. Single dose tolerance development was demonstrable with doses twice or more than the LD50 (median analgesic dose). Tolerance was maximal at about 12 h following the priming dose and disappeared within 48 h. Tolerance was accompanied by some degree of physical dependence as noted by signs of naloxone-precipitated withdrawal similar to those elicitable in the morphine-dependent state. Tolerance development to β-endorphin was blocked by the simultaneous administration of naloxone and also by pretreatment with actinomycin D or cycloheximide (the protein synthesis inhibitors). The authors (86) concluded that the single dose tolerance to β-endorphin appeared to be initiated by a process similar to those involved with tolerance resulting from chronic administration of morphine.

Tseng (87) observed the analgesic action of D-Ala^{2}D-Leu^{5}enkephalin (DADL) in rats with the tail-flick test, and reported that a chronic intrathecal infusion of DADL for 5 days caused a shift of dose response curves of both DADL and morphine injected intrathecally to the right. This result indicated that a tolerance to DADL analgesic action and cross-tolerance to morphine had developed in the chronically DADL infused animals. However, concomitant intrathecal infusion of naloxone which was more sensitive in blocking μ-receptors than δ-receptors blocked the development of cross-tolerance to morphine while that to DADL was left unaffected. This author (87) presented the evidence that two types of opiate receptors, δ- and μ-receptors, in the spinal cord of rats were involved in the development of tolerance by chronic DADL exposure.

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GENERAL ARTICLE

AFLATOXINS : TOXICITY AND CARCINOGENICITY

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Aflatoxins (AFs) (see Figure 1), a group of mycotoxins produced by the fungi *Aspergillus parasiticus* and their animal biotransformation products, have been considered as responsible for the deaths of large numbers of domestic animals and possibly for the high incidence of liver cancer among exposed human populations (1). These aflatoxin producing mold strains can grow at temperatures of 40-45°c with an optimum at 30°c, and a relative humidity of 75% or greater. These naturally occured toxins had a characteristic fluorescence pattern on thin layer chromatograms at four components designed as aflatoxins B_1, B_2,G_1 and G_2 ; the four components were distinguished by their blue or green fluorescence and by their Rf values on thin layer chromatograms. Twelve of structurally related components have been isolated and chemically characterized, ie. M_1, M_2, B_2, G_{2a},etc. The M_1 and M_2 fractions were first isolated from the milk of cows feeding on AFs contaminated food.

The chemical structure of the AFs contains a difurofuran or bisfuran ring system fused to a substituted coumarin moiety, with a methoxy group attach at the corresponding benzene ring. These functional groups all have definite effects on the toxicity of AFs (2-4).

The toxin that has been studied most extensively and constitutes by far the major components of most naturally produced mixtures of AFs is AFB_1. It is the most toxic member of the group and has also been shown to be the most potent hepatocarcinogen yet found for the rat and rainbow trout (3,4).

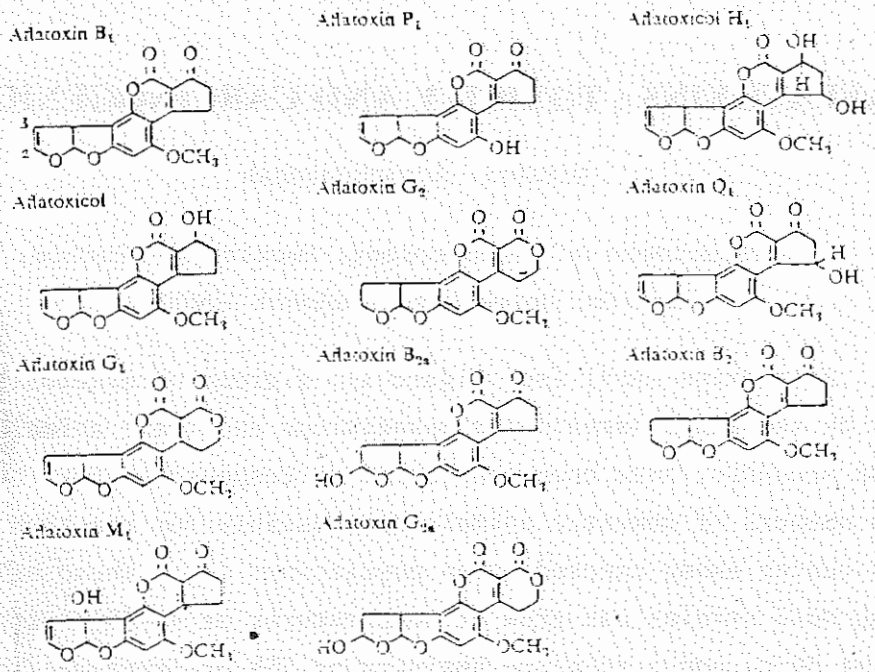

Figure 1. Structures of the naturally occuring aflatoxins and metabolites.

TOXICITY

The toxic actions of aflatoxins have been investigated in many kinds of biologic test systems. The aflatoxins are lethal to many animal species thus far studied, when administered acutely at suitable dose levels. Typical single dose LD_{50} values of aflatoxin B_1, is in the range of 0.5-10 mg/kg body weight (Table 1).

In all species, AFs toxicity main target organ is the liver ; other can be kidney, stomach, lung, salivary and lacrimal glands, colon and skin. In AFB_1 acute and subacute poisoning, liver is the main target organ. It cause centrilobular necrosis and/or bile duct proliferation. Toxicity depends on age, sex, species, strain and diet. Repeated dose or chronic feed AFB_1 can induce hepatocellular carcinoma and cholangioma in animal at least 3 species (rat, rainbow trout, duck). Sheep is resistant to chronic exposure to AFB_1. And in all

Table 1. Single dose lethality of AFB_1 in various animal species (5)

Animal	Age or Weight	Sex	LD50(mg/kg)
Duckling	1 day	male & female	0.40
Rabbit	weanling		0.50
Dog	adult	male & female	0.50
Trout*	100 g	male & female	0.50
Guinea pig	adult	male & female	1.40
Rat	1 day	male & female	0.56
	21 days	male	5.50
	21 days	female	7.40
Hamster	21 days	male	5.50
	30 days	male	10.20
Mouse	21 days	male	63.00
Sheep	adult	male & female	500.00

* Intraperitoneal; other values refer to the oral route.

species, young animals are more susceptible than adult, male are more susceptible than female to acute and subacute toxicity and develop tumor more quickly than female. Toxic and carcinogenic effects are enhanced by low protein diets or cirrhogenic diets prior to or during exposure to toxic compound.

In human, number of cases and evidence involving AFs in acute poisonings were reported in Thailand (6) Uganda (7) and Taiwan (8). Reye's syndrome is epidemic in northeastern Thailand and is limited to children up to adolescene; it is characterized by a short prodrome of several hours followed by vomiting, hypoglycemia, convulsions, hyperammoniemia, and coma usually ending in death within 24 to 48 hours after onset. Histopathologic examination revealed severe cerebral edema and extensive fatty accumulation in hepatocytes, renal tubular epithelium, and myocardial fibers. Autopsy specimens from 23 Thai children who died with Rey's syndrome were chemically assayed for aflatoxins; AFB_1 and AFB_2 were found in the liver specimens from 22 cases, usually at the levels

between 1 and 4 µg/kg tissue wet weight. In two cases, the concentrations of AFB_1 were of the same magnitude as the levels in the livers from monkeys in the acute toxicity study. At this level, AFB_1 intoxication in the monkey was remarkably similar to Rey's syndrome. However, other factors may be involved in this syndrome such as malnutrition status, viral infection and seasonal changes.

CARCINOGENICITY

In vitro stuies of the mode of action of AFs revealed that AFB_1 requires metabolic activation for toxic activity (3). Since distinct differences in drug metabolizing activity exist among various animal species, the differences in species susceptibility to carcinogenic aflatoxicosis have been considered with these metabolic differences. Correlations between the in vitro hepatic metabolism of AFB_1 and species susceptibility to aflatoxicosis have been shown (8-10). Under *in vitro* conditions as reviewed by Campbell and Hayes (3), AFB_1 is known to be oxidized by the mixed-function oxygenases of the microsomal fraction of homogenized liver to from the products of hydroxylation, AFM and AFQ_1; of o-demethylation, AFP_1; of hydration, AFB_{2a} ; and of epoxidation, the 2,3-epoxide of AFB_1. AFB_1 is also reduced by the reductase. In addition, the cytosol fraction contains numerous enzymes and other molecules involved in the conjugation of certain AF metabolite to render them water-soluble.

To determine the relative contributions of these metabolites toward the carcinogenic effect of AFB_1 and to seek the active form of AFB_1, Wong and Hsieh (4) performed the Ames mutagen assay on all of these metabolites except for the 2,3-epoxide of AFB_1, which has not yet been isolated. The results are summarized in Table 2. The 2,3-epoxide was postulate to be an ultimate carcinogenic metabolite of AFB_1 that can bind covalently to the DNA at the N^7 -position guanine base yielding N^7 -guanine AFB_1 adduct of DNA or RNA molecules (11).

Table 2. Correlation of *S.typhimurium* mutagenicity of aflatoxins to their animal carcinogenicity

Aflatoxin	Relative mutagenicity (%)[a]	Carcinogenicity
AFB_1	100	most potent hepatocarcinogen in rat and rainbow trout
AFL	22.8	possessed half the tumor activity of AFB_1 in rainbow trout
AFG_1	3.3	less tumorigenic in rat than AFB_1 less hepatocarcinogenic in rainbow trout than AFB_1
AFM_1	3.2	possessed one-third the tumor activity of AFB_1 in rainbow trout less tumorigenic than AFB_1 in rat
$AFLH_1$	2.0	
AFQ_1	1.1	nontumorigenic in rainbow trout
AFB_2	0.2	inactive in rat, weak activity in rainbow trout
AFP_1	0.1	
AFG_2	0.1	found inactive in rainbow trout
AFB_{2a}	0.00	
AFG_{2a}	0.00	

Data from Wong and Hsieh (4)
a = Slope value percent : compound slope divided by AFB_1 slope x 100.

The postmitochondrial supernatant (S-9) of the rat liver was used as the activation system for the AFs to cause reverse mutation to a sensitized frameshift auxotroph of *Salmonella typhimurium*, strain TA 98. The hepatic S-9 preparation was definitely required for the mutagenic activity of all the AFs tested, indicating that none of them is the

active from but that they are indeed *procarcinogens*(precarcinogens). Of all the AFs tested AFB_1 had the highest mutagenic activity, followed by AFL, AFG_1, and other derivatives Therefore,it appears that AFB_1 has the molecular structure optimal for mutagenic activity. Any alteration of either the 2, 3-vinyl ether double bond, the methoxy, or the cyclopentenone groups invariably results in a marked reduction in activity.

When these mutagenicity data were compared with the carcinogenicity data for various AFs obtained from animal-feeding experiments a remarkable correlation was found between the *in vitro* bacterial mutagenicity of these compounds and their *in vivo* animal "carcinogenicity, as shown in Table 3.

The difference in the carcinogenic potential of various AFB_1 metabolites suggests that the susceptibility of an animal to carcinogenic aflatoxicosis may be a reflection of the net bioactivation efficiency of the simultaneous action of various metabolic pathways in the liver. In other words, the probability that a biochemical lesion caused by AFB_1 may be a function of the concentration of the active form of AFB_1 formed at the target site. This critical concentration of AFB_1 in a particular liver can be estimated as Salmonella mutagenicity by the Ames assays using the hepatic S-9 preparation derived from the liver in question. Hsieh et al. (12) used the hepatic S-9 preparations derived from different animals for the Salmonella mutagenicity test to compare the net bioactivation efficiency for AFB_1 in their livers. Because of their distinct differences in susceptibility to the *in vivo* carcinogenic effect of AFB_1, four species, i.e., ducks, rats, monkey and mice were used as source of hepatic S-9 system for *in vitro* asssays of AFB_1 bioactivation. This data,including human liver S-9 preparation activity were shown in Table 3. The *in vivo* responses of each species to the carcinogenic effect of AFB_1, as available from the literature, are also tabulated for comparison.

From the limited data complied, the ranking of the relative

Table 3. Aflatoxin-B_1-induced in vitro *Salmonella* mutagenicity and in vivo animal carcinogenicity (12).

		AFB_1 exposure		
Species (no., sex)	*Mutagenic activity (rev./μg AFB_1/mg prot.)*	*level*	*time (y-v)*	*Hepatoma incidence (%)*
Duck (3, male)	8493 (6508–10,489)	35 ppb	1.2	72
Rat (9, male)	2005 (1262–2643)	15 ppb	1.3–1.5	100
		100 ppb	0.8–1.2	86
		100 ppb	2.0	48
Monkey (5, male)	814 (119–2056)	360 ppb	3.0	0
		1800 ppb	3.0	0
		99–842 mg	4–6.0	7
Mouse (9, male)	491 (420–605)	1000 ppb	1.3	15
		1000 ppb	1.7	0
		100 μg/day	1.0	0
Human (5, female)	286 (100–755)	?	?	?

net bioactivation efficiencies for AFB_1 of the livers of different animal species appears to be consistent with the ranking of their susceptibilities to the carcinogenic effect of AFB_1.

Based on the observations of long-term feeding experiments, mice and monkeys are shown to be relatively resistant to the carcinogenic effects of AFB_1, compared to the rat (the most wildly used animal for AF carcinogenicity determinations), which developed a 48% incidence of liver carcinoma after 2 years of continous feeding of 100 ppb of AFB_1 in the diet (13). Only 7% of monkeys developed liver tumors after 2 years of multiple subacute doses and multiple routes of exposure to AFB_1 (14), and no tumor was detectable in mice fed 1,000 ppb of AFB_1 for 80 weeks (2). Hsieh et. al. (12) concluded that the low in *in vitro* bioactivation activity of human S-9 preparations relative to that of the other species indicate that the human were less sensitive or more resistant than mice to the carcinogenic aflatoxicosis. So far, correlations have been presented between the levels of aflatoxins found in diets consumed in certain areas of Thailand (1) East Africa (15,16) and

the high frequency of occurence in these areas of primary liver cancer. However, liver tumors in these areas may arise from more than one etiological factor, i.e., alcohol consumption, low protein diet and liver fluke infection.

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จาก

ห้างหุ้นส่วนจำกัด โอสก

๒๑๑๕ ถนนรามคำแหง กรุงเทพ ฯ

โทร. ๑๑๔๗๐๑๘

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คำแนะนำสำหรับผู้เขียนเรื่องลงวารสาร

วัตถุประสงค์

"วารสารเภสัชวิทยา" เป็นวารสารทางวิชาการของสมาคมเภสัชวิทยาแห่งประเทศไทย มีวัตถุประสงค์เพื่อเผยแพร่วิชาการ ผลงานทางเภสัชวิทยาและวิทยาศาสตร์ที่เกี่ยวข้อง กับส่งเสริมการวิจัยและเสริมสร้างการติดต่อประสานงานระหว่างสมาชิกในสถาบันต่างๆ

เรื่องที่ตีพิมพ์

1. รายงานวิจัย (Original Article) เป็นรายงานผลงานวิจัยของผู้เขียนเอง ซึ่งยังไม่เคยตีพิมพ์หรือกำลังรอการตีพิมพ์ในวารสารอื่น
2. รายงานผู้ป่วย (Case Report) เป็นรายงานผลการศึกษาในผู้ป่วย ในเรื่องที่เกี่ยวข้องกับวิชาเภสัชวิทยา
3. บทความปริทัศน์ (Review Article) เป็นการรวบรวมข้อมูลและสรุปวิจารณ์ในเรื่องใดเรื่องหนึ่งอย่างละเอียด ลึกซึ้ง และก้าวหน้าในด้านนั้นๆ
4. บทความทั่วไป (General Article) อาจเป็นการสรุปความรู้ความเข้าใจในเรื่องใดเรื่องหนึ่ง ซึ่งมีประโยชน์ต่อการเรียนการสอน หรือต่อสมาชิกและประชาชนที่สนใจ
5. เวทีทัศน์ (Point of View) เป็นการวิจารณ์หรือเสนอข้อคิดเห็นในสาระสำคัญทางเภสัชวิทยา หรือที่เกี่ยวข้องกับการเรียนการสอนวิชาเภสัชวิทยา หรือการดำเนินงานของสมาคมเภสัชวิทยาแห่งประเทศไทย
6. จดหมายถึงบรรณาธิการ (Letter to Editor) เป็นการวิจารณ์หรือเสนอข้อคิดเห็นที่เกี่ยวข้องกับการจัดทำวารสารเภสัชวิทยา หรือเรื่องที่ตีพิมพ์ในวารสาร ซึ่งคณะบรรณาธิการอาจพิจารณาให้ผู้ที่เกี่ยวข้องตอบข้อวิจารณ์หรือข้อเสนอแนะนั้นๆ
7. วิจารณ์หนังสือ (Book Review) เป็นข้อวิจารณ์หรือแนะนำหนังสือที่ตีพิมพ์ทั้งภายในประเทศและในต่างประเทศ ซึ่งคณะบรรณาธิการเห็นว่าให้ประโยชน์ต่อผู้อ่าน
8. บทบรรณาธิการ (Editorial) เป็นบทความหรือข้อคิดเห็นในเรื่องที่เกี่ยวข้องกับวิชาเภสัชวิทยา วารสารเภสัชวิทยา หรือสมาคมเภสัชวิทยาแห่งประเทศไทย ซึ่งคณะบรรณาธิการจะพิจารณาเป็นเรื่องๆ ไป

เงื่อนไข

1. ต้นฉบับที่ส่งให้พิจารณาต้องไม่เคยตีพิมพ์มาก่อน หรือกำลังรอการตีพิมพ์ในวารสารหรือหนังสืออื่นๆ และจะต้องไม่ส่งไปตีพิมพ์ที่อื่นภายหลังจากที่คณะบรรณาธิการได้ตอบรับเรื่องดังกล่าวแล้ว
2. เรื่องที่ตีพิมพ์แล้วเป็นสมบัติของสมาคมเภสัชวิทยาแห่งประเทศไทย และเป็นผู้สงวนสิทธิ์ทุกประการ
3. ข้อความและความคิดเห็นในเรื่องที่ตีพิมพ์ในวารสารเป็นของผู้เขียน ซึ่งคณะบรรณาธิการไม่จำเป็นจะต้องเห็นพ้องด้วย
4. วารสารจะส่งสำเนาเรื่องที่ตีพิมพ์แล้ว จำนวน 25 ฉบับให้ผู้เขียนตามที่อยู่ที่ระบุไว้

การเตรียมต้นฉบับ

1. ต้นฉบับอาจเป็นภาษาไทยหรือภาษาอังกฤษก็ได้ ถ้าเป็นภาษาไทยจะต้องมีเรื่องย่อภาษาอังกฤษอยู่ด้วย และมีชื่อเรื่อง ชื่อ ชื่อสกุล และที่ทำงานของผู้เขียนอยู่ด้วยทั้งภาษาไทยและภาษาอังกฤษ ยกเว้นเรื่องที่เป็น เวทีทัศน์ จดหมายถึงบรรณาธิการ วิจารณ์หนังสือ และบทบรรณาธิการ อาจไม่ต้องมีเรื่องย่อก็ได้
2. ต้นฉบับควรพิมพ์ดีดบรรทัดเว้นครึ่งบรรทัด (1½ Space) บนกระดาษขาวอย่างสั้น และพิมพ์หน้าเดียว ภายในกรอบขนาด 15 x 20 cm. ตัวเลขควรใช้เลขอาราบิคทั้งหมด ตัวอย่างการพิมพ์ การเว้นบรรทัดและวรรคตอนต่างๆ โปรดดูจากเรื่องที่ตีพิมพ์ในวารสาร ปีที่ 4 บทความที่พิมพ์มาตามรูปแบบที่กำหนดไว้ดังกล่าวแล้วจะได้รับการพิจารณาตีพิมพ์เร็วขึ้น

3. รายงานวิจัยหรือรายงานผู้ป่วยควรมีโครงสร้างตามลำดับดังนี้ ชื่อเรื่อง ชื่อและสถาบันที่ทำงานของผู้เขียน เรื่องย่อ(SUMMARY) บทนำ(INTRODUCTION) วิธีการศึกษา(METHODS) ผลการศึกษา(RESULTS) วิจารณ์ผล(DISCUSSION) บทสรุป(CONCLUSION) คำขอบคุณ(ACKNOWLEDGEMENT) และเอกสารอ้างอิง (REFERENCES)

4. บทความปริทัศน์ควรมีโครงสร้างตามลำดับดังนี้ ชื่อเรื่อง ชื่อและสถาบันที่ทำงานของผู้เขียน เรื่องย่อ บทนำและเนื้อเรื่อง(ซึ่งไม่จำกัดลักษณะ) บทสรุป คำขอบคุณ และเอกสารอ้างอิง

5. บทความทั่วไป เวทีทัศน์ จดหมายถึงบรรณาธิการ และบทบรรณาธิการ ไม่จำกัดหัวข้อการเขียน และอาจใช้การอ้างอิงแบบบรรณานุกรม(BIBLIOGRAPHY หรือ READING LIST) ก็ได้

6. เอกสารอ้างอิง ให้ใช้ระบบตัวเลขเรียงตามลำดับการอ้างถึงในเนื้อเรื่อง

การอ้างอิงเอกสารจากวารสาร ถ้ามีชื่อผู้เขียนไม่เกิน 4 คนให้เขียนชื่อทุกคน ถ้ามีชื่อผู้เขียนเกิน 4 คน ให้เขียนชื่อเฉพาะ 3 คนแรก ตามด้วยคำ และคณะ(et al) และให้จัดลำดับดังนี้

ชื่อสกุลผู้แต่ง, อักษรย่อชื่อต้น (ชื่อซึ่งเขียนเป็นภาษาไทย ให้เรียง ชื่อต้น ชื่อสกุล) ชื่อเรื่อง ชื่อวารสาร (วารสารภาษาอังกฤษให้ใช้ชื่อย่อตาม Index Medicus) เล่มที่ หน้าแรก-หน้าสุดท้าย, ปี ดังตัวอย่าง

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7. ตาราง และ/หรือรูปประกอบการตีพิมพ์พร้อมคำอธิบาย ควรพิมพ์อยู่ในเนื้อเรื่องตามตำแหน่งที่ต้องการ รูปเขียนควรเขียนด้วยหมึกอินเดียนบนกระดาษขาวอย่างดี รูปถ่ายควรเป็นรูปขาวดำบนกระดาษอย่างเรียบ ทั้งตาราง รูป และคำอธิบายควรมีขนาดพอเหมาะที่จะตีพิมพ์ลงในกรอบขนาด 1 หน้าของวารสารได้โดยตรง (ไม่เกิน 15 x 20 cm.) ตาราง และ/หรือรูปซึ่งนำมาจากผลงานที่ตีพิมพ์แล้วจะต้องอ้างถึงแหล่งที่มาด้วย

การส่งต้นฉบับ

ให้ส่งต้นฉบับจำนวน 2 ชุด ต่อคณะบรรณาธิการได้ทุกคน หรือจะส่งทางไปรษณีย์ถึงบรรณาธิการ พ.ต. บพิตร กลางกัลยา ภาควิชาเภสัชวิทยา วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า ถนนราชวิถี กรุงเทพ 10400 บรรณาธิการจะส่งคำตอบรับ และ/หรือข้อเสนอแนะในการแก้ไขต้นฉบับมายังผู้เขียน ซึ่งในกรณีที่มีการแก้ไข ผู้เขียนอาจแก้ไขตามคำแนะนำ หรืออธิบายยืนยัน หรือเขียนเพิ่มเติมตามที่เห็นสมควร แล้วส่งคืนยังคณะบรรณาธิการโดยด่วน เพื่อพิมพ์ตามรูปแบบและตีพิมพ์ในวารสารต่อไป

อภินันทนาการ

จาก

บริษัท ไทยแจแปน ดิสทริบิวเตอร์ จำกัด

อาคาร D.T.C.

176 ซอยพงษ์เวชอนุสรณ์ ถนนสุขุมวิท (64) พระโขนง กทม.

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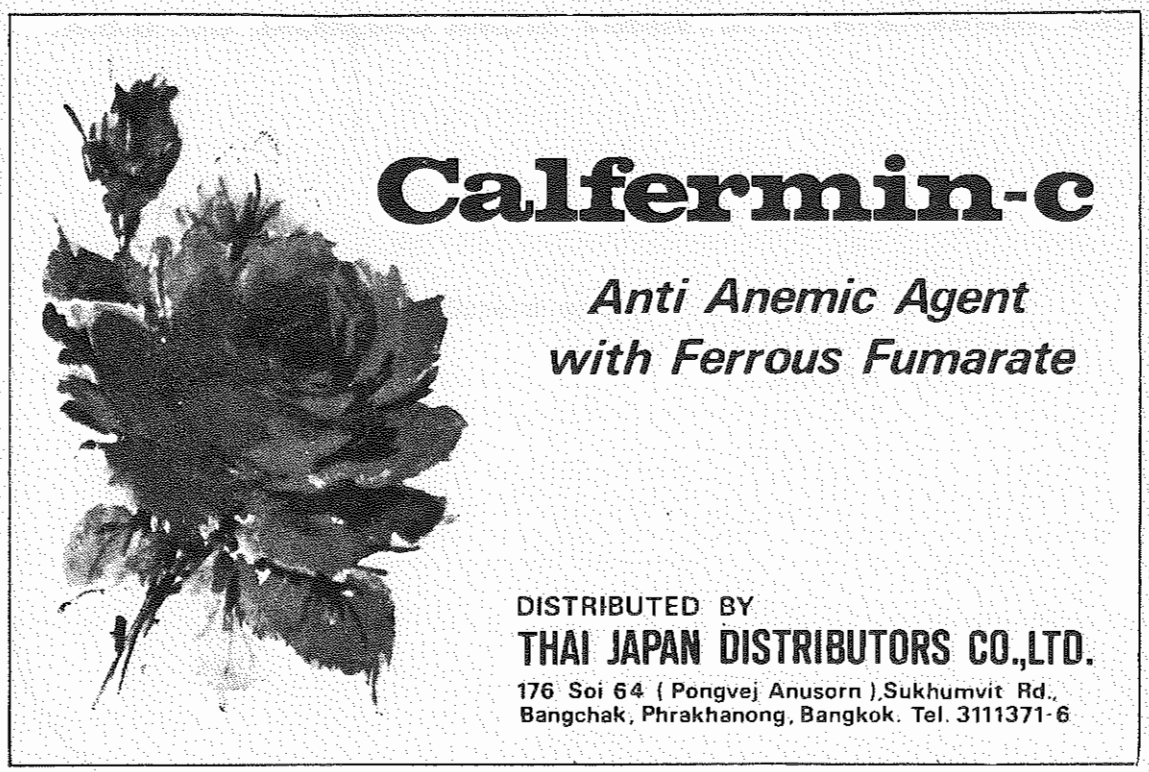

อภินันทนาการ

จาก

ห้างหุ้นส่วนจำกัด ชัชรีย์

จำหน่าย เคมีภัณฑ์ เครื่องแก้วและอุปกรณ์วิทยาศาสตร์

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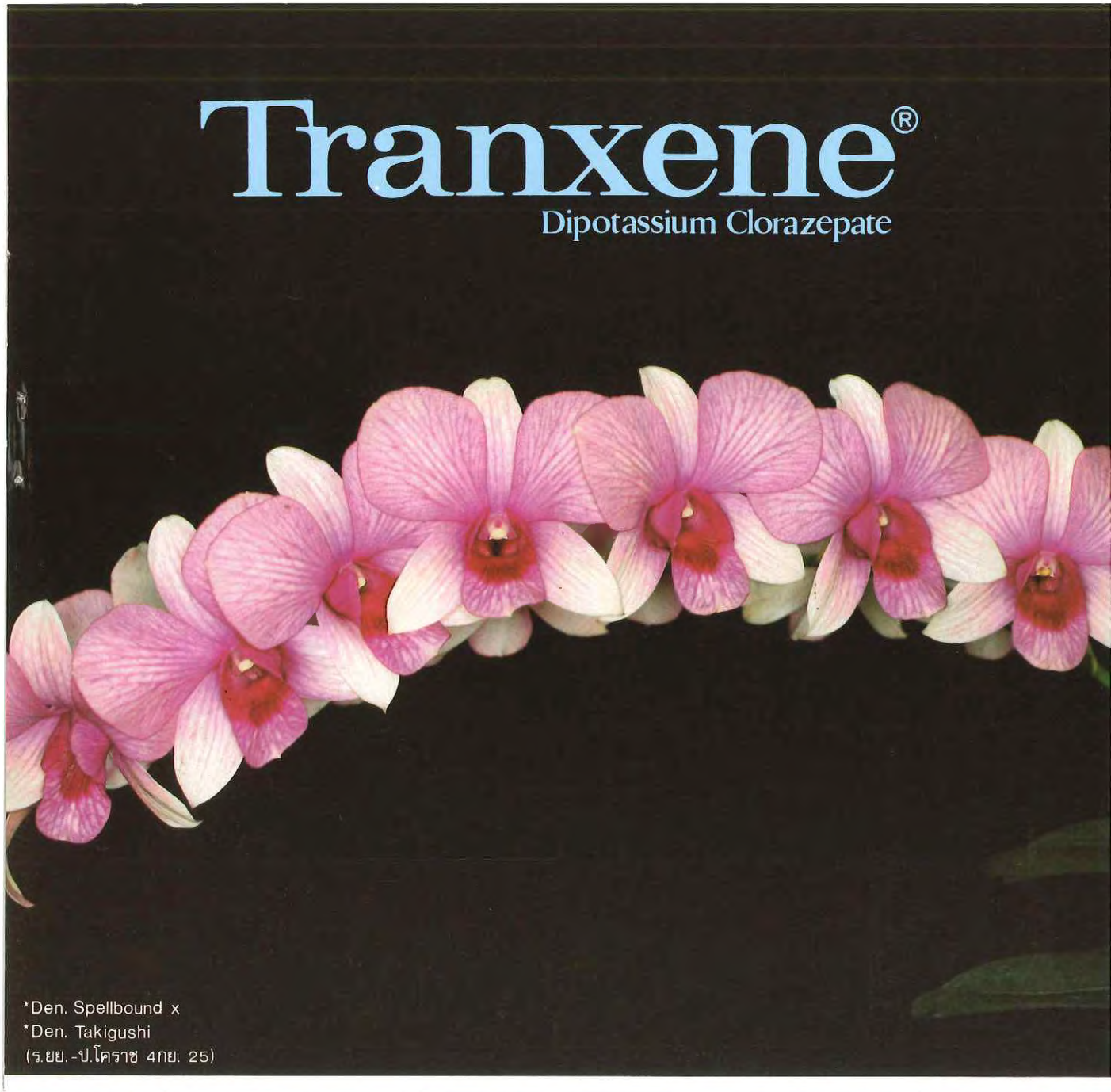

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