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CHANGES IN SERUM IMMUNOGLOBULIN LEVELS AMONG CYNOMOLGUS MONKEYS WITH LONG TERM MORPHINE TREATMENT

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การเปลี่ยนแปลงของระดับอิมมูโนโกลบูลินในซีรัมของลิงแสมที่ได้รับมอร์ฟีนเป็นระยะเวลานาน

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บทคัดย่อ

ได้ศึกษาการเปลี่ยนแปลงของภูมิคุ้มกันและฮอร์โมนคอร์ติซอลในซีรัมของลิงที่ได้รับมอร์ฟีนเป็นระยะเวลานาน โดยการฉีดมอร์ฟีนขนาด 3 และ 6 มิลลิกรัมต่อน้ำหนักตัวเป็นกิโลกรัมให้กับลิงแสมตัวผู้ กลุ่มละ 3 ตัวทุกวัน แล้วทำการเจาะเลือดเพื่อนำมาวิเคราะห์หาระดับของอิมมูโนโกลบูลินชนิด G (IgG) และ M (IgM) และฮอร์โมนคอร์ติซอลสัปดาห์ละครั้ง

การศึกษาพบว่าระดับของฮอร์โมนคอร์ติซอลลดลงในระยะแรกแล้วค่อย ๆ เพิ่มขึ้นในระยะต่อมา สำหรับระดับของอิมมูโนโกลบูลิน, IgG และIgM นั้น พบว่ามีระดับต่ำลงในกลุ่มที่ได้รับมอร์ฟีนในขนาดสูง (6 มิลลิกรัมต่อกิโลกรัม) เมื่อเปรียบเทียบกับกลุ่มที่ได้รับมอร์ฟีนในขนาดต่ำ (3 มิลลิกรัมต่อกิโลกรัม) โดยที่จะพบว่าระดับของอิมมูโนโกลบูลินจะเพิ่มขึ้นในกลุ่มที่ได้รับมอร์ฟีนในขนาดต่ำ และ IgG ลดลงในกลุ่มที่ได้รับมอร์ฟีนในขนาดสูง ดังนั้นการได้รับมอร์ฟีนในขนาดสูงอาจจะมีผลต่อการกดภูมิคุ้มกันและอาจเป็นผลนำไปสู่ภาวะการเกิดโรคขึ้นได้

ABSTRACT

Effects of chronic morphine exposure to monkeys on humoral immune status with reference to serum cortisol levels were studied. Male cynomolgus monkeys *(Macaca fascicularis)* were exposed to morphine daily (3mg/kg; n=3, 6mg/kg; n=3) and their blood specimens were collected every week to test serum levels of immunoglobulins and cortisol.

Serum cortisol levels decreased initially and then elevated gradually. Levels of immunoglobulins, IgG and IgM, in monkeys exposed to large dose (6mg/kg) of morphine was lower than that in monkeys with small dose (3mg/kg). An elevation in Igs levels were obsereved in monkeys treated with the small dose of morphine but consistent reduction in the serum IgG levels was observed in monkeys exposed to morphine with the large dose. Therefore, chronic exposure to the large dose of morphine may lead to immunosuppression and result in disease prone status.

INTRODUCTION

Morphine is an analgesic narcotic substance that exerts its pharmacological effects through interaction with selective opiate receptors in neurons.[1,2,3] There are dramatic heterogeneity in opiate receptors in the brain of mammals and higher levels of their expression have been found in the anterior amygdala, periaqueductal gray, thalamus, caudate head, hypothalamus and pituitary gland.[3,4,5] Therefore, opiates act on the hypothalamus to stimulate and/or to inhibit its function in hormone releases.[6,7]

It is noteworthy that drug abusers are concurrently prone to be suffered from infectious diseases and is readily conceivable that chronic drug addiction compromises immune status through direct and/or indirect pathways. Presence of the possible effects of opiates on lymphocyte functions has been recognized under certain circumstances,[8] however, there is still little direct evidence for the relations between addiction and immunological conditions.[9]

A tight relationship between endocrinological conditions and immune status has been pointed out[10] and our previous study has shown that certain amounts of chronic morphine administration leads a decrease in cortisol levels in cynomolgus monkeys.[11] Negative relationship between cortisol levels and immunological status is widely accepted[12,13,14,15] and the relationships between drug addiction and immune status with reference to endocrinological parameters status are of interest. This prompted us to design this work studying relations between chronic morphine administration and humoral immune status in cynomolgus monkeys.

MATERIALS AND METHOD

Subjects

Six adult male cynomolgus monkeys *(Macaca fascicularis)* maintained at Primate Research Unit, Chulalongkorn University were subjected. They were housed individually in a galvanized iron cage. The photo period was 0600-1800 h light. Temperature and humidity were slightly fluctuated according to the season. The animals were fed with monkey chow (purchased from Pokphan animal Feed Co. Ltd.) daily in the morning and with fresh fruit and vegetables supplemented occasionally with boiled chicken eggs in the afternoon.

Experimental design

The animals were divided into 2 groups, a large dose and a small dose morphine addiction groups. Each group included 3 monkeys. They were subcutaneously inoculated with saline during pre- and post- treatment periods. Morphine hydrochloride with a concentration of 6.0mg/kg was inoculated into monkeys of the large dose group daily for 110 days,

whereas, 3.0mg/kg was inoculated into monkeys of the small dose group daily for 74 days. All the injections were performed at 1200-1300 h. Fasting blood samples were drawn by femoral venupuncture from anaesthetized monkeys kept in the restraining chair at 0800-0900 h with an interval of 7 days in principle. Serum was separated immediately after the blood clotting at room temperature by centrifugation at 1000g for 20 min. Serum was then aliquoted and stored at -40C until cortisol and immunoglobulin assays.

Immunoglobulin assay

IgG and IgM levels were determined by the single radial immunodiffusion method. Serum (5ml) was pipetted into each well of IgG and IgM immunolpates (MBL Ltd., Nagoya Japan). The IgG and IgM plates were then incubated to complete reaction at room temperature for 48 and 72 hours, respectively. The diameter of each precipitation ring was measured and concentration of immunoglobulins was calculated according to the standard sera.

Cortisol assay

Serum cortisol was assayed following Sufi et al. (1986)[16] which employs homogenous RIA technique and the present data is based on Malaivijitnond (1995).[17]

RESULTS

As the original data fluctuated widely (Figure 1), it was difficult to find out the tendency in the effect of morphine treatment. Then, the original data were standardized. First, we set average concentration of cortisol, IgG and IgM values of 3 monkeys in each group during pre-treatment period as 1.0,. Then, subsequent periods were divided into sub-period and averages of original data were calculated in each sub-period and shown as relative values against control value.

Serum cortisol level (Figure 2a) decreased initially and then elevated gradually up to x1.6 of control level at the end of post-treatment period in both of the group. Large dose group showed quicker recovery and elevation of cortisol and the relative values were higher than that of small dose group throughout the experiment.

IgM level (Figure 2b) increased in both of the group during treatment and post-treatment period, however, the increase in the large dose group was very small and the level was nearly control level.

Consistent reduction in the serum IgG level was observed in the large dose group (Figure 2c). On the other hand, a slight elevation was found in the small dose group (Figure 2c).

No similar profiles were found among the fluctuation pattern in serum cortisol, IgG and IgM.

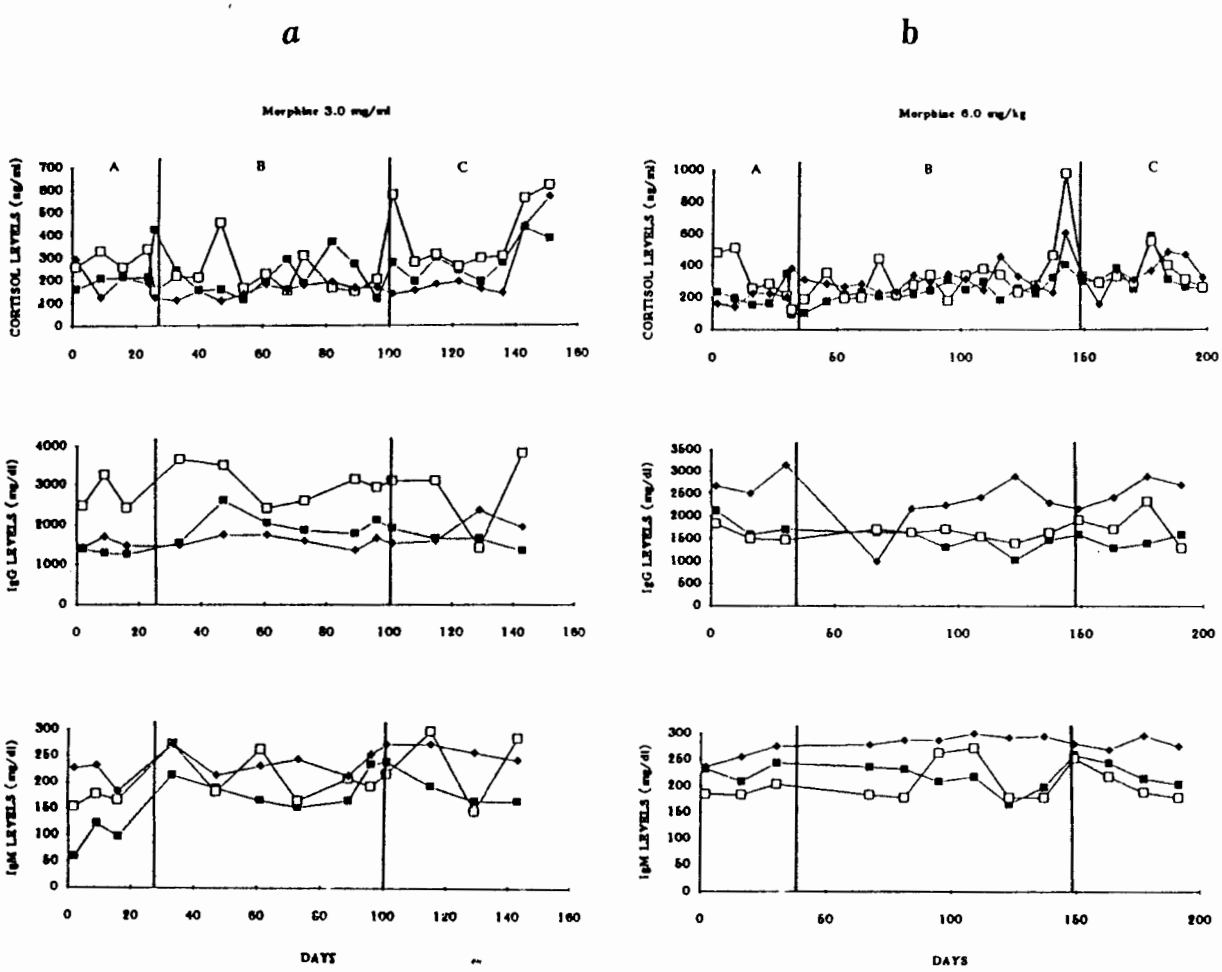

Figure 1: Individual alteration of serum cortisol, immunoglobulin G (IgG) and immunoglobulin M (IgG) concentration in monkeys treated with morphine hydrochloride with a concentration of

a) 3.0 mg/kg/day (monkeys no 505, —■—, no 509; —□—, no 511; —◆—)

b) 6.0 mg/kg/day (monkeys no 506; —■— ,no 508; —□—, no 704; —◆—)

Period A,B and C corresponds to pre-treatment, treatment and pos-treatment period, respectively,

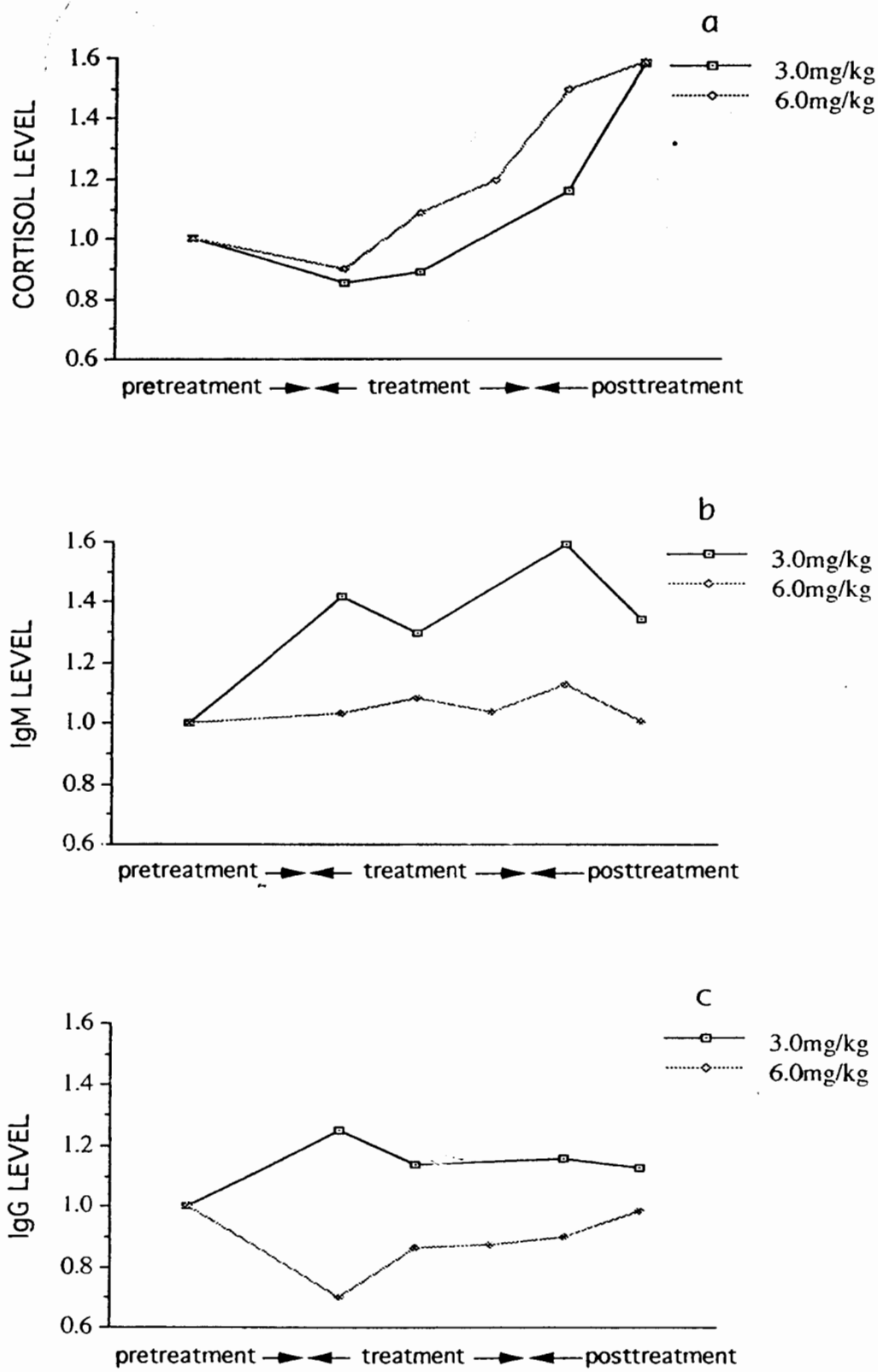

Figure 2: Alteration of serum cortisol (a), immunoglobulin M (IgM) (b) and immunoglobulin G (IgG) (c) in monkeys under daily exposure to morphine. Each value is shown as relative value of control period which is set as 1.0, respectively.

DISCUSSION

Chronic morphine treatment shows different effects on psychological, endocrinological and behavioral status. Our previous study on the effect of chronic morphine treatment in macaques showed alterations in serum prolactin, thyroid and sex hormone levels.[11,17] In this study, we examined effects of morphine addiction on the immune and stress conditions in monkeys.

It is generally agreed that immune status is subjected to stresses and a decrease of blood immunoglobulins levels by psychological stress is also documented in apes and monkeys.[18,19] Cortisol is a stress marker, which responses quickly to stressers. Therefore, its fluctuation range is wide and its level is unstable. Figure 2a shows changes in cortisol levels in different periods. Cortisol level in monkeys with a small dose was lowered during the treatment period probably by narcotic effect of the morphine. High dose of morphine treatment may quickly lead to a tight dependence on morphine in monkeys and this dependence induces stress in monkeys which results in a low cortisol level with a short period. Behavioral observation during the experiments also indicated this stressed conditions in the monkeys.

Recent study of immune alterations in chronic morphine-treated (3.2mg/kg) rhesus monkeys *(Macaca mulatta)* reported changes in T-cell subset ratio, increase in immunoglobulin production from cultured lymphocytes, but no differences in the plasma immunoglobulin levels.[9] Our data showed an increase in the immunoglobulin levels during treatment and post-treatment periods in monkeys with a small dose of morphine (3mg/kg) and this discrepancy may be resulted from different species of monkeys and the different experimental design. Increase in polyclonal IgG and IgM levels in monkeys treated with small doses of morphine corresponds to a decrease in cortisol during treatment period, since a lower cortisol level leads an increase in the basic Ig secretion from lymphocytes.[7] However, no clear patterns among cortisol, IgM and IgG levels have been identified.

The lower levels of IgM and IgG in monkeys treated with the large dose of morphine indicate that exposure to and dependence on morphine may induce immunosuppression and result in a tendency to acquire infectious diseases through direct or indirect way among tightly dependent drug abusers.

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References

1. Kuhar MJ, Pert CB and Snyder SH. Regional distribution of opiate receptor binding in monkey and human brain. Nature1973; 245: 447-450.
2. Pert CB, Snowman AM and Snyder SH. Localization of opiate receptor binding in synaptic membranes of rat brain. Brain Res 1974; 70: 184-188.
3. Snyder SH, Pert CB and Pasternak GW. The opioid receptor. Ann Intern Med 1974; 81: 534-540.
4. Simantov R and Snyder SH. Opiate receptor binding in the pituitary gland. Brain Res 1977; 124: 178-184.
5. Rossier J, Pittman Q, Bloom F and Guillemin R. Distribution of opioid peptides in the pituitary: a new hypothalamicpars nervosa enkephalinergic pathway. Fed Proc 1980; 39: 2555-2560.

6. Delitala G, Virdis R, Tomasi P, Palermo M and Grossman A. Enhancement of GRF(1-29)- induced GH release by an agonist of opiate receptors. Proc 8th Int Congr 1988: 198.
7. Grossman A. Opioid peptides and reproductive function. Sem. Reprod. Endocrin. 1987; 5: 115-124.
8. Johnson HM, Smith EM, Torres BA and Blalock JE. Neuroendocrine hormone regulation of *in vitro* antibody production. Proc Natl Acad Sci USA 1982; 79: 4171-4174.
9. Carr DJJ and France CP. Immune alterations in morphine treated rhesus monkeys. J Pharmacol Exp Therapeutics 1993; 267: 9-15.
10. Greenspan FS. Basic and Clinical Endocrinology. 3rd ed. Appleton & Lange USA. 1991.
11. Settheetham W, Varavudhi P and Yodyingyuad U. Influence of chronic morphine treatments on serum levels of P, E2 and cortisol in adult female cynomolgus monkeys. In Primatology Today. ed. A Ehara et al. Elsevier Sci. Publ. 1991: 409-410.
12. Allolio B, Schulte HM, Duess U, Kallabis D, Hamel E and Winkelmann W. Effect of oral morphine and naloxone on pituitary-adrenal response in man induced by human corticotorophinrele asing hormone. Acta Endocrin. 1987;114: 509-514.
13. Ben-Eliyahu S, Yirmiya R, liebeskind JC, Taylor AN. and Gale RP. Stress increases metastatic spread of mammary tumor in rats: evidence for mediation by the immune system. Brain Behav Immun 1991; 5: 193-205.
14. Ben-Eliyahu S, Shavit Y, Yirmiya R and Liebeskind JC. Stress induced suppression of natural killer cell cytotoxcity in rat: a naltrexone-insensitive paradigm. Behavioral Neurosci 1990; 104: 235-238.
15. Batuman OA, Sajewski D, Ottenweller JE, Pitman DL and Natelson BH. Effects of repeated stress on T cell function in rats. Brain Behav. Immun 1990; 4: 105-117.
16. Sufi S, Donaldson L and Jeffcoate R. Method Manual. WHO special programme of research, development and research training in human reproduction in programme for the provision of matched assay reagents for the radioimmu noassay of hormone in reproductive physiology. 1986.
17. Malaivijitnond S.Pharmacokinetics of morphine related to the regulation of gonadal and thyroidal functions in pubertal and adult male cynomolgus monkeys. Ph.D thesis, Chulalongkorn Univesity, 1995.
18. Scanlan JM, Coe CL, Latts A and Suomi SJ. Effects of age, rearing, and separation stress on immunoglobulin levels in rhesus monkeys. Am J Primatol 1987; 13: 11-22.
19. Masataka N, Ishida T, Suzuki J, Matsumura S, Udono S and Sasaoka S. Dominance and immunity in chimpanzees (Pan troglo dytes). Ethology 1990; 85: 147-155.