Original Article

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The Biochemical Study of Intermaxillary Fixation (IMF) Stress in Oral Surgery Inpatients

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Summary: Although intermaxillary fixation (IMF) is performed to treat the patients with maxillary fracture, this procedure is very stressful to the patients. IMF has been reported to increase noradrenaline (NA) release in the brain and elevate plasma corticosterone contents in the rat. These changes were significantly attenuated by diazepam, an anxiolytic of the benzodiazepine family. These results suggest that IMF could greatly affect the pituitary-adrenal system as a stress. In the present study, in order to examine the influence of IMF on the human body function, we measured levels of 17-hydrocorticosteroids (17-OHCS) and 17-ketosteroid (17-KS), which are metabolites of the adreno-cortical hormone cortisol, in the urine of inpatients undergoing IMF. The subjects were requested to fill out a questionnaire on irritableness caused by IMF. In these patients, urinary 17-OHCS levels were significantly increased after IMF and well correlated to the results of the questionnaire. The finding suggested that urinary 17-OHCS levels reflect stress related to IMF, and that such stress mainly causes an irritated feeling. Natural killer cell activity (NK activity), which is considered to be related to stress, was measured in these patients. The relationship between 17-OHCS levels and NK activity was examined in reference to the results of the questionnaire. Questionnaire showed that most patients noted insomnia and an irritated feeling during IMF. To examine the influence of anxiolytic agents on stress related to IMF, an anxiolytic agent, ethyl loflazepate, was administered during IMF, and urinary 17-OHCS levels were measured. There was no correlation between 17-OHCS levels and NK activity in the patients. Furthermore, no correlation was observed between visual analogue scale (VSA) and NK activity. Increases in 17-OHCS levels in the group treated with ethyl loflazepate, an anxiolytic of the benzodiazepine family, were significantly lower than in the untreated group. This suggests that ethyl loflazepate reduced stress responses to IMF. It has been reported that NK activity is reduced inpatients with depression or chronic fatigue syndrome. However, NK activity may not be affected by mechanical stress such as IMF. The finding that an anxiolytic agent, ethyl loflazepate, inhibited stress responses to IMF further suggests that anxiolytic drugs are very useful for treatment of irritated feeling of the patients undergoing IMF.

Key words stress, intermaxillary fixation (IMF), ethyl loflazepate, 17-hydrocorticosteroids (17-OHCS), 17-ketosteroid (17-KS), natural killer cell activity (NK activity)

INTRODUCTION

Intermaxillary fixation (IMF) is a treatment modality used in oral surgery. However, it is very stressful to the patients since during the course of IMF, various restrictions in the patient's daily life, such as inconvenience in eating and speaking can occur. In order to study the stress caused by IMF, Koga et al. [1,2] performed IMF in rats, and measured levels of noradrenaline (NA) and its major metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) in the rat brain. They found that IMF enhanced NA

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release in the rat brain along with increased plasma corticosterone levels and adrenal gland weight, which showed that IMF greatly affected the pituitary-adrenal system as a stressor. Further, Koga et al. [3] found that increases in both MHPG-SO_4 and plasma corticosterone levels in the rats undergoing IMF were significantly attenuated by the administration of diazepam. From these results, they suggested that anxiolytic agents could be useful for relieving stress in the patients undergoing IMF.

In the present study, in order to evaluate the influence of IMF on the human body, we measured urinary levels of catecholamines (adrenaline and NA) and of 17-hydroxycorticosteroid (17-OHCS) and 17-ketosteroid (17-KS), which are metabolites of the adreno-cortical hormone cortisol, in patients undergoing IMF. Simultaneously, the patients were requested to fill out a questionnaire on irritability.

Further, we measured the activity of natural killer cells (NK activity) in these patients, since natural killer (NK) cells have been reported to play a principal role in nonspecific human immunity [4,5] and it was reported that NK activity is influenced by various types of stresses [6-9]. Further, we examined the effects of ethyl loflazepate [10,11], a long-acting anxiolytic agent of the benzodiazepine family, on changes in 17-OHCS levels in patients undergoing IMF.

SUBJECTS AND METHODS

Subjects

The subjects consisted of 59 inpatients (40 males and 19 females) who underwent IMF for the treatment of fracture or deformity of the jaw at the Department of Dentistry and Oral Surgery in Kurume University Hospital. The patient age ranged from 16 to 54 years (mean, 27.2 years). NK activity was measured in 19 of 59 patients, and ethyl loflazepate, a long-acting anxiolytic agent, was administered per os to 12 of 59 patients.

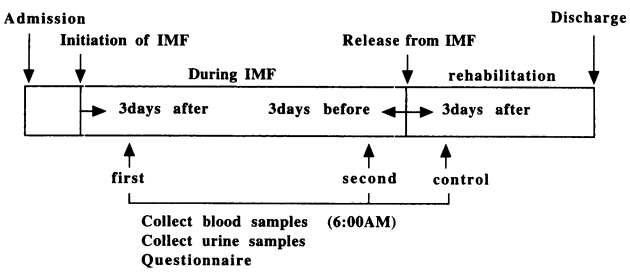


Fig. 1. Experimental schedule.

Experimental schedule

As in the previous study [12], a 24-hour urine sample was collected twice during the course of IMF (3 days after the initiation of IMF and 3 days before the release from IMF) and once 3 days after the release from IMF to measure urinary levels of 17-OHCS and 17-KS. A questionnaire was filled simultaneously with the biochemical examinations twice during IMF and once after the release from IMF (Fig. 1).

NK activity was measured using blood samples collected three times early in the morning (6:00 AM) simultaneously with biochemical examinations. In addition, 1 mg of powdered ethyl loflazepate, was administered every day once after supper during the course of IMF.

All the patients were under the stress of traumatic pain and anxiety during hospitalization. Control urine samples were collected after the release from IMF.

Measurement of 17-OHCS and 17-KS

Urine samples were collected for 24 hrs between 0:00 a.m. and 0:00 a.m. the next day. After completion of 24-hour urine collection, 50 ml of urine was transferred to a test tube, and stored at -20 ℃ until assayed.

After dephosphorylation, a steroid conjugate of 17-OHCS was hydrolyzed using gluphatase, and then the fluorescence that developed after reaction with phenylhydrazine (Porter-Silber reaction) was measured at wave lengths of 370 nm, 410 nm, and 450 nm [13]. After a similar procedure, the levels of 17-KS were measured on the basis of the fluorescence developed after reaction with m-dinitrobenzene (Zimmermann reaction) at wave lengths of 460 nm, 520 nm, and 580 nm [14]. All data were corrected by creatinine levels.

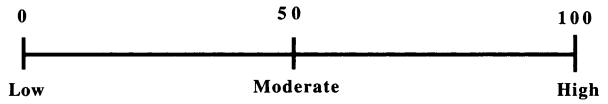
Measurement of NK activity

Heparinized blood samples (5 ml each) were stored at 4 ℃ and NK activity was measured by the cytotoxicity test using the Cr-release method within 24 hrs. Briefly, the target cells (K562) were labeled with $_{51}Cr$, and then impaired by NK cells. Subsequently, the level of $_{51}Cr$ release from the target cells was measured [15,16]. We entrusted the measurement of NK activity to SRL Co., Ltd.

TABLE 1.
Questionnaire

1. Do you have an irritated feeling?
   a. potently irritated feeling. b. markedly irritated feeling.
   c. slightly irritated feeling. d. do not have an irritated feeling.

2. How do you feel now?
   Mark the figure below



Visual Analogue Scale (VAS)

Questionnaire

Table 1 shows the details of questionnaire. In question 2, the feeling of the patients during the examinations was numerically expressed using the visual analogue scale (VSA).

Statistical analysis

Statistical analysis was performed using the analysis of variance (ANOVA). Correlation analysis was performed using Pearson's correlation analysis [17,18].

RESULTS

Relationship between 17-KS and 17-OHCS levels and VAS values

The correlation between the VAS values, that reflect the feeling, and the levels of 17-KS and 17-OHCS was evaluated in the 59 patients used in this study. There was positive correlation between 17-OHCS ($r=-0.524$) levels and between VAS values and 17-KS levels ($r=-0.492$) (Figs 2 and 3). In patients not treated with ethyl loflazepate, the 17-OHCS levels obtained in the first and second measurements were significantly higher than the control values ($p<0.001$, Fig. 4A), whereas only the levels of 17-KS obtained in the first measurement were significantly higher than the control value ($p<0.01$, Fig. 4B).

Relationship between NK activity and levels of 17-OHCS and 17-KS

Mean values for NK activity in 19 patients were 28.42% 3 days after the initiation of IMF, 34.11% 3 days before the release from IMF, and 28.74% 3 days after the release from IMF. That is, the mean value for NK activity obtained 3 days after the initiation of IMF was similar to that obtained 3 days after the release from IMF, and both were within the range of standard values (18-40%). There were no significant correlation between 17-OHCS levels and NK activity (r=0.1) (Fig. 5) or between 17-KS levels and NK activity (r= 0.088). There was no significant correlation between VAS values and NK activity (r=0.269) (Fig. 6).

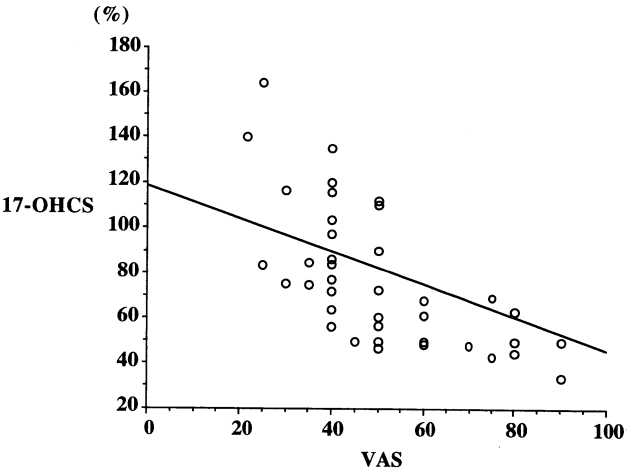


Fig. 2. Correlation between VAS values and 17-OHCS levels. Correlation: $r=-0.524$ ($p<0.001$) (n=59)

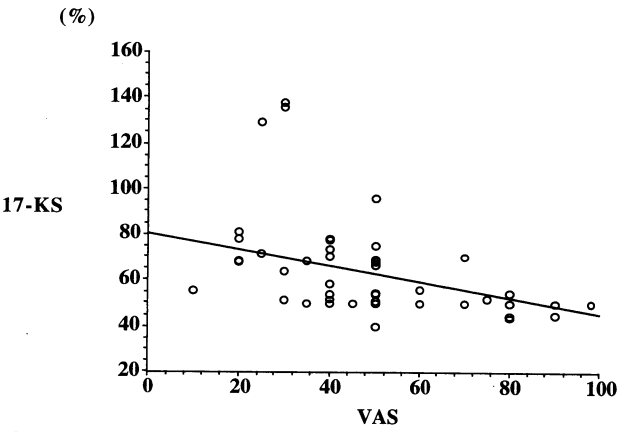
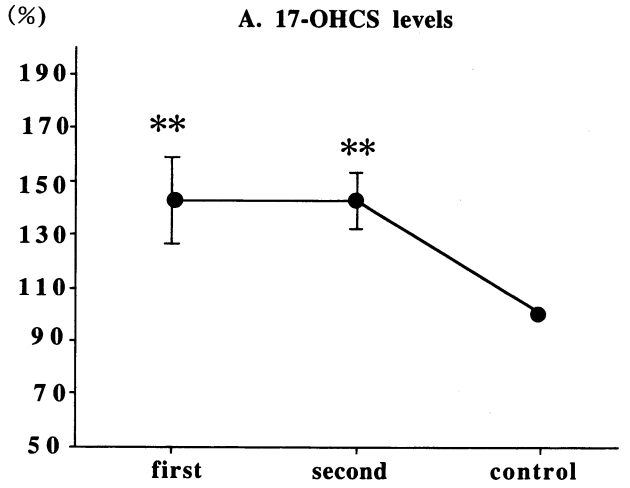


Fig. 3. Correlation between VAS values and 17-KS levels. Correlation: $r=-0.492$ ($p<0.05$) (n=59)

Effects of the ethyl loflazepate administration

The levels of 17-OHCS were significantly lower ($p<0.01$) in the ethyl loflazepate-treated group than in the untreated group 3 days after the initiation of IMF (Fig. 7).



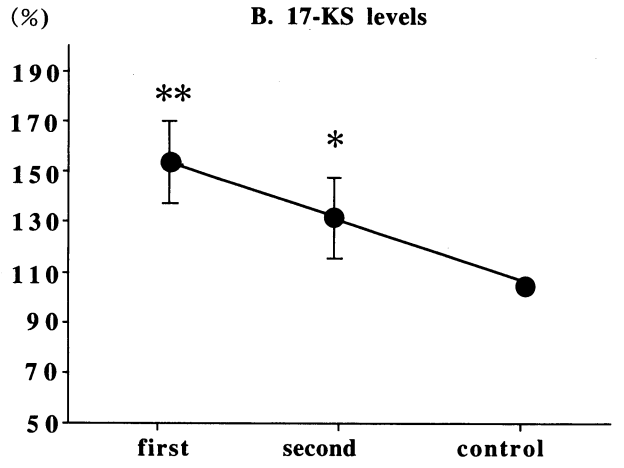


Fig. 4. Time course of 17-OHCS and 17-KS levels.
*: $p<0.05$ **: $p<0.01$ vs control (n=47)
Each value is %/control. Error bar is Mean ± S.E.M.

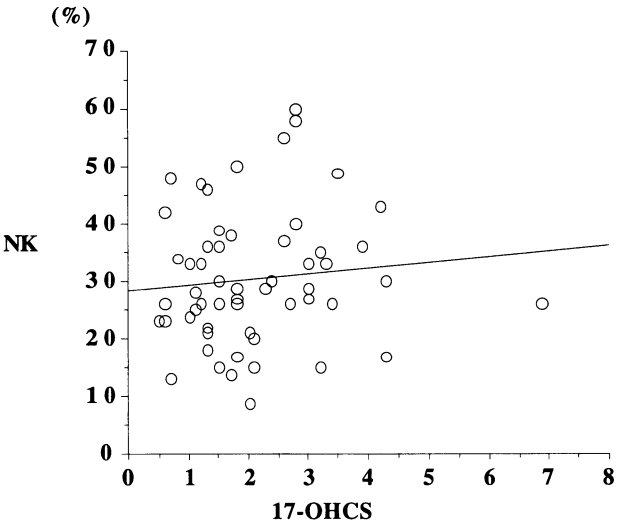


Fig. 5. Correlation between 17-OHCS and NK activity. r=0.1 p=0.4601

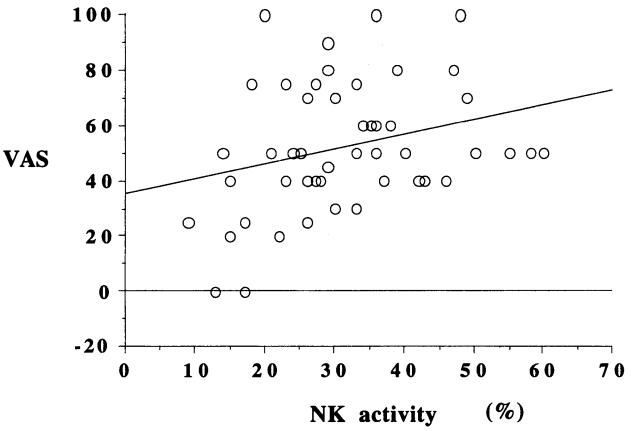


Fig. 6. Correlation between VAS values and NK activity. r=0.269 p=0.042

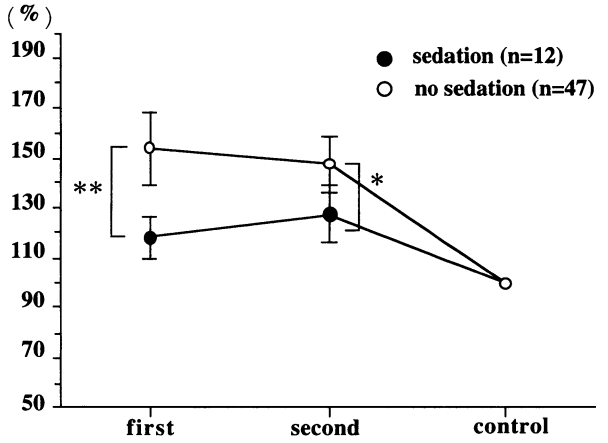


Fig. 7. Effect of ethyl loflazepate on 17-OHCS.
*: $p<0.05$ **: $p<0.01$
Each value is %/control. Error bar is Mean ± S.E.M.

Results of a questionnaire

The results of a questionnaire revealed that most patients noted an irritated feeling during the course of IMF. In addition, most patients replied that the inconvenience in eating was the most stressful issue during the course of IMF. Subsequently, when answers to the questions regarding an irritated feeling during the course of IMF were compared between ethyl loflazepate-treated and untreated groups, only 9 of 47 patients in the untreated group replied that they did not have an irritated feeling, whereas 13 of 47 patients replied that they had a potently or markedly irritated feeling. In contrast, 8 of 12 patients in the ethyl loflazepate-treated group replied that they did not have an irritated feeling, and 4 of 12 patients replied that they had a slightly irritated feeling.

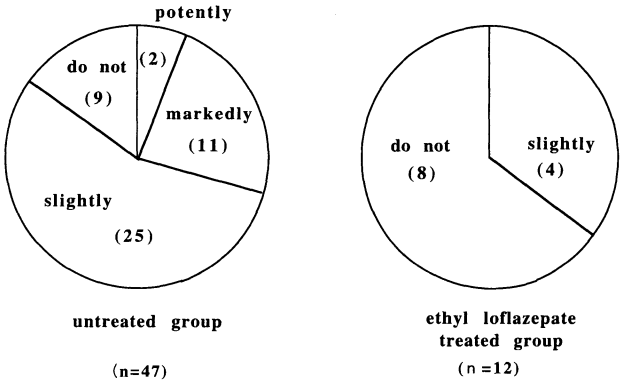


Fig. 8. Effect of ethyl loflazepate on irritated feeling.

However, no patients in the ethyl loflazepate-treated group reported potently or markedly irritated feelings (Fig. 8).

DISCUSSION

Currently, IMF is widely used to treat maxillofacial fracture, bone defects after the removal of cysts or tumors in the oral cavity, bone defects due to mutilation or segmental resection of the maxillary bone, or for gnathoplasty [19]. Physiologic mandibular movement is naturally lost in patients undergoing IMF, making food intake by mastication impossible and ordinary conversation difficult. The duration of IMF is approximately 1 month, but it can be shortened or sometimes prolonged for more than 2 months according to the patient's condition. Therefore, IMF can be extremely painful and very stressful to patients. It should be noted that the term, "stress" was originally used in the field of engineering to mean mechanical stress. Selye [20] contributed to the widespread use of the term "stress" in the field of medicine. Currently, the word, "stress" is frequently used as a generic medical term that means factors involved in the etiology and pathologic conditions of mental and physical disorders. Such a widespread use of the term, "stress" even in a daily conversation reflects the stressful modern society where various types of stress exist. Under such circumstances, psychological care for relieving patients from various types of stress is important in addition to the medical treatment of diseases. Selye [20] first proposed the concept of "stress" in 1936, and various studies have been conducted since. The recent advancement of neurochemistry, neuropharmacology, and immunohistochemistry revealed the presence of various neurotransmitters and neurotransmission modifiers in the brain. In addition, the development of microanalyses for these substances and their metabolites provided many novel findings regarding the intracerebral neurotransmission mechanism. Thereafter, many studies have been conducted to clarify the stress-induced biochemical changes in the brain [21-27]. Based on the theory that stress causes mental disorders such as psychosomatic disease and schizophrenia, and that brain noradrenergic neurons are closely involved in the pathologic conditions of these mental disorders, we evaluated changes in brain noradrenergic neurons under various types of stress. As a result, it was found that NA is involved in the action of adreno-cortical hormones via CRH [28,29]. Both 17-OHCS and 17-KS measured in this study are metabolites of the adreno-cortical hormone cortisol which are excreted in the urine. Therefore, it was considered that changes in intracerebral NA levels, that is, the severity of stress, can be estimated by measuring urinary17-OHCS and 17-KS levels [12].

In this study, the levels of 17-OHCS and 17-KS were significantly higher than the control values in patients undergoing IMF. However, the percent increase in 17-KS was lower than that in 17-OHCS, and 17-OHCS was positively correlated with stress caused by IMF. Therefore, it was suggested that 17-OHCS would be a useful parameter for evaluating stress in IMF.

Because NK cells play the principal role in nonspecific human immunity, NK activity is naturally decreased by various types of stress [30,31]. It was also reported that NK activity is decreased in patients with depression or chronic fatigue syndrome [32-34]. When NK activity in outpatients was measured in our Department, it was decreased in those who consulted our Department because of anxiety about malignant tumors, suggesting the involvement of psychological stress, such as anxiety or fear of cancer, in NK activity [35-37]. To clarify whether there is any difference in the various types of stress that decrease NK activity, we measured the levels of 17-OHCS and NK activity in patients undergoing IMF, and compared the results. However, as reported in this study, 17-OHCS levels were increased in patients who underwent IMF to treat fracture or deformity of the jaw during the course of IMF, but they were decreased after the release from IMF. In contrast, NK activity did not tend to increase even after the release from the stress of IMF. Moreover, there were no correlation between levels of 17-OHCS and 17-KS, and NK activity ($r = -0.235$).

Although the prolonged duration of IMF becomes a chronic stress to the patients, NK activity did not significantly change in this study during the course of IMF. This finding suggested that a potent physical stress such as IMF only slightly influences the immune system because such stress is not closely associated with NK activity, showing the presence of various types of stress that affect the patient differently.

To explore a procedure for managing stress caused by IMF, the influence of an anxiolytic agent on 17-OHCS levels and the results of a questionnaire survey were evaluated in this study. Benzodiazepine anxiolytic agents such as ethyl loflazepate exhibit their actions over a prolonged period of time after binding with benzodiazepine receptors [38]. However, benzodiazepine receptors couple to $GABA_A$ receptors, a subtype of γ-aminobutyric acid (GABA) receptors, and thus exhibit their actions via $GABA_A$ receptor/benzodiazepine receptor/chloride ion channel supramolecular complex [39]. That is, the function of $GABA_A$ receptors is enhanced by the binding of ethyl loflazepate with benzodiazepine receptors, which increases the opening frequency of the chloride ion channel, resulting in hyperpolarization of the cell membrane and potent suppression of the stress. Suppression of stress via $GABA_A$ receptors may play a leading part in the biochemical mechanism of the expression of anxiolytic and anticonvulsive actions of benzodiazepine agents [11].

Levels of 17-OHCS were significantly lower in patients treated with ethyl loflazepate during the course of IMF. In addition, a questionnaire survey revealed that the proportion of patients who complained of insomnia or of an irritated feeling was lower in those treated with ethyl loflazepate during the course of IMF, demonstrating the subjective antistress action of ethyl loflazepate.

These findings suggested that the prolonged duration of IMF is a chronic stress to patients undergoing IMF, and 17-OHCS is a useful parameter for evaluating the severity of stress in IMF. Moreover, 17-OHCS levels were significantly lower in patients treated with ethyl loflazepate during the course of IMF. Therefore, it was suggested that ethyl loflazepate relieves the stress caused by IMF.

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