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EXPERIMENTAL STUDIES ON A NEW ANTI-INFLAMMATORY AGENT OF NON-STEROID GROUP, "DA-398", AND ITS CLINICAL EXPERIENCES IN ORAL SURGERY

BY

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ABSTRACT

The effect of DA-398, a new anti-inflammatory agent of the non-steroid group, on carrageenin-induced edema was examined experimentally, and its inhibitory effect on rats, weighing 120–140 g, by administration of 30 to 50 mg/kg was recognized.

The clinical effect of this drug on inflammatory and postoperative cases in oral surgery was estimated, dividing the 70 subjects into five groups whose members were administered 150–600 mg of DA-398 or placebo. It was thereby found that a daily administration of more than 300 mg would be clinically effective. As side effects produced by DA-398, some slight symptoms like stomachache and nausea were found in 4 cases (7.3%) out of the total 55 cases.

INTRODUCTION

Although medical treatment has made a remarkable progress in recent years, there is still a high frequency of inflammatory cases in the field of oral surgery. Fever, pains, swelling, and trismus arising from various types of diseases cause, in turn, functional disturbance of various kinds such as difficulty of chewing, speaking, or swallowing. Hence, treatment of such inflammatory cases is considerably important in the clinics of oral surgery.

In the field of oral surgery, most of inflammatory cases are due to dental infection while there are very rarely a few cases of allergic inflammation. Acetylsalicylic acid agents, sulfa drugs, or antibiotics have been usually used in the treatment of such cases of inflammation. In addition to them, streptokinase, Oxyphenbutazone, chymotrypsin, and Phenylbuta-

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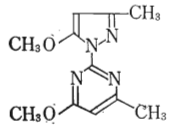
zone have recently become to be used clinically. Nevertheless, when a more effective anti-inflammatory treatment is required, we have had to use adenocortical steroids. As steroids have disagreeable side effects, we are required to reduce the dose of steroids or replace it with other kind of agents sooner or later when they are given to patients for a longer period.

Recently, mephenamic acid, Indomethacin, Bucholome, and Benzydamine hydrochloride have been developed as new anti-inflammatory agents and some of them are already being used clinically. Although such agents have a more effective anti-inflammatory function than Phenylbutazone, which is said to be the most effective anti-inflammatory agent now available and to be equal to adenocortical steroids, we look forward to anti-inflammatory agents that are more effective but with less side effects.

In the present report, we describe the result of experimental studies on the effect of DA-398 on carrageenin-induced edema, and its clinical effect for inflammatory and postoperative cases. DA-398 is a new anti-inflammatory agent named "Mebron" of the non-steroid group developed by Daiichi Seiyaku Co., Ltd.

DA-398, 1-(4-methoxy-6-methyl-2-pyrimidinyl)-3-methyl-5-methoxypyrazole, has the following structural formula:



$C_{11}H_{14}O_2N_4$
Mol. wt.: 234.27
m.p. 87.5–89.0°C

According to basic experiments, this drug has a powerful analgesic action, based on its immediate effect on the process of inflammation, and also has anti-edema, anti-granulation, and antipyretic actions, and is almost free from toxicity.

Inhibitory Effect on Experimental Edema

Effect of DA-398 on experimental edema produced by carrageenin, was examined.

1. *Materials and Methods*

Male rats of Wistar strain, weighing 120~140 g, were divided into five groups, each group consisting of five rats. As an inflammatory agent, 0.1 ml of 0.5% solution of HL-1 carrageenin (Nitto Kaiso (Seaweeds) Co.,) was injected into the right hind paw of the rats to induce edema. A solution containing 398.5 mg/ml of DA-398, was injected intrapenitoreally to 4 groups of rats and administered orally to one group. Three groups of rats

were injected intrapenitoreally with the anti-inflammatory agent, DA-398, 30 min before injection of the phlogogen. The dose of DA-398 injected to the 3 groups was 10, 30 and 90 mg/kg, respectively. The fourth group was injected intrapenitoreally 50 mg/kg of the anti-inflammatory agent at the same time as the phlogogen injection. The 5th group was administered orally 100 mg/kg of the anti-inflammatory agent at the same time as the phlogogen injection. Under the same conditions, a control group was injected the physiological saline. The degree of experimental edema of the hind paw of rats during 7 hr after the administration of DA-398 was measured and recorded by the mercurial method.

2. *Results*

Fig. 1 shows the hourly relative rate of inhibitory effect on carrageenin-induced edema in the hind paw of the rats which were administered 10, 30, or 90 mg/kg of DA-398, 30 min before carrageenin injection.

In the group of rats injected 10 mg/kg of DA-398 intraperitoneally, inhibitory effect was recognized in the first hour but was not observed thereafter. However, distinct inhibitory effect was observed for many hours, in the rats of other two groups injected 30 and 90 mg/kg of DA-398 intraperitoneally.

Fig. 2 shows changes in the rate of inhibitory effect on the carrageenin-induced edema in the hind paw of the rats injected intraperitoneally with 50 mg/kg and those which were given 100 mg/kg orally at the same time

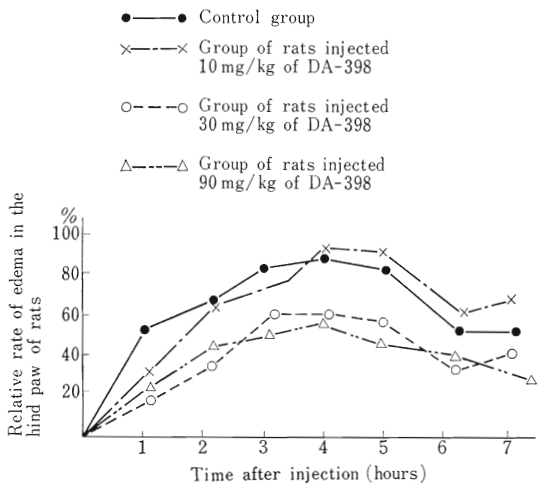


Fig. 1. Inhibitory effect of DA–398 on carrageenin-induced edema (DA–398 was administered into abdomen 30 minutes before the injection of carrageenin.)

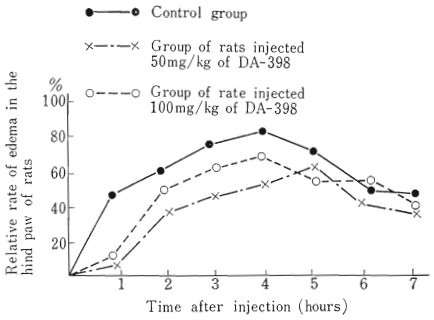


Fig. 2. Inhibitory effect of DA–398 on carrageenin-induced edema (DA–398 was administered at the same time as carrageenin injection.)

when carrageenin was injected. These two groups were compared with the control and it was found that the inhibitory effect in these two groups given DA-398 and carrageenin at the same time was smaller than that of the previously mentioned two groups in which DA-398 was given 30 min before the injection of carrageenin.

In addition general stature of the rats was observed throughout this experiment and it was evident that the rats administered 90 mg/kg were exhausted in their motion, becoming dull and that those administered 100 mg/kg orally were less exhausted.

Clinical Studies

1. *Materials and Methods*

The cases for our studies on DA-398 consisted of 70 subjects, 37 males and 33 females, who were treated at the First Department of Oral Surgery, Tokyo Medical and Dental University Hospital. These cases were classified into two groups; one group consisting of 36 inflammatory cases and the other consisting of 34 postoperative cases. Table 1 shows the entities of the diseases and the procedures. DA-398 was used in cases in which relatively minor operations were performed and who did not suffer from severe inflammatory diseases in the field of oral surgery. The cases in each group are shown in Table 1.

2. *Method of Administration*

Among the total of 70 subjects, 55 cases were divided into four groups according to the daily dose of DA-398. Group 1 of 14 cases were given 150 mg daily; 23 cases in group 2, 300 mg; 9 cases in group 3, 450 mg; and

Table 1. Cases administered DA–398

Group of inflammatory cases		Group of postoperative cases	
Diagnosis	No. of cases	Names of operation	No. of cases
Mandibular osteomyelitis	5	Bilateral osteotomy of ascending ramus of mandible	1
Maxillary osteomyelitis	2	Cheiloplasty	1
Pericoronitis of withdom-tooth	9	Vessel ligature of lingual hemangioma	2
Alveolar ostitis	10	Excision of mucocele	2
Parulis	2	Apicoectomy	1
Infectious disease after extraction of teeth	2	Sequestrectomy	1
Dry socket	1	Extraction of teeth	26
Parotitis	1		
Temporomandibular arthritis	3		
Temporomandibular arthrose	1		

9 cases in group 4, 600 mg. The control group induced 15 cases who were administered placebo. Evaluation of the results was made according to the single blind test method.

In order to determine the minimum effective dose of DA-398, the clinical effect was compared among these groups. Table 2 shows the cases and the doses administered. Antibiotics were used for most of the cases in combination with the tested medicine.

As a rule, a daily given dose was administered to the cases 30 min after each meal. The cases in the operative groups were not administered DA-398 before surgery. The duration of administration ranged from 2 to 8 days.

3. *Evaluation of the Effect and Clinical Results*

1) Evaluation of effectiveness

The cases in this study were suffering from inflammatory diseases or were postoperative cases, and there were only a few cases in which DA-398 was the chief drug. It was administered in combination with other agents,

Table 2. Daily dose of DA–398 classified by groups

Case	Daily dose of DA–398	No. of cases
Group 1	150 mg	14
Group 2	300 mg	23
Group 3	450 mg	9
Group 4	600 mg	9
Control group	6 tabletes of placebo	15

so that it was difficult to set a standard for evaluating of the effectiveness of DA-398 alone. For this reason, the effectiveness in analgesic and anti-swelling action of DA-398 was evaluated on the third day after its administration, those in which the symptoms disappeared were marked +, those in which the symptoms became less apparents ±, and those in which the symptoms were still observed as −. Considering all this together, these cases were classified clinically into three groups of effective, fairly effective, and ineffective. The antipyretic effect of DA-398 was examined in 8 cases of inflammation who developed fever.

2) Clinical results

Clinical results of DA-398 in 5 groups are shown in Tables 3–5. As the analgesic effect was influenced by the site and duration of the operation, evaluation was directed to the postoperative pain. DA-398 was not administered before but after operation in all the cases. Its analgesic effect was examined whether the patients had used other analgesics medicine or not.

(a) Group 1:

Group 1 given 150 mg of DA-398 consisted of 4 inflammatory and 10 postoperative patients. Of a total of 14 cases, 10 were marked + concerning the analgesic effect, while 7 cases were also graded + regarding anti-swelling effect. By our composite evaluation, DA-398 was effective in 57.1%, fairly effective in 14.3%, and ineffective in 28.6% of cases in Group 1. Postoperative cases accounted for six out of the total 10 cases who were marked + regarding analgesic effect. A total of 4 cases, including one case each of packing of lingual hemangioma, cheiloplasty, bilateral osteotomy of ascending ramus of mandible for improvement of occlusion, and extraction of mandibular impacted wisdomtooth, failed to show any response to analgesic effect. As for anti-swelling effect, four inflammatory cases were relieved of swelling in 3~5 days from the start of administration while one case of perimaxillitis was freed from swelling after DA-398 was administered for 8 consecutive days.

(b) Group 2:

The number of cases which were administered 300 mg of DA-398 was 23, including 17 inflammatory cases and 6 postoperative cases. Of these 17 cases were marked + with regard to analgesic effect while 16 cases were marked at + concerning anti-swelling effect. By our composite evaluation, DA-398 was effective in 73.9%, fairly effective in 8.7%, and ineffective in 17.4% of cases in Group 2.

Analgesic effect was recognized in 5 of 6 postoperative cases, but the remaining one case of extraction of mandibular impacted wisdom-tooth

suffered from dry socket and failed to be relieved of pain even after the administration of DA-398 for 4 successive days. DA-398 was administered to one case of temporo-mandibular arthrose in order to relieve the pain felt at the time of opening of the mouth and found that DA-398 was remarkably effective in relieving such a pain. DA-398 showed anti-swelling effect, had effect on all postoperative cases, but its anti-swelling effect was not seen in 4 of the total 17 inflammatory cases even after administration for 3~6 days.

(c) Group 3:

Among a total of 9 cases of this group including 6 inflammatory and 3 postoperative cases, 7 cases were marked + regarding analgesic effect while 6 cases were graded + concerning anti-swelling effect. By our composite evaluation, DA-398 was effective in 66.7%, fairly effective in 11.1%, and ineffective in 22.2% of cases in Group 3. Analgesic effect was recognized in 2 of 3 postoperative cases, one case each of sequestrectomy and excision of cyst, but not in the remaining one case of extraction of mandibular impacted wisdomtooth. Antibiotics and sulfa agents were given to all the cases in this group in combination with DA-398, and DA-398 failed to show anti-swelling effect on each case of inflammation and postoperation.

(d) Group 4:

Of a total of 9 cases in this group, including 4 inflammatory and 5 postoperative cases, 7 cases were marked + concerning analgesic effect while eight cases were graded + with regard to anti-swelling effect. By our composite evaluation, DA-398 was effective in 77.8%, fairly effective in 22.2% of cases in Group 4. This means that DA-398 was effective on all the case in this group. Analgesic effect was seen in all of postoperative cases, including 3 cases of severe jaw operation and 2 cases of soft tissues operation. DA-398 was used for one case of temporomandibular arthrose in order to relieve the pain felt at the time of opening of the mouth and it was remarkable effective. Anti-swelling effect was recognized in all of the cases who had suffered swelling.

(e) Control group:

This group given placebo included 5 inflammatory and 10 postoperative cases. Analgesic effect + was found in 4 of the total 15 cases but anti-swelling effect was not recognized. By our composite evaluation, DA-398 was effective in 26.7%, and ineffective in 73.3% of cases in control group. It is interesting to note, however, that placebo had an analgesic effect in 4 cases, all of which underwent an operation in the jaw region (see Table 3).

Comparison of the effectiveness of DA-398 in the four groups is shown in Table 4. Notable is the fact that it was less effective in group 1 given

Table 3. Effects of placebo on 15 cases
5 inflammatory cases
10 postoperative cases

Effects	Analgesic effect			Anti-swelling effect		
	+	±	−	+	±	−
Total	4		11			15
Inflammatory cases			5			5
Postoperative cases	4		6			10
Evaluation						
Effective	0					
Fairly effective	4 cases (26.7%)					
Ineffective	11 cases (73.3%)					

Table 4. Evaluation of effect by administered dose

Group No.	No. of total cases	Effective No. of cases (%)	Fairly effective No. of cases (%)	Ineffedtive No. of cases (%)
Group 1	14	8 (57.1)	2 (14.3)	4 (28.6)
Group 2	23	17 (73.9)	2 (8.7)	4 (17.4)
Group 3	9	6 (66.7)	1 (11.1)	2 (22.2)
Group 4	9	7 (77.8)	2 (22.2)	0
Total	55	38 (69.1)	7 (12.7)	10 (18.2)

150 and that effective cases accounted for about 70% in the other three groups administered over 300 mg. It is also worthy to note that Group 2 given 300 mg had 70% of effective cases, which is almost the same level as those given 450 or 600 mg.

Table 5 indicates comparison of analgesic and anti-swelling effect in the four groups. All the groups, except Group 1 showed a high percentage of effectiveness.

(f) Antipyretic effect

Antipyretic effect of DA-398 was recognized in 6 of a total of 8 inflammatory cases who had fever. The oral administration of 50 to 150 mg of DA-398 caused their body temperature of about 38°C to decrease to the level of 36°C.

Table 5. Comparisons of analgesic and anti-swelling effects

Group No.	No. of total cases	Analgesic effect			Anti-swelling effct		
		+	±	−	+	±	−
Group 1	14	10		4	7	3	2
Group 2	23	17		6	16		2
Group 3	9	7	1	1	6	1	2
Group 4	9	7	1	1	8		
Total	55	41	2	12	37	4	6

4. *Side Effects*

Side effects, which might have been due to the oral administration of DA-398, were seen in 4 (or 7.3%) of the total 55 cases. These side effects included 2 cases of stomachache and one case each of nausea and exanthema, which were relieved by withdrawal of DA-398. Reduction in the dose or suspention of administration of DA-398 was not taken but it was withdrawn immediately. There were no abnormal laboratory data in the cases with side effects from DA-398.

Discussion

In testing the clinical effect of DA-398, we first examined its effect on carrageenin-induced experimental edema by using rats. It was found that DA-398 had no inhibitory effect on such experimental edema of rats in a dose of 10 mg/kg but was effective in a dose of 30 mg/kg or more. Our observations on general stature of rats throughout the process of testing made it apparent that a group of rats administered 90 mg/kg became slow in motion and were most exhausted. Judging from these findings, a suitable amount of administration to inhibit the experimental edema ranges from 30 to 50 mg/kg in the case of rats weighing 120–140 g. A greater inhibitory effect was found when DA-398 was given 30 min before the injection of carrageenin than to administer it at the same time as the injection of carrageenin.

Based on these test findings, clinical effect of DA-398 was examined[3] in a total of 70 cases. In making which were divided into four groups according to the doses given, i.e., 150, 300, 450, 600 mg, so as to evaluate the difference in the effect depending on the dose and to determine the effective amount of administration.

Analgesic effect was recognized in 74.5% of all the cases examined and anti-swelling effect in 78.7% of the total.

As for the percentage of effective cases in each of the four groups, the 150 mg group showed the lowest rate of 57.1% and the 300 mg group represented a rate of more than 70%, while the 450 mg and 600 mg groups also indicated a similar rate of about 70%.

Statistical analysis of these data, however, failed to show significant difference among the groups due to inadequate number of subjects. Furthermore, antibiotics or other agents had to be used in combination with DA-398 because the present cases included inflammatory and postoperative cases, and it was difficult to evaluate the effect of DA-398 independently. Judging from the clinical experiences, we consider that a daily administra-

tion of 150 mg is too small to ensure a remidial effect and it is recommended that at least 300 mg be given daily. Therefore, we believe that six to nine tablets of DA-398 are the effective amount of daily administration.

Concerning the side effects of DA-398, stomachache, nausea, and exanthema were found in four (or 7.3%) of the total 55 cases. These side effects, however, were not serious and were relieved rapidly on stopping medication. Antibiotics and other agents were used in combination with DA-398 in most of the subjects and frequently samples of target-cases were drawn at random but no stomachics were given to them. In view of these fact, we assume that DA-398 produces side effects less frequently than the conventional anti-inflammatory agents. In the future, however, we intend to examine whether it is good or not to resume administration of DA-398 later or reduce the dose of administration to cope with such a side effect.

CONCLUSION

Evaluation of the results of using DA-398 gave the following conclusions.

1) Apparent inhibitory effect of DA-398 was recognized on carrageenin-induced experimental edema of rats, each weighing 120–140 g, by the administration of 30~50 mg/kg.

2) In clinical experiments, the subjects were divided into five groups according to the dose administered of 150, 300, 450 and 600 mg of DA-398, and placebo, and the effective percentage in each of the five groups was estimated to examine the effective amount of administration. It was concluded that a daily administration of more than 300 mg would be effective clinically and it is recommended that 6–9 tablets of DA-398 be given after each meals daily.

3) As a side effect produced by DA-398, slight symptoms such as stomachache and nausea were recognized in 7.3% of the total cases, but such side effects were relieved by stopping of the administration of DA-398.

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