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A Comparative Study of Anti-Inflammatory Activity of Diflunisal and its Copper Complex

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ABSTRACT

The research work was undertaken to compare the anti-inflammatory activity of diflunisal and its copper complex in rats. For this purpose, Cu-diflunisal complex was prepared by the interaction of copper sulphate and sodium salt of diflunisal, and anti-inflammatory activity of diflunisal and its copper complex was compared in albino rats by producing carrageenan induced paw oedema and measuring the zone of inflammation at different time intervals i.e. 30 min, 1, 2, 3 and 4 h after carrageenan injection. Results indicated that copper complex of diflunisal inhibits carrageenan induced paw oedema more than the parent drug at all the time scales studied.

Key Words: Anti-inflammatory; Oedema; Diflunisal; Copper complex

INTRODUCTION

Diflunisal, 5-(2,4-difluorophenyl) salicylic acid is a non-steroidal anti-inflammatory drug (NSAID), which exerts its anti-inflammatory, analgesic and anti-pyretic effects by inhibiting the enzyme prostaglandin synthetase. The drug is a moderately active reversible cyclooxygenase inhibitor with minimal platelet inhibitory effect at therapeutic dose. Diflunisal is four times more potent and less irritating than aspirin, and has a duration of action eight hours in human (Shen, 1981). There are many observations that there is an elevation of total blood copper levels during acute as well as chronic inflammatory state (Milanino *et al*., 1985). Copper concentrations increase in association with the onset and persistence of the active disease but return to normal with remission. This rise in copper contents is thought to proceed from the natural anti-inflammatory response of the host to the inflammation (Sorenson, 1982a, 1982b). This interpretation is consistent with the fact that copper complexes of anti-inflammatory substances have been observed to be more effective than their parent drugs (Sorenson, 1982b).

It is well known that clinically used non-steroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal irritation and ulcers (Sorenson, 1982b; Borda, 1992) and diflunisal is no exception. There are some reports, which show that copper complexes of these drugs are void of gastric irritating activity of parent drugs (Oga, *et al*., 1991). Copper complexes of these drugs have anti-ulcer activity in animal models of ulcer (Sorenson, 1989; Allison, 1992). The observations that copper complexes of these drugs are more active and void of gastric irritating activity of parent drugs, distinguish these complexes from their parent drugs as being safer and potentially much more therapeutically useful. Following the original demonstration of Sorenson (1976) that copper complexes of NSAIDs were more active anti-inflammatory agents than their parent drugs. In an other report (Ferrari *et al*., 1989) acute carrageenan induced paw oedema in rats was also dramatically reduced following oral administration of $Cu(II)_2$-$(aspirinate)_4$. A 0.1 mmol/kg dose produced 56% inhibition while a 0.6 mmol/kg dose of aspirin produced only 33% inhibition. Zhiqiang *et al*. (1998) compared the anti-inflammatory activity of Cu-(aspirinate) with that of aspirin and reported that 50 mg/kg of complex decreases the acute inflammation comparable to 200 mg/kg dose of aspirin. Other recent reports on the effectiveness of Cu complexes of NSAIDs as compared to that of their parent drugs include copper complexes of diclofenac sodium (Kesharwani & Sing, 1996) and salicylic acid (41% to 57%) (Blahova *et al*., 1998).

The present study was undertaken to compare the anti-inflammatory activity of diflunisal, a derivative of salicylic acid and its copper complex in rats. The anti-inflammatory activity of the drug and its copper complex was studied using carrageenan induced paw oedema in rats, and measuring the zone of inflammation. This method is most widely used by various research groups as this method is reliable and cost effective.

MATERIALS AND METHODS

Chemicals and drugs. All the chemicals were of analytical grade and used as such without any further purification. The chemicals were purchased from Sigma Chemical Company. Merck Sharp and Dohme (MSD) of Pakistan Ltd., Karachi, Pakistan donated Diflunisal drug powder.

Preparation of copper complex of diflunisal. The copper complex of diflunisal was prepared following the published procedure (Razi *et al*., 1997). In brief, 0.50 g (2 mmol) of diflunisal was dissolved in 50 mL of distilled

water containing 0.08 g (2 mmol) of NaOH. The pH of the solution was adjusted to 10.5. The 0.25 g (1 mmol) of copper sulphate was dissolved in 50 mL of distilled water in a separate beaker. The copper sulphate solution was added into the drug solution drop-wise with constant stirring. The solution was stirred well for about half an hour. A dirty green precipitated product was obtained, which was filtered off, washed with distilled water and dried. The formation of the complex was confirmed by metal analysis using atomic absorption spectroscopy.

Comparative anti-inflammatory activity in animals. Albino rats of either sex, weighing 100 to 110 g were used for this study. They were fed on standard chow and tap water ad libitum. The animals were housed at room temperature.

Carrageenan induced paw oedema. To evaluate the anti-inflammatory activity of the test compounds, carrageenan induced paw oedema assay was carried out as described by Winter *et al.* (1962). Rats were divided into three groups. Each group contained six rats. One of the groups acted as control group. To induce oedema (acute inflammation), a freshly prepared 0.1 mL of 1% suspension of carrageenan in saline solution was injected subcutaneously in the right hind paw of rats. The drug and its copper complex were suspended in saline solution and administered (50 mg/kg body weight of rat) orally one hour before the carrageenan injection. The control group was treated with saline solution. The zone of inflammation was measured at 30 minutes, 1, 2, 3, and 4 h after carrageenan injection using vernier caliper. The percentage inhibition of the degree of inflammation for each group of rats treated with diflunisal and its copper complex was calculated as:

Percentage inhibition = [1- (a-x / b-y)] x 100

a and *x* are the mean diameter of the right hind paw of the rats after and before carrageenan injection in the test group, *b* and *y* are the mean diameter of the right hind paw of the rats after and before carrageenan injection in the control group.

RESULTS AND DISCUSSION

A comparative study of anti-inflammatory activity of copper complex of non-steroidal anti-inflammatory drug (NSAID), diflunisal with that of its parent drug was carried out using carrageenan induced paw oedema in rats as described in experimental section (Winter *et al*., 1962). The results of inhibition of paw oedema caused by diflunisal and its copper complex at 30 min, 1, 2, 3 and 4 h after the carrageenan injection are summarized in Table I and shown in Fig. 1.

The results show that percentage inhibition of inflammation after 30 min of carrageenan injection was 11 and 17 with the oral administration of diflunisal and its copper complex, respectively, and after one hour it was 29% and 38%. After two hours of carrageenan injection, the percentage inhibition of inflammation caused by diflunisal was 40, whereas its copper complex was able to decrease the inflammation up-to 51%. After three hours of carrageenan injection, the percentage inhibition of inflammation by diflunisal was 48, whereas with its complex was 60%. The percentage inhibition of inflammation after four hours of carageenan injection was 43 and 54 with the treatment of the drug and its copper complex respectively.

The mean response of the treatment group was compared statistically to that of control group at its respective time points. ANOVA was used when multiple means (drug and its copper complex) were compared with a control.

The results of anti-inflammatory activity studies of diflunisal and its copper complex show (Fig. 1, Table I) that the drug and its copper complex reduced the paw oedema and the observed inhibition of paw oedema caused by the drug was highly significant when compared to that of the control. The inhibition caused by copper complex of the drug was also highly significant when it was compared to that of the control. The maximum inhibition was observed after 3 h of carrageenan injection which is in agreement with results that suggest that the peak plasma concentrations occur about two to three hours after the oral administration of a single dose of diflunisal.

The comparative study of inhibition of paw oedema by the copper complex and the parent drug (diflunisal) shows that there was a significant increase in anti-inflammatory activity when the drug was given orally as copper complex as compared to the drug itself. The increase in the percentage inhibition of paw oedema for the copper complex as compared to the drug was 6, 9, 11, 12 and 11% at 30 min, 1, 2, 3, and 4 h after the carrageenan injection, respectively. These results are consistent with the reports

Fig. 1. Percentage inhibition of paw oedema at respective time

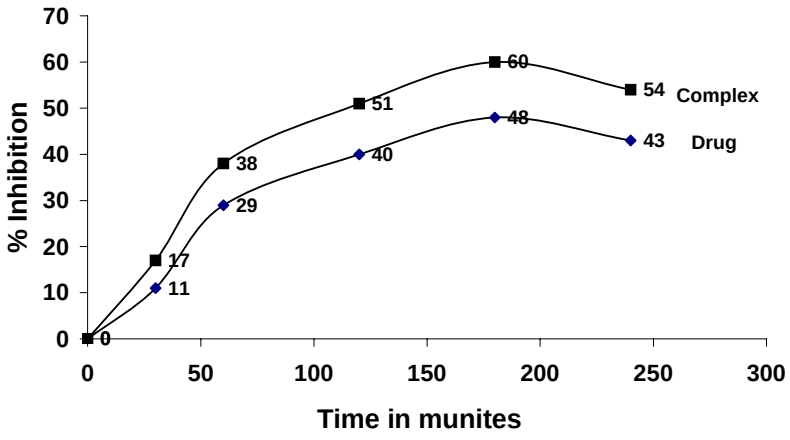

Table I. Effect of diflunisal and its copper complex on carrageenan-induced paw oedema in rats

Treatment (50 mg / kg) Body weight	Mean paw zone of inflammation (cm) (Mean ± SD)				
	30 min	60 min	120 min	180 min	240 min
Control	0.18 ± 0.02	0.34 ± 0.01	0.47 ± 0.02	0.42 ± 0.01	0.35 ± 0.01
Diflunisal (drug)	0.16 ± 0.02 (11%)	0.24 ± 0.01(29%)	0.28 ± 0.01(40%)	0.22 ± 0.01 (48%)	0.20 ± 0.01 (43%)
Cu (II) $(diflunisal)_2$ (complex)	0.15 ± 0.02 (17%)	0.21 ± 0.01 (38%)	0.23 ± 0.01 (51%)	0.17 ± 0.01 (60%)	0.16 ± 0.01 (54%)

Values given in the parentheses are of percentage inhibition

that copper complexes of NSAIDs, Cu(II)-aspirinate (Ferrari *et al*., 1989; Zhiqiang *et al*., 1998), Cu(II)-diclofenac sodium (Kesharwani & Sing, 1996), and Cu(II)-salicylate (Blahova *et al*., 1998) are more active anti-inflammatory agents than their parent drugs.

It was expected that diflunisal copper complex would be much more effective anti-inflammatory agent than the drug itself as copper complexes of salicylic acid (Blahova *et al*., 1998) and its derivatives are about 20% more effective than their parent drugs. Diflunisal, (2,4-difluorophenyl salicylic acid) has two fluorine atoms which may have an effect on the activity of its copper complex, as the flurbiprofen copper complex has similar anti-inflammatory activity as that of drug itself, however the complex produced less gastric irritation than did the parent drug (Oga *et al*., 1991).

The study of toxicity of the copper complex and its effect on gastrointestinal track is required to determine whether the complex is therapeutically more beneficial than its parent drug. Further study would also be needed to look into the possible mechanism of action of the copper complexes.

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