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The role of copper in preventing gastrointestinal damage by acidic anti-inflammatory drugs

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The ability of several non-steroidal acidic anti-inflammatory drugs to cause ulceration when given as copper complexes has been examined. The damage caused by clopirac, niflumic acid and aspirin was virtually abolished when they were given as copper complexes whereas the damage caused by indomethacin, ketoprofen and (+)-naproxen was unaltered. The lack of ulceration with three of these preparations appeared to be correlated with a much reduced ability to inhibit prostaglandin synthesis as determined using an *in vitro* enzyme system.

One major drawback to the treatment of inflammatory conditions, particularly rheumatoid arthritis, with oral acidic non-steroidal anti-inflammatory drugs (NSAID) is the degree of gastric intolerance, bleeding, erosions and ulceration caused by this group of compounds. Removal of this irritation would constitute a good therapeutic advance.

Recently Sorenson (1975, 1976) has proposed that copper complexes of the NSAID may be more potent than the parent acids and also claimed that they show anti-ulcer activity in certain tests.

The present investigation was made to see whether copper complexes of several NSAID were as ulcerogenic as the parent acids. The drugs chosen were acetylsalicylic acid (aspirin), clopirac, niflumic acid, ketoprofen, indomethacin and (+)-naproxen. We have further investigated the ability of these compounds to inhibit prostaglandin synthesis *in vitro* since this activity of the NSAID has been suggested as a possible reason for their ulcerogenic activity (Robert, 1974; Gaut, Baruth & others, 1975; Main & Whittle, 1975).

The results indicate that only with clopirac, aspirin and niflumic acid would it be beneficial to administer the drug as a copper complex to reduce the ulceration caused by these compounds. Furthermore this prevention of ulceration appears to correlate with a reduced ability to inhibit prostaglandin synthesis *in vitro*.

MATERIALS AND METHODS

Materials

The cupric salt of each anti-inflammatory acid was prepared by mixing a solution of its sodium salt with aqueous cupric sulphate in a 2:1 molar ratio. The preparation of the cupric salt of clopirac is given as a typical example.

A solution of the sodium salt of clopirac [1-*p*-chlorophenyl-2,5-dimethyl-3-pyrroleacetic acid (2·85 g, 0·01 mol)] in water (20 ml) was added to a stirred solution of cupric sulphate pentahydrate (1·25 g, 0·005 mol) in water (10 ml) at 20°. The green precipitate which formed immediately was collected by filtration, washed with water and dried under vacuum to give pure 1-*p*-chlorophenyl-2,5-dimethyl-3-pyrroleacetic acid cupric salt monohydrate (2·9 g, 96% yield), m.p. 151–8° with decomposition. In a similar manner the cupric salts of niflumic acid, indomethacin, (+)-naproxen and ketoprofen were prepared and shown by elemental analysis to have structures consistent with a 2:1 molar ratio of organic carboxylate to copper. Copper aspirinate was prepared according to Manojlovic-Muir (1967).

Methods

Anti-inflammatory activity. Anti-inflammatory activity was assessed in the carrageenan-induced oedema test. Female OLAC Wistar rats (150–170 g) were randomly allocated into groups of 8 and dosed orally with test compounds suspended in 0·7% methyl cellulose. A control vehicle-dosed group was included. One h after dosing each rat received a subcutaneous injection into the plantar surface of each hind foot. The right foot received 0·1 ml of a 2% carrageenan suspension in saline and the left foot 0·1 ml of 0·9% saline. Three h after carrageenan injection the foot volumes were measured using a mercury displacement differential volume meter. The left and right hind feet were immersed in the mercury to reference marks made over the lateral malleolus bone of each foot. The difference between the readings of right and left foot volumes gives a

* Correspondence.

measure of the oedema of the carrageenan-injected paw. Readings from the volume meter are then used to calculate values for individual, total and mean oedemas for each group. The percentage inhibition for any compound is calculated by the following equation:—

$$\% \text{ inhibition} = 1\left[-\left(\frac{\text{Test mean}}{\text{Control mean}}\right)\right] \times \frac{100}{1}$$

Effects of compounds on the stomach of fasted rats. The method used to assess the effects of compounds on the rat stomach was as follows.

Food was withheld from groups of 8 female OLAC Wistar rats (130–160 g) and 18 h later compounds were dosed orally suspended in methyl cellulose. Four h later (for groups dosed with niflumic acid, (+)-naproxen, indomethacin and ketoprofen and their copper chelates) or 1 h later (for aspirin and its copper chelate) the rats were killed, the stomachs excised and inflated with 10 ml of 0·9% saline. After 30 min the stomachs were opened and the glandular portion examined for mucosal erosions. For clopirac and its copper chelate the rats were examined as above but were only starved for 8 h and the compound was administered and the animals killed 16 h later. This is because clopirac was found not to be ulcerogenic at 4 h.

Doses of the acids were given which had previously been found to consistently cause at least 6 out of 8 rats to exhibit at least one area of gastric erosions. The copper complexes were then administered at doses which resulted in the same amount of parent acid being given. Results in Fig. 1 are expressed as the percentage of rats which showed at least one area of erosions at the stated doses.

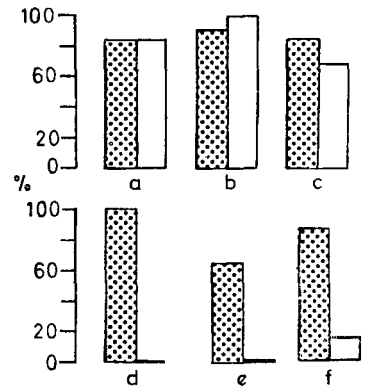

FIG. 1. Percentage of rats with ulcerations caused by NSAID (stipuled columns) and the copper complexes (open columns). a—Ketoprofen (6 mg kg^{-1}), b—indomethacin (16 mg kg^{-1}), c—(+)-naproxen (24 mg kg^{-1}), d—niflumic acid (60 mg kg^{-1}), e—clopirac (150 mg kg^{-1}), f—aspirin (300 mg kg^{-1}).

Inhibition of prostaglan dinsynthesis in vitro. The ability of compounds to inhibit the conversion of [^{3}H]arachidonic acid into PGE_2 was measured in a microsomal enzyme preparation isolated from bovine seminal vesicles, by the method of Yoshimoto, Ito & Tomita (1970) and the resulting microsomal pellet was deep frozen before use.

The amount of enzyme used for each incubation was essentially that of Yoshimoto & others (1970) the freeze-dried material being dissolved in 0·2 M tris/HCl buffer, pH 8·0, with 1 mM EDTA and 4% Triton-X, to give a concentration of 10 mg ml^{-1}. Aliquots of 0·5 ml were then incubated for 10 min with substrate (10 μM ^{3}[H]arachidonic acid) and cofactors (reduced glutathione, 2·0 mM, beef haemoglobin, 1·0 μM, and hydroquinone, 0·5 mM) in a net-volume of 2·5 ml, with or without inhibitor.

The reaction was terminated by reducing to pH 3·0 with 0·2 M citric acid, then lipids were extracted with diethyl ether, washed (water), and dried down under nitrogen. The residues were taken up in methanol and applied to t.l.c. plates, then developed to a height of 15·2 cm in ethyl acetate–acetone–glacial acetic acid [90:10:1]. The plates were then cut into strips corresponding to arachidonic acid and prostaglandins E_2 and $F_{2\alpha}$, and the activity counted in a liquid scintillation counter using a commercial scintillant, NE 260.

RESULTS AND DISCUSSION

In all cases the copper complexes of the NSAID were as active, given orally, as the parent acid in reducing the carrageenan-induced oedema in rat paws. No increase in activity was found with any of the complexes compared to the parent acids. Recently Rainsford & Whitehouse (1976) claimed that orally administered copper salicylates were no more effective than aspirin or salicylate as anti-inflammatory drugs. Copper acetate given orally was found to be inactive but active when given subcutaneously, confirming the work of Sorenson (1975).

When the ulcerogenic activity was examined, however, clear differences were found between compounds. (+)-Naproxen and its copper complex were equi-active in causing gastric damage and the damage caused by indomethacin and ketoprofen was not reduced by their administration as copper complexes (Fig. 1). On the other hand, the copper complexes of clopirac, niflumic acid and aspirin were less able to cause gastric damage than their respective parent acids (Fig. 1).

When the ability of the compounds and their copper complexes to inhibit prostaglandin synthesis *in vitro* was examined, again differences were found between compounds (Fig. 2). (+)-Naproxen, keto-

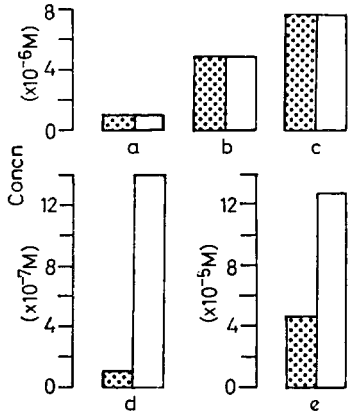

FIG. 2. Inhibition of prostaglandin biosynthesis *in vitro* by NSAID (stipuled columns) and their copper complexes (open columns). a—Ketoprofen, b—indomethacin, e—(+)-naproxen, d—niflumic acid, e—clopirac. y—axis ID50 concentration.

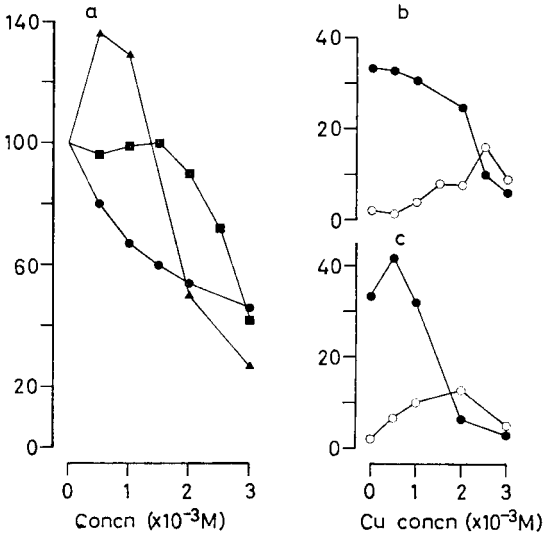

FIG. 3. Inhibition of prostaglandin synthesis by a—sodium aspirin ●, cupric aspirin ■ and cupric chloride ▲. y axis—Incorporation as a percentage of the control value. b—Cupric aspirin, ● PGE_2, ○ $PGF_{2\alpha}$. c—Cupric chloride, ● PGE_2, ○ $PGF_{2\alpha}$. b and c—y axis—Percentage incorporation.

profen and indomethacin were equi-active in inhibiting prostaglandin synthesis either as the free acids or as the copper complexes. The copper complexes of niflumic acid and clopirac, were, however, much less potent inhibitors than their parent acids. The situation with aspirin is more complicated (Fig. 3). The copper complex of aspirin was far less inhibitory than sodium aspirin, but since the potency of aspirin as a prostaglandin synthetase inhibitor is much less than the other NSAID examined, much more copper is added when copper aspirin is used compared to the other copper complexes. This high concentration of copper as cupric chloride stimulated total prostaglandin synthesis over a certain range of concentrations (Fig. 3a). Since Maddox (1973) claimed that cupric ions stimulate $PGF_{2\alpha}$ synthesis and inhibit PGE_2 synthesis we examined the effects of cupric chloride and cupric aspirin on both PGE_2 and $PGF_{2\alpha}$ synthesis. Fig. 3b shows that we confirmed the findings of Maddox (1973) with cupric chloride and showed that a similar pattern of $PGF_{2\alpha}$-increased and PGE_2-decreased synthesis occurred with cupric aspirin. However the increased $PGF_{2\alpha}$ synthesis does not compensate for the decreased PGE_2 synthesis with either cupric chloride or cupric aspirin. It is probable that the copper is acting in two ways in this system. In the first at low concentrations it acts catalytically to produce $PGF_{2\alpha}$ from the endoperoxide in a non-enzymatic manner. Secondly, since the amount of $PGF_{2\alpha}$ produced does not compensate for the amount of PGE_2 inhibited, the copper could be inhibiting at some stage either the oxygenase or the isomerase components of the enzyme system.

Initially, we thought that the reason why copper altered the ability of one acid, but not another, to cause mucosal erosions was related to the ability of one acid, but not another, to form chelates with the copper. Accordingly we examined clopirac and (+)-naproxen as representatives of the two groups of acids. However several attempts to show strong complex formation between clopirac and copper by physical chemical methods failed to provide such evidence and no differences have been seen between clopirac and (+)-naproxen in these tests (Marshall, A.C.—personal communication).

We can only assume, therefore, that when the copper salts of clopirac, niflumic acid and aspirin are given orally to rats the free acid is not availabe to produce erosions. Absorption and distribution of these compounds must be different from the other group of acids, (+)-naproxen indomethacin and ketoprofen, in that these free acids must be readily available to cause damage. A similar pattern of distribution may also occur in the *in vitro* microsomal enzyme preparation used to assess the ability of the

compounds to inhibit prostaglandin synthetase activity.

Presumably once absorbed the acids become available in the blood stream either free or bound to albumin and act at the site of inflammation as free acids. This is supported by two pieces of evidence. Firstly we have never been able to show copper chloride or acetate to be active as anti-inflammatory drugs when given orally although both are active given subcutaneously, thus presumably insufficient copper is absorbed by the oral route. Secondly the copper salts and parent acids have been found to be equi-active in reducing inflammation in the rat paw carrageenan assay.

In conclusion we have shown that by administering certain NSAID as their copper complexes, i.e. clopirac, niflumic acid and aspirin their propensity to cause ulceration is reduced. On the other hand the ability to cause gastric erosions and ulceration is not reduced when either indomethacin, ketoprofen or (+)-naproxen is given as a copper complex. This lack of ulceration with certain compounds appears to be related to a reduced ability to inhibit prostaglandin synthesis.

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