Concerning the merits of copper aspirin as a potential anti-inflammatory drug

K. D. RAINSFORD*, M. W. WHITEHOUSE, *Department of Experimental Pathology, John Curtin School of Medical Research, The Australian National University, Canberra, Australia 2601*

Recently Sorenson (1974, 1975, 1976) has proposed that copper complexes of the non-steroid anti-inflammatory drugs (N.S.A.I.D.) may be more potent than the parent N.S.A.I.D. and of greater pharmacological interest since they may show anti-ulcer activity in certain assays. Simple copper salts (basic carbonate, acetate, chloride) certainly show anti-inflammatory activity in rats (Bonta, 1969; Sorenson, 1974; Whitehouse, Field & others, 1975) but they are also highly irritant (Bonta, 1969; Whitehouse & others, 1975), a property which may possibly be associated with their anti-inflammatory potential.

The poorly soluble copper(II) salt of aspirin is currently being considered for clinical trials in arthritis and other rheumatic disorders (Sorenson, private communication). Since Sorenson (1974) had observed the increased anti-inflammatory activity of copper aspirin (compared to aspirin) after parenteral administration while the drug would most likely be given orally, it seemed that the oral route should be considered in assays of anti-inflammatory activity. We have now compared the activity of copper aspirin with copper salicylate (which is fairly water-soluble) and aspirin itself for (i) efficacy against acute oedema formation after oral and subcutaneous administration, (ii) irritancy at a site of parenteral administration (measured by acute oedemic tissue swelling) and (iii) effects on the gastric mucosa—all in rats. The results indicate that orally administered copper salicylates may be no more effective than apirin or salicylate as anti-inflammatory drugs.

Materials. Samples of copper aspirin were generously provided by Dr. J. R. J. Sorenson and Prof. W. R. Walker. Copper and zinc salicylates were prepared by mixing aqueous solutions of sodium salicylate with $CuCl_2$ or $ZnCl_2$ in the molecular proportions of 2·2: 1·0.

In the assays of anti-inflammatory activity the compounds were administered subcutaneously (in neck) in isotonic saline solutions/suspensions, or orally in 1% gum acacia 1 h before an injection of 4 mg sodium urate monohydrate crystals in 0·2 ml saline, or 1 mg sodium carrageenan in 0·1 ml saline into the rear paws of male Wistar rats. The increase in paw thickness (Δpt in mm) was measured by a micrometer screw gauge and the mean values were obtained from six measurements.

In the gastric mucosal response assays, the numbers of gastric mucosal lesions and the mucous effusion were assessed 2 h after administering compounds orally in 1 ml H_2O to starved rats using the procedure of Rainsford (1975). The lesion index (= L.I.) was assessed according to Robert, Nezamis & Phillips (1968) as modified by Rainsford (1975). The results are from five animals in each group. Mucous effusion was assessed on an arbitrary scale of 0 to 3+. The neutral copper solutions were first acidified (final concn = 20 mM HCl) before administration to prevent gastric emptying.

Parenteral irritancy was assessed by paw swelling 2h after injecting 0·2 ml isotonic solutions, pH 6, containing 20 mM Cu(II) into the subplantar surface of the rear paws of rats. The irritancy score = increase in foot paw thickness after 2 h and was graded on the following scale: 1 mm = 0; 1–2 mm = +; 2–3 mm = ++; 3–4 mm = +++; >4 mm = ++++.

* Present address: Department of Biochemistry, University of Tasmania, Hobart, Australia, 7001.

The results obtained show the following:

(a) Copper aspirin by mouth is no more active than aspirin in preventing acute oedema caused by soluble carrageenan or urate crystals and was just as irritating to the gastric mucosa as aspirin itself (Table 1). Since it dissolves in dilute HCl, its drug action is primarily due to liberated aspirin.

(b) Copper salicylate by mouth may be slightly less potent than copper aspirin in both of these anti-oedemic assays but was approximately equipotent with salicylic acid. No gastric lesions that could be seen with the naked eye were caused by copper salicylate. However, histological examination of the glandular gastric muscoa (stained with the periodic acid-Schiff reagent) revealed that even copper salicylate damaged the mucosa by causing some sloughing of both the superficial and the upper gastric pit mucosal cells concomitant with mucous discharge.

(c) Parenteral copper salicylate was much more potent than sodium salicylate (administered by either the oral or subcutaneous routes) or zinc salicylate (s.c. by way of comparison) in these anti-oedemic assays (Table 1).

(d) Parenteral copper salicylate was irritant, causing acute local oedema at sites of injection (paw, neck) in contrast to sodium salicylate (Irritancy score: Cu salicylate = 3+ at 1·0 μmol, Na salicylate = 0 at 10·0 μmol).

(e) Several other copper preparations including $CuCl_2$ had anti-oedemic activity when given subcutaneously but were virtually inactive when given orally. They also exhibited parenteral irritancy which varied from the maximal irritancy exhibited by $CuCl_2$ to negligible values from tightly bound Cu complexes thus indicating that irritancy may depend, in part, on the stability constants (K_1) of the Cu-ligand complex. It was also found that $CuCl_2$ and several copper complexes did not cause lesions in the gastric mucosa but they showed a range of activities in eliciting an acute discharge of mucus from the underlying mucosa. Mucous effusion was most pronounced with copper salicylate even at low doses (5–10 μmol kg^{-1}) and copper chloride (266 μmol kg^{-1} = 3+) whereas tight-binding complexes [e.g. Cu-triethylenetetramine, 133 μmol kg^{-1} = 1+] had little effect on discharge of mucus. Also, gastric swelling was most evident with the copper compounds that caused prolific mucus discharge.

Sorenson (1974) showed that copper aspirin given orally prevented the formation of ulcers induced in rats by ligation of the pylorus ('Shay preparation') and thus

Table 1. *Anti-inflammatory activity and tissue irritancy of copper salicylates in wistar rats.*

		Rat paw assay		Gastric mucosal assay			
Compound	Route	Δ pt (mm) 6 h after urate	Δ pt (mm) 2 h after carrageenan	Treatment	Number of lesions	Lesion Index	Mucous Effusion
Control saline		2·40 ± 0·30	2·40 ± 0·25				
Control acacia		2·35 ± 0·15	2·50 ± 0·35	Control(H_2O)	0	0	0
$Cu(aspirin)_2$	s.c.	1·50 ± 0·15	1·00 ± 0·20	None***	6·5 ± 3·1	15·0	+
	oral	1·45 ± 0·20	1·15 ± 0·15	+ stress***	7·0 ± 1·9	15·9	+++
Aspirin	oral	1·85 ± 0·05	1·30 ± 0·10	None**	4·0 ± 1·7	13·0	+
$Cu(salicylate)_2$	s.c.	1·25 ± 0·15	0·95 ± 0·20	None	0	0	+++
	oral	1·55 ± 0·20	1·45 ± 0·25	+ stress	0	0	+++
Salicylic acid	oral	1·60 ± 0·25	1·40 ± 0·15	None	0	0	0
				+ stress	3·3 ± 1·3	14·1	0
$Na(salicylate)_2$	s.c.	2·10 ± 0·15	1·30 ± 0·25	None	0·4 ± 0·4	2·6	0
	oral	2·10 ± 0·20	1·75 ± 0·15	+ stress	0·4 ± 0·4	2·6	±
$Zn(salicylate)_2$	s.c.	1·60 ± 0·30	1·45 ± 0·15	None	0	0	0
	oral	2·00 ± 0·15	1·75 ± 0·35				

Notes: In the anti-inflammatory assays the dose given orally was 200 mg kg^{-1} and the dose given subcutaneously was 100 μmol kg^{-1} (except for Na salicylate at 200 μmol kg^{-1}). All doses in the gastric mucosal assays were given orally at 100 mg kg^{-1}.

* Not in solution, dispersion in saline.

** No significant difference ($P > 0.05$) between aspirin and Cu aspirin-treated groups in lesion numbers (as assessed by the Mann-Whitney U test) or in the occurrence of lesions (as determined by Fishers' Exact Probability test).

*** No significant difference ($P > 0.05$) between aspirin + stress and Cu aspirin + stress groups in lesion numbers (determined by the Mann-Whitney U test) or in the occurrence of lesions (determined by Fishers' Exact Probability test).

claimed that copper aspirin had anti-ulcer activity. However, aspirin and salicylate alone are known to reduce the incidence of ulcers in this preparation (Lish, Dungan & Robbins, 1959; Djahanguiri, Abtahi & Hemmati, 1973), thus making it difficult to assess the anti-(stress) ulcer effect of copper with aspirin in this system. Since the ulcerogenic activity of aspirin has been recently shown to be much enhanced by brief exposure to physical stresses, e.g. cold, restraint (Rainsford, 1976), it was decided to investigate the effects of copper salicylates in rats exposed to both physical (cold) and disease stresses. It was found that animals which had received copper aspirin + cold stress (Rainsford, 1976) showed no more gastric damage than unstressed animals receiving copper aspirin (Table 1). In this action therefore copper aspirin was superior to aspirin inasmuch as additional physical stress caused no further damage.

In contrast, animals dosed with copper salicylate and then cold stressed had no gastric lesions that were observble by the naked eye (Table 1). However, microscopic observations showed that mucosal cell damage had occurred to the same extent as that seen in animals treated with copper salicylate (100 mg kg^{-1}) only (i.e. without superimposed stress), but considerable oedema was evident in the mucosae of the stressed animals. These latter findings further indicate that while Cu(II) in rats may not protect the gastric tissue from the effects of aspirin, it does seem to prevent the extra damage due to stress (acting synergistically with aspirin). Since mucous effusion was still pronounced in copper aspirin or copper salicylate + stress treated rats it appears that the reduction or absence in gastric damage (seen in comparison with animals given the parent compounds plus stress) may be a specific protective response elicited by Cu(II) ions. Oral administration of copper aspirin caused much greater acute gastric mucosal irritation in rats (inbred Hooded) with established arthritis (17 days post-adjuvant) than it did in normal (i.e. non-arthritic) littermates. By contrast, aspirin itself caused only the same degree of gastric irritation in both arthritic and normal animals. Copper salicylate (200 mg kg^{-1}) caused *no* gastric damage at all in these disease-stressed animals and in this respect was clearly superior to both aspirin and copper aspirin.

These results show that copper aspirin and copper salicylate are no more effective than the parent compounds in anti-inflammatory activity when administered orally. Since the putative anti-ulcer action of copper aspirin is so difficult to demonstrate and copper aspirin itself is undoubtedly injurious to the gastric mucosa, we are not convinced that copper aspirin offers much therapeutic advantage over aspirin.

Copper salicylate being soluble, less deleterious to the gastric mucosa than copper aspirin and as effective as salicylic acid or sodium salicylate by mouth as an acute anti-inflammatory agent in rats, may be considered more promising than copper aspirin. However, there is also the problem of the toxicity associated with administration of high doses of copper compounds which should be considered in relation to the long term effects of these compounds.

We are indebted to Dr. J. R. J. Sorenson (Cincinnati, U.S.A.) and Prof. W. R. Walker (Newcastle, Australia) for gifts of copper aspirin and valuable discussions, to Mrs. G. Robinson for technical assistance. This study was performed when K.D.R. was on study leave and supported by the University of Tasmania Research Committee and the NH & MRC (Canberra).

October 5, 1975

REFERENCES

BONTA, I. L. (1969). *Acta physiol. pharmac. Neerl.*, **15**, 188–222.

DJAHANGUIRI, B., ABTAHI, F. S. & HEMMATI, M. (1973). *Gastroenterology*, **65**, 630–633.

LISH, P. M., DUNGAN, K. W. & ROBBINS, S. R. (1959). *Archs int. Pharmacodyn. Thér.*, **119**, 389–397.

RAINSFORD, K. D. (1975a). *Gut*, **16**, 514–527.

RAINSFORD, K. D. (1976). *Agents and Actions*, in the press.

ROBERT, A., NEZAMIS, J. E. & PHILLIPS, J. P. (1968). *Gastroenterology*, **55**, 481–487.

SORENSON, J. R. J. (1974). In: *Trace Substances in Environmental Health—VIII.* pp. 305–311. Editor: Hemphill, D. D., Columbia: University of Missouri.

SORENSON, J. R. J. (1975). Abstracts, Am. Chem. Soc. Meeting, Philadelphia Medi. No. 32.

SORENSON, J. R. J. (1976). *J. medl Chem.*, in the press.

WHITEHOUSE, M. W., FIELD, L., DENKO, C. W. & RYALL, R. G. (1975). *Scand. J. Rheumat.*, **4**, Suppl. 8, Abstract 183.

An inhalation aerosol of Δ^9-tetrahydrocannabinol

J. L. OLSEN, J. W. LODGE, B. J. SHAPIRO*, D. P. TASHKIN*, *Division of Pharmaceutics, School of Pharmacy, University of North Carolina, Chapel Hill, N.C. 27514 and *Inhalation Therapy Department, UCLA Hospital and Clinics, University of California, Los Angeles, CA, U.S.A.*

The most common method of Δ^9-tetrahydrocannabinol [Δ^9-THC] administration is by smoking marijuana leaf. This route achieves a rapid and reproducible absorption as reflected by consistent increases in pulse, psychotropic effects and bronchodilation. However, during smoking, other pyrolysed products are inhaled which may be bronchial irritants and possibly even carcinogens. Hence a study of Δ^9-THC aerosols was instigated to see if it is an advantageous method of administration.

Formulations of Δ^9-THC are difficult to prepare because of water insolubility and also because of the tacky [sticky] nature of the pure material at room temperature. Early experiments demonstrated excellent solubility of Δ^9-THC in conventional ethanol–difluorodichloromethane [propellant 12]–tetrafluorodichloroethane [propellant 114] solvent systems. Attempts at evaluation of these dosage forms in animals, however, indicated excessive tack of the spray and hence poor transport to the lungs.

The current preparation includes sorbitan trioleate [Arlacel 85] as a detacifier, and a higher pressure solvent system to generate smaller aerosolized particles. The Arlacel 85 is a surface-active agent currently used in at least two medicinal inhalation aerosol products.

For a single aerosol container of 15 ml capacity fitted with 67 microliter metered dose valve (Emson Research Inc. Bridgeport Conn 06605) the formula giving 1 mg per actuation is: Δ^9-THC 2% in alcohol U.S.P., 6·0 ml; alcohol U.S.P., 3·0 ml; Arlacel 85, 0·068 g; propellant 114, 2·5 g; propellant 12, 5·0 g.

The Δ^9-THC alcohol solution is measured into a clean aerosol container and the alcohol evaporated with filtered N_2. The Arlacel 85 is added as a suspension in the alcohol called for in the formula. Propellant 114 is cold filled, followed by the propellant 12. The cooled valve is fitted and crimped.

The resulting aerosol is a true solution with apparent good stability if protected from light. Weight loss measurements indicate an average 78 mg delivery per actuation of which 1 mg is the drug. This concentration can easily be adjusted if desired.

Preliminary results in man show a marked increase in specific airway conductance without a marked increase in heart rate following administration of aerosolized Δ^9-THC. Ten mg of aerosolized Δ^9-THC increased specific airway conductance 89 $\pm$16% and increased pulse 17 $\pm$ 4% in four subjects. No placebo controls were used, however more comprehensive studies are currently underway. The values we found compare favourably to an increase in specific airway conductance of 53 $\pm$ 10% and an increase of 56 $\pm$ 11% in pulse rate following marijuana smoking of approximately 10 mg of Δ^9-THC. Furthermore, the delivery of aerosolized Δ^9-THC is potentially easier to quantify than delivery by pyrolysis since pyrolysis may convert Δ^9-THC into other compounds.

June 19, 1975