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Zinc-aspirin complex:synthesis, physicochemical and biological evaluation

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Abstract

Aspirin has a propensity to cause ulcers and Zn^{2+} is known to possess anti-ulcer as well as anti-inflammatory activity. The zinc complex of aspirin was therefore synthesised and its structure was determined by IR, ^{1}H-NMR, ^{13}C-NMR, UV, DSC, atomic absorption spectroscopy and elemental analysis. The physicochemical properties of the zinc complex such as the pH-solubility profile at 25°C and partition coefficient were determined and compared with that of aspirin. Anti-inflammatory studies (using carrageenan-induced hind paw edema method) showed that the zinc complex is 2.64-times more potent than aspirin and 1.73-times more potent than the physical mixture of aspirin and zinc sulphate. ANOVA followed by Duncan's new multiple range test indicated a significant difference ($p < 0.01$) among them. Ulcerogenic effects of these and of zinc sulphate were observed in rats. The lesion indices obtained were statistically evaluated using the Kruskal-Wallis rank test and found to be statistically different ($p < 0.005$). The zinc complex of aspirin has been found to be the least ulcerogenic. These studies indicate that the zinc complex has a better therapeutic index than aspirin with better physicochemical properties.

Key words: Aspirin; Zinc-aspirin complex; pH-solubility profile; Partition coefficient; Anti-inflammatory activity; Antiulcerogenic activity; Zinc sulfate

1. Introduction

The principal limiting side effect of acidic NSAIDs is the gastrointestinal damage which occurs as a result of a dual insult, NSAID-mediated direct acidic damage (primary insult) caused by the free carboxyl group, followed almost simultaneously by the deleterious systemic effect of inhibition of prostaglandin synthesis (secondary insult) (Shoen and Vender, 1989). Gastrointestinal blood loss, in the case of aspirin, is mainly due to its local effect which has been confirmed by the absence of gastric erosions or bleeding after parenteral administration of aspirin in doses that caused significant gastric damage when given by the oral route (Leonards and Levy, 1970). Therefore, it is reasonable to assume that the gastrointestinal bleeding liability of aspirin preparations can be reduced or even eliminated either by appropriate pharmaceutical formulation designs,

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e.g., buffered aspirin, effervescent aspirin or by masking the free carboxyl group of aspirin or by administering it along with a gastroprotective agent.

Zinc, on the other hand, is known to possess anti-ulcer properties besides having anti-inflammatory activity. Zinc sulphate is given internally in doses of up to 220 mg (equivalent to 50 mg of zinc) t.i.d. to assist wound healing (Wade, 1977). However, in overdosage it produces corrosive effects on gastric mucosa including ulceration which may be explained by its conversion to zinc chloride in the stomach (Reynolds, 1989). Clinical studies have shown that oral administration of zinc sulphate at the same dose is quite beneficial in the treatment of human gastric ulcers (Fraser et al., 1972; Frommer, 1975). Laboratory animal investigations have also demonstrated the protective effect of zinc compounds against experimental ulcers, including gastric ulceration induced by electrical vagal stimulation (Cho and Ogle, 1977), methacholine (Cho et al., 1978), restraint stress (Cho and Ogle, 1978a), pylorus occlusion (Ogle and Cho, 1977), ethanol (Wong et al., 1986) and acetic acid (Kong, 1990).

Zinc reduces the inflammatory processes through different mechanisms (vide infra) which has been confirmed by the use of zinc sulphate in rheumatoid arthritis (Simkin, 1976) and in psoriatic arthritis (Clemmensen et al., 1980) at the dose already mentioned. Simkin (1976) also reported that if zinc is complexed with an absorbable chelating agent, it will not only prevent emesis caused by zinc sulphate but will also enhance the gastrointestinal absorption of ionic zinc as zinc sulphate is otherwise poorly absorbed (Reynolds, 1989).

All these observations prompted us to synthesise and to evaluate the zinc complex of aspirin which may have the following advantages: (a) the free carboxyl group of aspirin will be masked; (b) the ulcerogenicity of aspirin may be further reduced by the direct gastroprotective action of zinc; (c) zinc may add to the anti-inflammatory effects of aspirin; (d) gastrointestinal absorption of aspirin as well as of zinc may be enhanced from the complexed form; and (e) zinc will be present in a better tolerated form.

2. Materials and methods

2.1. Materials

Aspirin of Indian pharmacopoeial grade, acetonitrile of spectrophotometric grade, while sodium bicarbonate, zinc sulphate heptahydrate and all other chemicals of analytical reagent grade were used. Carrageenan Lambda, type IV, was purchased from Sigma Chemical Co., U.S.A. and 1-octanol was procured from Fluka, A.G. Triple-distilled water was used in all studies. Male albino rats (Porton strain) obtained from the Central Animal House, Panjab University, Chandigarh (India) were used for biological studies.

2.2. Preparation of zinc complex of aspirin

Acetylsalicylic acid (18.0 g, 0.1 mol) was dissolved in a solution of sodium bicarbonate (8.4 g, 0.1 mol) in water (120 ml). The solution was filtered and to it was added slowly and with constant stirring a solution of zinc sulphate heptahydrate (14.4 g, 0.05 mol) in water (40 ml). Turbidity appeared which on keeping at 10–15°C led to the formation of white crystals. The crystals were filtered, washed with a minimum quantity of cold water, and dried under vacuum to constant weight to give the zinc complex of aspirin (14.8 g, 64.6%); m.p. 116–118°C.

2.3. Instrumental methods of analysis

Infrared (IR) spectra up to 400 cm^{-1} were recorded on a Perkin Elmer 882 infrared spectrophotometer using potassium bromide pellets. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on Varian EM 390-NMR (90 MHz) and Bruker AC 300 F-NMR (300 MHz) spectrometers using tetramethylsilane as internal standard and $CDCl_3$, DMSO-d_6 as solvents.

Elemental analysis was performed on a Perkin Elmer 2400 CHN analyser. Zinc content was determined using a Pye Unicam SP 2900 atomic absorption spectrophotometer. A Karl-Fischer moisture automatic titration apparatus was used for the determination of water content. Ultraviolet (UV) spectra were recorded on a Milton Roy

Spectronic 1201 UV-Vis spectrophotometer while differential scanning calorimetry (DSC) was conducted on a DuPont 2000 apparatus. The sample size used for DSC was about 5 mg and the scanning speed was 10°C/min.

2.4. pH-solubility studies of aspirin and its zinc complex

Solubility measurements were carried out for both the compounds at 25°C in Britton and Robinson modified universal buffer solutions (combination of acetic acid/sodium acetate, phosphoric acid/sodium phosphate and boric acid/sodium borate, 0.05 M each) adjusted to a constant ionic strength (μ) of 0.175 with potassium chloride. Buffers were prepared at intervals of 1.0 pH unit within the range of 1.75–8.75. The pH measurements were performed with a Control Dynamics pH meter. An excess of the drug was added to 10 ml of each buffer and agitated vigorously for 3 min in an ultrasonicator. It was then filtered immediately in test tubes previously placed in a Block Thermostat (Grant, BT3, 20–140°C) maintained at 25°C. The equilibrium pH of the filtrate was recorded. Appropriate dilutions were carried out with 0.1 N HCl and analysed at 275 nm using a standard plot of aspirin in the same media.

2.5. Determination of partition coefficient

Partition coefficients of aspirin and its zinc complex were determined in an octanol-0.1 N HCl system. The two phases were mutually saturated before use. The octanol layer was used to prepare a stock solution, 2 ml of which was shaken vigorously, in triplicate with 4 ml of saturated 0.1 N HCl on a vortex mixer for 5 min. The mixtures were then centrifuged at 2000 rpm for 20 min and the octanol layer was carefully removed, suitably diluted with acetonitrile and analysed at 275 nm using a standard plot of aspirin in the same media. The stock solution, which was used for partitioning, was also analysed in a similar manner. The partition coefficient was calculated from the equation:

$$P = \frac{C_o}{(C_i - C_o)} \times \frac{V_a}{V_o}$$

where C_i and C_o represent the initial (i) and equilibrium (o) solute concentrations of the octanol phase, respectively, V_a is the volume of the aqueous phase and V_o denotes the volume of the 1-octanol phase.

2.6. Determination of anti-inflammatory activity

The method of 'carrageenan-induced hind paw edema' in rats developed by Winter et al. (1962) was used. Young male rats weighing 100–140 g were randomly divided into different groups, each consisting of a minimum of six rats. Food was withdrawn 16 h before the experiment while, during the test, both food and water were withdrawn. Test animals were administered orally aqueous suspensions (0.5 ml 100 g^{-1}) of aspirin, its zinc complex and a physical mixture of aspirin and zinc sulphate, at 11.1–300 mg kg^{-1} of aspirin, on an equimolar basis. The doses to be used were ground to fine powder and suspended in 5 ml of distilled water using a drop of Tween 80 as the suspending agent. The vehicle alone in an equivalent quantity was used as a placebo for the control group. Paw edema was induced 1 h after drug or placebo administration in the right hind paw of each rat by a subplantar injection of 0.1 ml of a 1% w/v suspension of carrageenan in distilled water. The volume of injected paw was measured immediately (0 min) and at 30, 60, 120 and 180 min after the injection using a plethysmometer (UGO Basile 7150, Comerio, Italy), and the amount of paw swelling determined and expressed as per cent edema relative to the initial (0 min) hind paw volume. The mean value of per cent edema ± standard error of the mean (SE) was determined for each time interval and per cent inhibition of edema produced by each drug-treated group was calculated with respect to the control group as:

$$\frac{\%\text{edema(control)} - \%\text{edema(drug)}}{\%\text{edema(control)}} \times 100$$

The plots of per cent inhibition at 3 h and at 3 h minus 1 h after injection vs log dose were used to calculate the ED_{50} values for the time periods mentioned.

2.7. Determination of ulcerogenic effects

The approach of Dearden and Nicholson (1984) was used. Since the ED_{50} of aspirin (as determined above) was greater than that of its zinc complex and the physical mixture, the lesion index (LI) was determined for all three at the ED_{50} of aspirin (189.56 mg kg^{-1}), on an equimolar basis. LI was also determined for pure zinc sulphate administered in a quantity equivalent to the zinc present in the zinc complex and in the physical mixture (34.60 mg kg^{-1} of zinc). Vehicle alone in an equivalent quantity was used as a placebo for the control group.

Male rats weighing 170–240 g were randomly divided into five homogeneous groups, each consisting of five to seven rats. They were put into individual cages and fasted for 36–48 h (depending on their weights) before the start of the experiment. The drug (in suspension form as already described) or placebo was administered orally four times over a 2 day period. Both food and water were withdrawn during this period. On the day after the final dose, the animals were killed with chloroform fumes. Their stomachs were removed, opened along the length of greater curvature and cleaned of debris. The gastric walls were carefully washed with physiological saline, placed on a support and photographed under identical conditions with a camera (Nikon N 4004) with an extension ring located about 25 cm from the stomach. Gastric mucosal damage was also examined by focussing under a dissecting microscope (10 ×) and scored according to the severity of the damage. A lesion index was computed for each rat by counting the number of lesions (x) in each of different size classes (y) and adding the products of x and y.

2.8. Statistical analyses

For parametric data obtained from the carrageenan-induced paw edema test, statistical significance of the mean per cent edema of each drug treated group at each time interval was determined with respect to that of the control group, using Students's t-test (unpaired, two-tailed). Linear regression analysis was performed on log dose-per cent inhibition of edema (at 3 h as well as at 3 h minus 1 h after carrageenan injection) data to calculate the correlation coefficient (r), slope and intercept (y-axis) and ED_{50} with 95% confidence limits. Statistical significance of the correlation coefficient was also determined using two-tailed Student's t-test. Analysis of variance (ANOVA) one-way was performd at each dose level to evaluate the statistical significance of the differences in edematic responses caused by different treatments. In cases where the F ratio was found to be statistically significant, it was followed by Duncan's new multiple range test to determine which of the multiple treatment(s) was actually responsible for making the F ratio significant.

For non-parametric data obtained from tests to determine ulcerogenic effects, the Kruskal-Wallis rank test was used to evaluate the statistical significance of the differences in lesion indices obtained with different treatments.

3. Results and discussion

3.1. Characterisation of zinc complex of aspirin

Preliminary examinations by IR, NMR, UV, DSC, atomic absorption spectroscopy and elemental analysis were conducted. The major IR peaks (Fig.1) obtained for the two compounds are as follows:

IR (aspirin in KBr): 3300–2500 (carboxyl OH stretching), 1755 (ester C = O), 1690 (carboxyl C = O), 1416 (carboxyl OH bending in plane), 1305 (carboxyl C-O), 1218 (ester C-C(= O)-O), 1188 (ester O-C-C), 916 (carboxyl OH bending out of plane) cm^{-1}.

IR (zinc-aspirin in KBr): 1740 (ester C = O), 1588 (asymmetrical stretching of carboxylate anion), 1405 (symmetrical stretching of carboxylate anion), 1227 (ester C-C(= O)-O), 1198 (ester O-C-C), 483 (Zn-O) cm^{-1}.

Examination of the IR spectra of aspirin and its zinc complex revealed a definite shift in absorption for the carboxyl group and a slight shift for the acetoxy group. The shifts occurred in the direction of longer wavelengths for both the groups, indicating that the carboxyl group of aspirin is strongly involved in complexation with zinc, while the involvement of the acetoxy group to some extent cannot be ruled out. Donation of electrons to metal produces lower excitation states and therefore shifts to longer wavelengths (Williams et al., 1976).

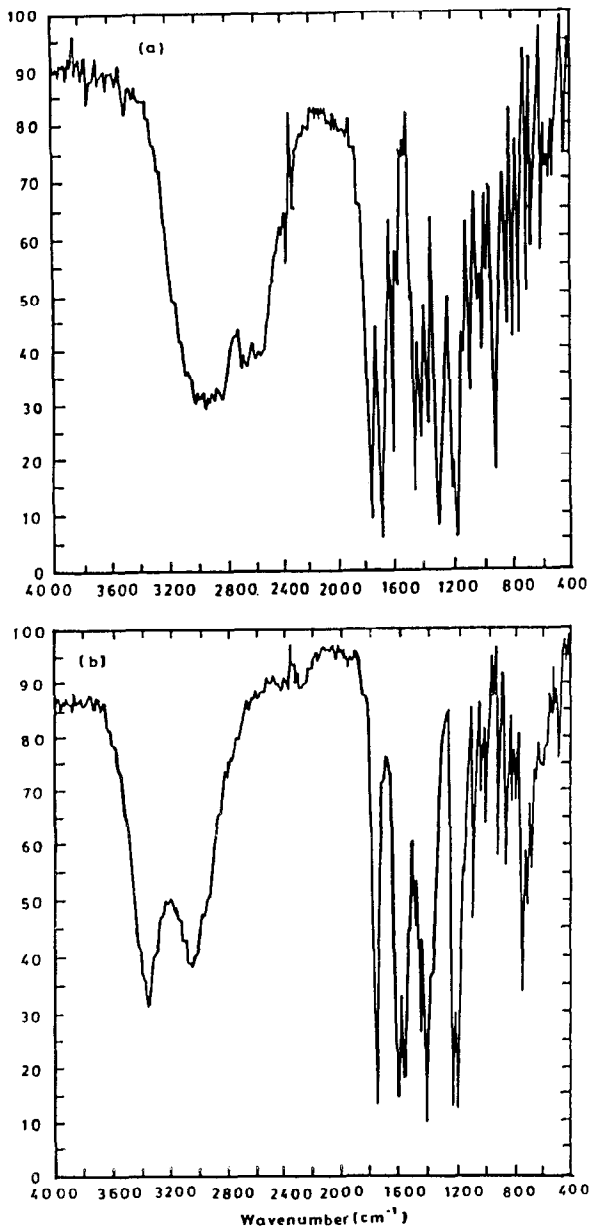

Fig. 1. IR spectra of (a) aspirin and (b) zinc-aspirin complex.

Comparison of the ^{1}H-NMR spectra of the two compounds showed the disappearance of the proton of the carboxyl group at δ 12.0–12.1 ppm and slight shifts in the peaks of 4 C-H, 5 C-H and 3 C-H in the case of the zinc complex of aspirin. The formation of a new compound was further confirmed by the ^{13}C-NMR spectra of the two compounds which revealed a strong shift in the absorption of -COOH and slight shifts in the case of -O-CO-CH_3, C-4, C-5 and C-1 as clear from the data given below:

^{13}C-NMR (aspirin in $CDCl_3$): δ (ppm) 169.09 (-O-CO-CH_3), 165.77 (-COOH), 150.44 (C-2), 133.45 (C-4), 131.53 (C-6), 125.78 (C-5), 124.09 (C-1), 123.58 (C-3) and 20.87 (-O-CO-CH_3).

^{13}C-NMR (zinc aspirin in $CDCl_3$-DMSO-d_6): δ (ppm) 170.56 (-O-CO-CH_3), 169.63 (-COOH), 150.31 (C-2), 132.05 (C-4), 131.83 (C-6), 127.54 (C-5), 125.31 (C-1), 123.09 (C-3) and 21.09 (-O-CO-CH_3).

Elemental analysis of the zinc complex of aspirin showed it to possess a ligand-metal ratio of 2:1 with two molecules of water as water of crystallisation.

Anal.: (Calc. for $C_{18}H_{18}ZnO_{10}$) C, 47.01; H, 3.94 and Zn, 14.25. Found: C, 46.85; H, 3.39 and Zn, 14.63.

The presence of two molecules of water in the zinc complex was further confirmed by Karl-Fischer testing for water determination which gave the water content equal to 8.08% (Calc. for $2H_2O$: 7.83%).

The ultraviolet spectra of aspirin and the zinc complex of aspirin in 0.1 N HCl showed maximum absorption at 275 nm. The linearly regressed equation calculated from the standard plot of aspirin in 0.1 N HCl at 275 nm gave the content of aspirin equal to 77.67% in the zinc complex (Calc. for $(C_9H_7O_4)_2$ Zn $2H_2O$: 77.94%)

Fig. 2 shows DSC curves of aspirin, the physical mixture of aspirin and zinc sulphate, and the

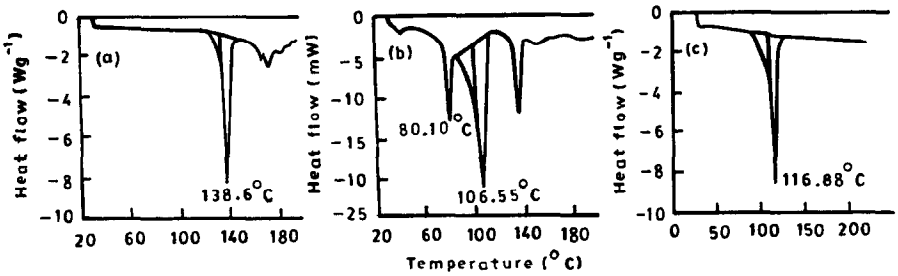

Fig. 2. DSC curves of (a) aspirin, (b) physical mixture of aspirin and zinc sulphate and (c) zinc-aspirin complex.

zinc complex of aspirin. The endothermic peak at 138.60°C equivalent to aspirin (Fig. 2a) disappeared completely in the complex (Fig. 2c) with the appearance of a new endothermic peak at 116.88°C, confirming the formation of a new compound.

3.2. Physicochemical properties of zinc complex of aspirin

3.2.1. pH-solubility profile

The buffer pH-solubility profiles of aspirin and its zinc complex at 25°C shown in Fig. 3 indicate that in the buffer pH range 1.75–4.70, the solubility of the zinc complex, in terms of aspirin, was 1.5–2.5-times that of aspirin. Beyond pH 4.70, the solubility of the complex not only began to decrease, but also became closer to that of aspirin at buffer pH 6.0 and then was reduced further. It was also observed that, unlike aspirin, the zinc complex raised the buffer pH 1.75 and 2.62 to 2.78 and 3.58, respectively.

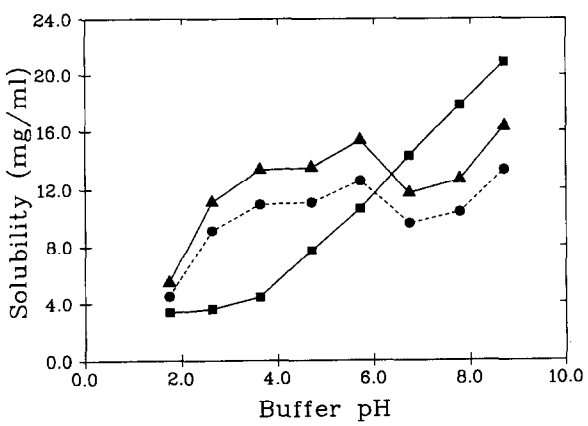

Fig. 3. pH-solubility profiles at 25°C: (■) aspirin, (▲) zinc-aspirin complex, (●) zinc-aspirin complex in terms of aspirin.

It has already been reported by Davison et al. (1962) that the disadvantages of acetylsalicylic acid as an analgesic and antipyretic agent are low solubility, which delays absorption (Leonards, 1963) and gastric irritation and bleeding which may result from the erosive action of the crystals of the drug or possibly from its acidity (Davison et al., 1962). To minimise these problems, a number of proprietary buffered products containing aspirin are marketed. As can be seen from the pH-solubility profile, the increased solubility at gastric pH and the rise in pH of the most acidic buffers make the zinc complex of aspirin as advantageous as a buffered preparation.

3.2.2. Octanol-0.1 N HCl partition coefficient

The partition coefficients using octanol-0.1 N HCl were determined for both aspirin and its zinc complex and were found to be 11.67 (log P 1.07) for the drug and 4.25 (log P 0.63) for the zinc complex. The decrease in partition coefficient of the zinc complex can be attributed to its increased solubility (2.31-times in terms of aspirin) in 0.1 N HCl determined separately.

It has been proposed that the transport of organic ligands into the cells can be facilitated by the formation of metal complexes (Albert, 1968) and this concept was invoked to explain the transport and storage of catecholamines. This finding supports the idea that in spite of a reduction in log P, metal ion complexation may be of importance in the transport of aspirin into the cells.

3.3. Biological activity of zinc complex of aspirin

3.3.1. Anti-inflammatory activity

The effects of aspirin, its zinc complex and the physical mixture of aspirin and zinc sulphate, when administered orally at 11.1, 33.3, 100 and 300 mg kg^{-1} (equivalent amounts of aspirin) on the time course of development of edema after subplantar injection of carrageenan, are shown in Fig. 4. All three treatments showed inhibition of edema at 3 h and in the second phase (3 h minus 1 h) in a dose-dependent manner. The values of ED_{50} for each treatment and other statistical parameters, calculated after linear regression of log dose vs per cent inhibition of edema (3 h and 3 h minus 1 h) data, are listed in Table 1.

One-way ANOVA analysis, when carried out at each dose level, indicated a significant difference ($p < 0.01$) in the edematic responses for different treatments, as shown by the F ratios in Table 2. This was followed by Duncan's new multiple range test, which showed that per cent edema obtained with the zinc complex of aspirin was significantly different ($p < 0.05$) from the control at all four doses, from aspirin at all doses except at 33.3 mg kg^{-1} and from the physical mixture at 11.1 and 100 mg kg^{-1}. Per cent edema in the case of aspirin and the physical mixture was significantly different from the control at all doses except at 11.1 mg kg^{-1}. The difference between the per cent edema obtained with aspirin and that with the physical mixture was not statistically significant.

Carrageenan induced edema is a biphasic event. The first phase accounts for approx. 40% of the total edema produced in 3 h. It begins immediately after carrageenan injection and is characterised by a rapid rise in foot volume, which diminishes in 1 h. The second phase of edema formation begins to develop slowly at the end of the first hour. 30 min later, a strong acceleration occurs which tapers off 3 h after carrageenan injection (Vinegar et al., 1969). This second phase is characterised by infiltration of

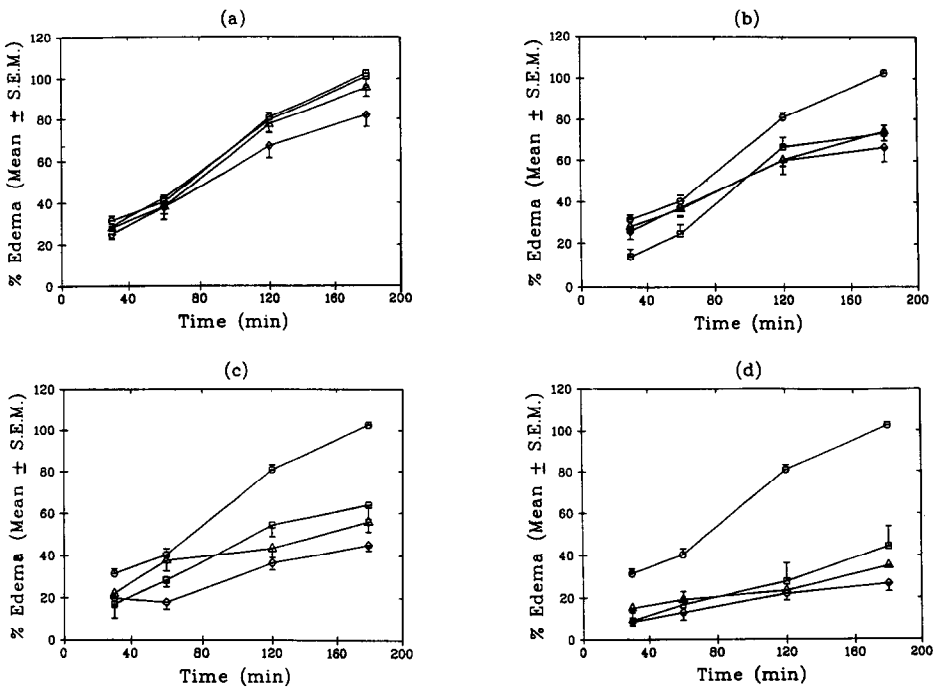

Fig. 4. Effects of oral administration of (□) aspirin, (◇) zinc complex of aspirin and (△) physical mixture of aspirin and zinc sulphate, on carrageenan-induced paw edema in rats at doses equivalent to : (a) 11.1 mg kg^{-1}, (b) 33.3 mg kg^{-1}, (c) 100 mg kg^{-1} and (d) 300 mg kg^{-1} of aspirin. (○) Control group.

Table 1
Effects of oral administration of aspirin, zinc complex of aspirin and physical mixture of aspirin and zinc sulphate on carrageenan-induced paw edema in rats

Treatment	Dose (mg/kg)	Number of rats	3 h after carrageenan injection				3 h minus 1 h after carrageenan injection			
			Edema (%) ± SE	Inhibition (%) ± SE	Statistical parameters after linear regression of log dose-% inhibition data	ED_{50} (mg kg^{-1}) with 95% confidence limits	Edema (%) ± SE	Inhibition (%) ± SE	Statistical parameters after linear regression of log dose-% inhibition data	ED_{50} (mg kg^{-1}) with 95% confidence limits
Control	–	31	102.53 ± 1.44	–	–	–	62.22 ± 3.36	–	–	–
Aspirin	11.1	6	100.97 ± 4.30	1.52 ± 4.19	% inhibition =		58.76 ± 3.47	5.56 ± 5.57	% inhibition =	
	33.3	12	73.33 ± 3.83 [d]	28.48 ± 3.73	36.6034 log dose − 33.3730		48.69 ± 4.19 [b]	21.74 ± 6.74	35.9046 log dose 31.7677	
	100.0	7	63.88 ± 6.83 [d]	37.69 ± 6.66	$r = 0.9828$	189.56 ± 2.66	35.49 ± 7.82 [c]	42.96 ± 12.57	$r = 0.9958$	189.56 ± 1.61
	300.0	6	44.41 ± 9.42 [d]	56.68 ± 9.19	($p < 0.02$)		27.62 ± 6.35 [d]	55.61 ± 10.21	($p < 0.01$)	
Zinc complex of aspirin	14.24 (11.1) [a]	6	81.91 ± 5.82 [d]	20.10 ± 5.67	% inhibition =		44.12 ± 5.54 [b]	29.09 ± 8.90	% inhibition =	
	42.72 (33.3)	8	66.46 ± 7.03 [d]	35.17 ± 6.86	38.2606 log dose − 21.0181		29.14 ± 4.82 [d]	53.16 ± 7.75	31.5711 log dose − 1.3233	
	128.16 (100.0)	12	44.88 ± 2.68 [d]	56.23 ± 2.61	$r = 0.9982$	92.12 ± 1.62	26.92 ± 2.92 [d]	56.74 ± 4.70	$r = 0.9684$	54.18 ± 3.46
	384.48 (300.0)	7	26.70 ± 3.74 [d]	73.96 ± 3.65	($p < 0.01$)	(71.81 ± 1.26)	13.61 ± 4.75 [d]	78.13 ± 7.64	($p < 0.05$)	(42.23 ± 2.70)
Aspirin plus zinc sulphate	11.1 + 8.9	7	95.65 ± 4.52	6.71 ± 4.41	% inhibition =		57.40 ± 6.36	7.74 ± 10.22	% inhibition =	
	33.3 + 26.7	10	74.34 ± 4.57 [d]	27.49 ± 4.46	40.8682 log dose − 35.5934		37.77 ± 3.13 [d]	39.29 ± 5.02	48.4117 log dose − 37.2084	
	100.0 + 80.1	6	55.72 ± 4.78 [d]	45.65 ± 4.67	$r = 0.9996$	124.27 ± 1.12	18.04 ± 1.18 [d]	71.00 ± 1.89	$r = 0.9575$	63.30 ± 3.09
	300.0 + 240.3	9	35.18 ± 1.15 [d]	65.68 ± 1.12	($p < 0.001$)		16.06 ± 1.70 [d]	74.19 ± 2.74	($p < 0.05$)	

[a] Figures in parentheses are the equivalent quantities of aspirin. [b] $p < 0.05$, [c] $p < 0.01$, [d] $p < 0.001$: significantly different from control (when determined by Student's t-test).

leucocytes and phagocytic cells and release of appreciable amounts of prostaglandins and superoxide anions (Frechilla et al., 1990). The data in Table 1 show that the ED_{50} of aspirin (189.56 mg kg^{-1}) was the same for the second phase and the combined first and second phase. On the other hand, the ED_{50} of the zinc complex of aspirin for the combined first and second phase was 71.81 mg kg^{-1} equivalent of aspirin. The increase in potency (2.64-times) of the zinc complex can be attributed to the inhibition of prostaglandin biosynthesis by aspirin as well as by zinc (Nugteren et al., 1966) plus the anti-inflammatory action of zinc mediated through impairment of the phagocytic capacity of macrophages and increase in their viability (Karl et al., 1973), interference with the complement system (Yamamoto and Takahashi, 1975) and stabilisation of lysosomal membranes as measured by the release of β-glucuronidase, probably through an inhibitory effect of zinc on lipid peroxidation (Chvapil et al., 1972; Chvapil, 1973). Finally, superoxide dismutase, which plays a key role in the protection of tissues against the toxic

Table 2
Analysis of variance (ANOVA) and Duncan's new multiple range test for the values of mean percent edema produced by different groups

Treatment	Dose (mg/kg)	Analysis of variance (ANOVA) one way					Duncan's new multiple range test: A_k (5% allowance) $= \frac{t_k}{\sqrt{2}} [S^2(1/n_i + 1/n_j)]^{1/2}$	
		Source	Sum of squares	Degrees of freedom	Mean square	F ratio		
Control	–	treatment	2238.6282	3	746.2094	7.869	$X_{control} - X_{complex}$	$= 20.615 > 9.5839$ [b]
Aspirin	11.1	error	4362.1372	46	94.8291	($p < 0.01$)	$X_{aspirin} - X_{complex}$	$= 19.055 > 12.0856$ [b]
Zinc-aspirin	14.24 (11.1) [a]	total	6600.7654	49			$X_{phys.mixt.} - X_{complex}$	$= 13.735 > 11.0728$ [b]
Aspirin plus zinc sulphate	11.1 + 8.9						$X_{control} - X_{aspirin}$	$= 1.56 < 8.8774$
							$X_{control} - X_{phys.mixt.}$	$= 6.88 < 8.7619$
							$X_{aspirin} - X_{phys.mixt.}$	$= 5.32 < 11.0728$
Control	–	treatment	14681.46	3	4893.82	32.7555	$X_{control} - X_{complex}$	$= 36.07 > 10.6952$ [b]
Aspirin	33.3	error	8516.0428	57	149.4043	($p < 0.01$)	$X_{aspirin} - X_{complex}$	$= 6.87 < 11.40$
Zinc-aspirin	42.72 (33.3)	total	23197.503	60			$X_{phys.mixt.} - X_{complex}$	$= 7.88 < 12.4634$
Aspirin plus zinc sulphate	33.3 + 26.7						$X_{control} - X_{aspirin}$	$= 29.20 > 8.9335$ [b]
							$X_{control} - X_{phys.mixt.}$	$= 28.19 > 9.0855$ [b]
							$X_{aspirin} - X_{phys.mixt}$	$= 1.01 < 10.6942$
Control	–	treatment	35878.98	3	11959.66	112.4858	$X_{control} - X_{complex}$	$= 57.65 > 7.7345$ [b]
Aspirin	100.0	error	5528.72	52	106.3215	($p < 0.01$)	$X_{aspirin} - X_{complex}$	$= 19.00 > 10.5419$ [b]
Zinc-aspirin	128.16 (100)	total	41407.70	55			$X_{phys.mixt.} - X_{complex}$	$= 10.845 > 10.5358$ [b]
Aspirin plus zinc sulphate	100 + 80.1						$X_{control} - X_{aspirin}$	$= 38.65 > 8.8199$ [b]
							$X_{control} - X_{phys.mixt.}$	$= 46.805 > 9.8878$ [b]
							$X_{aspirin} - X_{phys.mixt}$	$= 8.155 < 11.7246$
Control	–	treatment	59691.90	3	19897.30	184.6446	$X_{control} - X_{complex}$	$= 75.834 > 9.586$ [b]
Aspirin	300.0	error	5280.37	49	107.76	($p < 0.01$)	$X_{aspirin} - X_{complex}$	$= 17.714 > 12.4163$ [b]
Zinc-aspirin	384.48 (300)	total	64972.27	52			$X_{phys.mixt.} - X_{complex}$	$= 8.484 < 10.6913$
Aspirin plus zinc sulphate	300 + 240.3						$X_{control} - X_{aspirin}$	$= 58.12 > 9.4633$ [b]
							$X_{control} - X_{phys.mixt.}$	$= 67.35 > 8.4502$ [b]
							$X_{aspirin} - X_{phys.mixt}$	$= 9.23 < 11.181$

[a] Figures in parentheses are the equivalent quantities of aspirin.
[b] $p < 0.05$.

effects of superoxide anions, has been shown to be a zinc metallo-enzyme (Chvapil, 1973).

As shown in Table 1, the ED_{50} of the zinc complex was reduced appreciably for the second phase, i.e., from 71.81 to 42.23 mg kg^{-1} (equivalent quantity of aspirin), demonstrating a stronger inhibition of the second phase of edema. This is because the factors responsible for the second phase are specifically countered by zinc as mentioned above. The same holds true in the case of the physical mixture whose ED_{50} for the second phase was reduced from 124.27 to 63.30 mg kg^{-1} equivalent of aspirin. The superiority of the zinc complex over the physical mixture may be due to enhanced gastrointestinal absorption of zinc as well as of aspirin from the complexed form.

3.3.2. Ulcerogenic effects

Fig. 5 presents photographs of rats' stomachs as seen after the oral administration of aspirin, its zinc complex, the aspirin-zinc sulphate mixture and zinc sulphate (all in a dose corresponding to 189.56 mg kg^{-1} of aspirin and/or 34.60 mg kg^{-1} of zinc). The order of ulceration was aspirin-zinc sulphate mixture > aspirin > zinc sulphate > zinc-aspirin complex. The complex was the least damaging with an average lesion index (LI) of one-tenth of that of the physical mixture, about one-third of that of aspirin, about half of that of

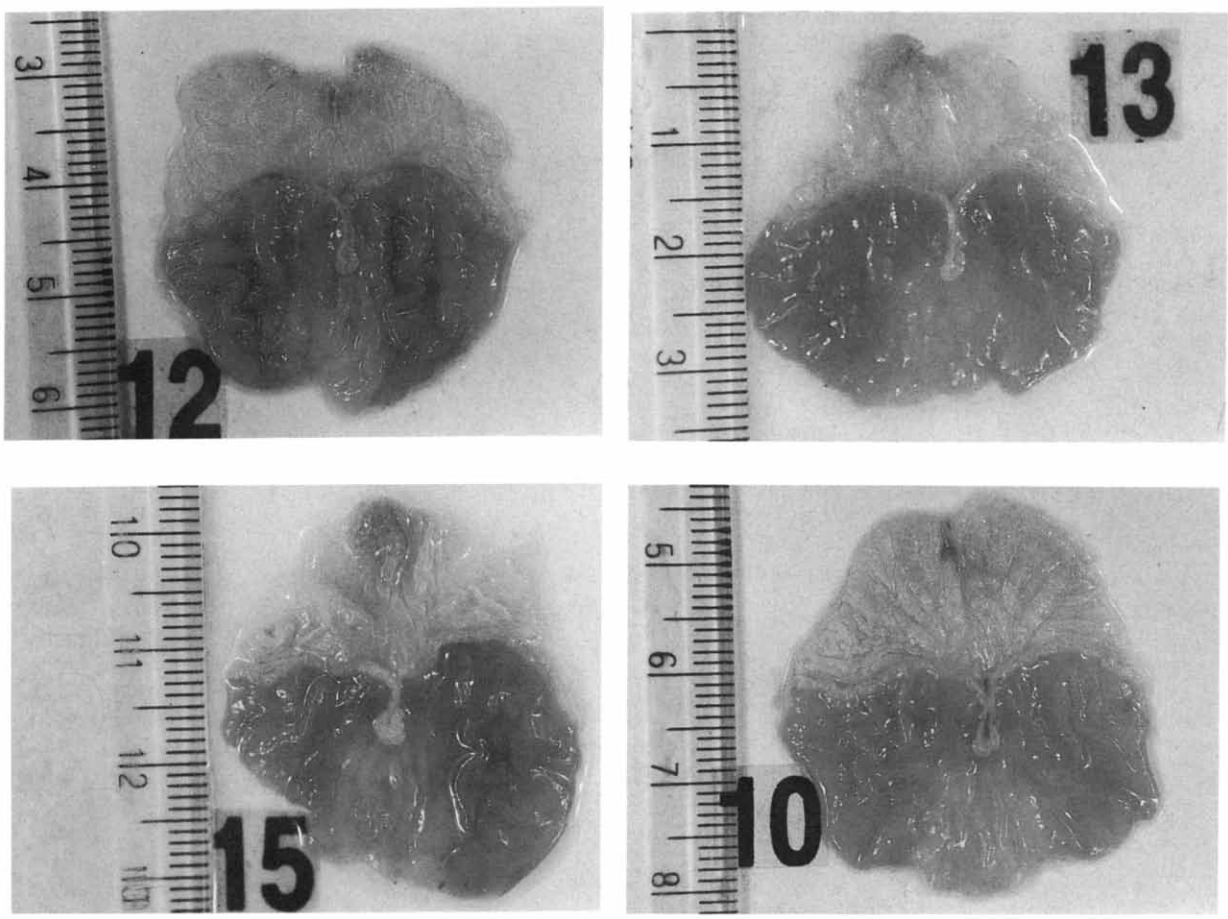

Fig. 5. Effects of oral administration of (12) zinc sulphate, (13) aspirin, (15) physical mixture of aspirin and zinc sulphate and (10) zinc complex of aspirin, on rats' stomachs at a dose corresponding to 189.56 mg kg^{-1} of aspirin and/or 34.6 mg kg^{-1} of zinc, given four times over a 2 day period. Note the flattening of folds with measly appearance in the case of aspirin.

zinc sulphate and was closer to the control value (Table 3).

The values of LI obtained with different treatments were analysed statistically for significance by the Kruskal-Wallis rank test, which is a non-parametric test used for analysing qualitative data expressed in quantitative terms and involving more than two treatments (Shefler, 1980). The values of LI were assigned ranks starting from 1 for the lowest value of LI (Table 3) and the Kruskal-Wallis statistic (H) was calculated as:

$$H = \frac{12}{n_T(n_T+1)}\left[\frac{(\sum R_a)^2}{n_a} + \frac{(\sum R_b)^2}{n_b} + \frac{(\sum R_c)^2}{n_c} + \frac{(\sum R_d)^2}{n_d} + \frac{(\sum R_e)^2}{n_e}\right] - 3(n_T+1)$$

where ΣR_a, ΣR_b, ΣR_c, ΣR_d and ΣR_e are the sums of ranks for aspirin, zinc complex, physical mixture, zinc sulphate and control, respectively, n_a, n_b, n_c, n_d and n_e denote the respective number of rats used and n_T is the total number of rats. A value of H equal to 20.43 was obtained ($H_{crit.} = 14.86$) which indicated the existence of statistically significant differences between the groups at the 0.005 probability level.

A reduction in the LI of the zinc-aspirin complex can be explained on the basis of the direct gastroprotective action of zinc through an increase in mucus synthesis (Cho and Ogle, 1978b; Kong, 1990), reduction of H^+ back-diffusion into gastric mucosa (Wong et al., 1986), stabilisation of biomembranes (Chvapil, 1973) and a direct action on the enzyme system of damaged tissues (Kong, 1990). Mucus secretion plays an important role in the protection of gastroduodenal mucosa from gastric lesions of diverse etiology (Frechilla et al., 1991). Aspirin disrupts the mucus-bicarbonate barrier by inhibiting the synthesis and secretion of gastric mucus (Menguy and Masters, 1965; Dekanski et al., 1975) and also of bicarbonate ions (Garner, 1978). On the other hand, zinc treatment increases adherent mucus in rat stomach (Cho and Ogle, 1978b; Kong, 1990) and promotes ulcer healing by strengthening the gastric mucosal barrier. It has also been shown that increased back-diffusion of H^+ from the gastric lumen into the gastric mucosa and subsequent cell damage occur (Davenport, 1965) following a change in mucosal cell permeability due to ion-trapping of aspirin in them (Martin, 1963). Treatment with zinc results in a reduction of H^+ back-diffusion into the gastric mucosa (Wong et al., 1986).

Table 3
Values of lesion index (LI) and their corresponding ranks, obtained for each rat in different groups

Rat no.	Aspirin		Zinc aspirin		Aspirin plus zinc sulphate		Zinc sulphate		Control	
	($n = 6$)		($n = 7$)		($n = 6$)		($n = 5$)		($n = 5$)	
	LI	Rank	LI	Rank	LI	Rank	LI	Rank	LI	Rank
1	31.50	23.0	16.50	14.0	169.25	29.0	18.25	15.5	1.00	4.0
2	15.75	13.0	0.50	2.5	24.00	18.0	21.25	17.0	3.25	6.5
3	15.00	12.0	18.25	15.5	43.50	25.0	3.25	6.5	5.00	9.0
4	41.00	24.0	12.00	11.0	117.50	28.0	24.25	19.5	4.00	8.0
5	29.00	21.0	0.50	2.5	73.25	26.0	24.25	19.5	0.00	1.0
6	30.25	22.0	1.50	5.0	75.50	27.0	–	–	–	–
7	–	–	9.50	10.0	–	–	–	–	–	–
AV. LI	27.08		8.39		83.83		18.25		2.65	
$\sum R$		115.00		60.50		153.00		78.00		28.50
$\bar{R}$		19.17		8.64		25.50		15.60		5.70

In addition, there is an evidence that oxygen-derived free radicals play an important role in endothelial and epithelial damage. Zinc has been postulated to stabilise biomembranes by forming stable mercaptides with the thiol groups of intrinsic macromolecules of the membrane (Chvapil, 1973), thus providing protection against the damaging action of highly reactive free radicals on the gastric mucosa (Kong, 1990). Inhibition of mucosal mast cell degranulation with a concomitant decrease in histamine levels in the gastric secretions, thereby preventing micro-circulatory changes in the gastric mucosa, is another mechanism proposed for the anti-ulcer action of zinc sulphate (Cho and Ogle, 1978a).

Acute damage caused to the gastric mucosa by the physical mixture of aspirin and zinc sulphate may be due to the ulcerogenic effect of aspirin along with the corrosive effects of overdosage of zinc sulphate on gastric mucosa (Reynolds, 1989). The latter effect was confirmed by the presence of lesions after the oral administration of zinc sulphate alone in an equivalent quantity. In the case of the zinc-aspirin complex, the LI was very low due to three main reasons; masking of the free carboxyl group of aspirin responsible for its direct acidic damage, the presence of zinc in a complexed form which is better tolerated and lastly due to the gastroprotective action of zinc.

The LI was determined only at the ED_{50} of aspirin, since what is important clinically, so far as gastric irritancy is concerned, is not the dosage required to produce a certain level of irritancy, but rather the level of irritancy produced at the clinically effective dose (Dearden and Nicholson, 1984). Since the LI for the zinc complex was quite low even at the ED_{50} of aspirin, it will be much less at its own ED_{50} which has been found to be one-third of that of aspirin.

4. Conclusion

The zinc complex of aspirin has been synthesised and characterised and has been found to be a 2:1 complex (aspirin:zinc). It possesses the advantages of enhanced solubility at gastric pH, increased potency (about 3-times) as anti-inflammatory agent and much reduced ulcerogenic liability, making it superior to aspirin.

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