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Bioavailability of indomethacin from zinc-indomethacin complex

Anil K. Singla, Dinesh K. Mediratta and Kamla Pathak

Department of Pharmaceutical Sciences, Panjab University, Chandigarh 160014 (India)

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Summary

Indomethacin has a propensity to cause ulcers, and Zn^{2+} is known to possess an antiulcerogenic profile. Preparation and investigation of the zinc-indomethacin complex were therefore envisaged. The complex was prepared via the interaction of indomethacin and zinc acetate in ethanol. The bioavailability on oral administration of indomethacin and zinc-indomethacin complex (corresponding to 100 mg indomethacin) capsules, as measured by the value total for urinary excretion at 32 h, was determined in healthy male volunteers. Bioavailability from the zinc complex is significantly lower (ANOVA, $p < 0.05$). Comparison of their urinary peak rates, biological half-life, and elimination rate constants showed a significant difference ($p < 0.05$). Nevertheless, use of the complex may be preferable to indomethacin alone in order to guard against the formation of ulcers. It was found that the zinc-indomethacin complex in a dose corresponding to indomethacin, 8 mg kg^{-1} p.o., exerts an antiulcerogenic effect on pyloric ligation-induced gastric ulcers in rats. It also reduces gastric volume, and the free and total acidity.

Introduction

One of the major limitations of anti-inflammatory drug therapy with indomethacin is that of gastro-intestinal and centrally mediated side effects (O'Brien, 1968). On a number of occasions, several attempts have been made to reduce its gastric intolerance either by enteric coating or by using different drug delivery techniques. Another approach involves the incorporation of suitable material in the formulation, which can reduce gastric intolerance without interfering with the anti-inflammatory activity of the drug. Zinc preparations, on the other hand, are known to possess antiulcer properties besides having anti-inflammatory activity. Clinical studies have shown that oral administration of zinc sulphate can be beneficial in the therapy of human gastric ulcers (Fraser et al., 1972; Frommer, 1975). Laboratory animal investigations have also demonstrated the protective effect of zinc compounds against experimental ulcer, including gastric ulceration induced by electrical vagal stimulation (Cho and Ogle, 1977), methacholine (Cho et al., 1978), restraint stress (Cho and Ogle, 1978), pylorus occlusion (Cho et al., 1976) and reserpine (Ogle and Cho, 1978). In addition, Zn^{2+} has been shown to have anti-arthritic effects of its own (Simkin, 1976).

It therefore appeared reasonable to develop a zinc-indomethacin complex and to estimate the bioavailability of indomethacin from it in humans.

Correspondence: A.K. Singla, Department of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India.

Materials and Methods

Materials

Indomethacin was supplied by Ranbaxy Labs (New Delhi, India) and its purity was checked according to USP XXI (1985) specifications. $ZnSO_4 \cdot 7H_2O$ was obtained from E. Merck and other chemicals from Sarabhai M. Chemicals.

Preparation of zinc-indomethacin complex

To a solution of indomethacin (3.24 g, 9 mmol) in ethanol (250 ml) cooled in a freezing mixture was added slowly with stirring zinc acetate (1.02 g, 4.6 mmol) solution in 50% v/v alcohol (100 ml) cooled to 0 °C. The mixture was allowed to stand for 2 h. The resulting white precipitate was filtered, washed with water followed by ethanol (50% v/v), and dried under vacuum to give 2.85 g (81.34% w/w yield) of the zinc complex; m.p. 232–234 °C (decomp.). Anal: Calc. for $C_{38}H_{30}N_2O_8Cl_2Zn$; C, 58.6; H, 3.88; N, 3.6; Cl, 9.1 and Zn, 8.38. Found C, 58.53; H, 4.1; N, 3.07; Cl, 9.21 and Zn, 8.12.

Pyloric ligation-induced gastric ulceration

After food had been withdrawn from rats for 24 h, Wistar strain albino rats (number used in each experiment is shown in Fig. 1 in parentheses) of either sex, 150–200 g, were dosed subcutaneously with indomethacin (8 mg kg^{-1}), zinc sulphate (equivalent to 0.672 mg Zn^{2+} kg^{-1}) and their physical mixture (same dose level), and zinc complex of indomethacin (8.96 mg equivalent to 8 mg indomethacin and 0.672 mg zinc kg^{-1}). Pyloric ligation was performed according to the method of Shay et al. (1945). To determine the ulcer index, the stomachs of the animals were removed and opened along the line of greatest curvature, washed and examined under a microscope at low magnification. Scoring of ulcers and estimation of acid were performed as described by Kunchandy et al. (1985). Gastric secretion was collected in pylorus-ligated rats and the volume was measured and expressed in ml 100 g^{-1}. Free and total acid content was measured by titration with 0.01 N NaOH to pH 3.5 for the free and pH 8.5 for the total acidity and expressed as mequiv. l^{-1} 100 g^{-1}. Statistical analysis of data was performed by Students's *t*-test.

Drug formulation

Hard gelatin capsules containing indomethacin (100 mg) and the zinc complex (112 mg, equivalent to 100 mg indomethacin), previously powdered through 100 gauge mesh, were prepared for study.

Subject selection and dose administration

Six non-obese adult male subjects between 22 and 26 years of age, ranging from 55 to 70 kg in weight, were selected for the study which was carried out under medical supervision. No history of sensitivity or allergy to any drug was the condition for joining the study. All participants voluntarily signed an informed consent after its contents were fully explained. The subjects were instructed to abstain from any kind of medication for at least 1 week and during the course of the study and were required to fast from all food and drink except water beginning 8 h before administration of the drugs. Drug administration had no detectable effect on their health.

The participants were randomly divided into two groups (I and II). On the first dosing drug, three of the subjects (group I) each received orally, on fasted stomach, a single dose of 100 mg indomethacin in capsule form. On the same day, the remaining three subjects (group II) each received 112 mg (equivalent to 100 mg indomethacin) of zinc-indomethacin complex with 200 ml water in the morning, after collecting a blank by fully voiding the bladder. No food or liquid other than water was allowed for 2 h following ingestion of the dose. After a period of 7 days, the procedure was repeated except that group I received a zinc-indomethacin capsule, with group II receiving a capsule of indomethacin.

Urine samples, collected at 0, 1, 2, 3, 4, 6, 8, 12, 16, 24 and 32 h following drug administration, were measured and a portion of each was frozen until analysis in duplicate. In each study, subjects were allowed to continue normal activities during the urine sampling periods.

Measurement of urine drug concentrations

Concentrations of indomethacin in urine were determined using a UV spectrophotometer (Hitachi-Perkin Elmer, Lambda 3 Model) (Hucker et al., 1966). Calibration lines of indomethacin

concentration to absorbance in distilled water and human urine were linear over the range 2–80 μg ml^{-1}. The recovery of indomethacin was 95.04% ± 5.188 (S.D.) over the calibration range.

Data analysis

Optimal calibration lines were calculated via least-squares regression analysis. Cumulative amounts excreted in urine and urinary excretionary rate were evaluated for all subjects for both treatments. The elimination rate constant was determined (Nelson, 1961) by carrying out linear regression of the terminal linear portion of the sigma-minus plot, i.e. log fraction unexcreted vs time. Calculation of the absorption rate constant was based on the equation suggested in the method of Wagner (1971). Bioavailability parameters were subjected to analysis of variance for cross-over design (Wagner, 1975) with subjects, groups, time periods and treatments as factors in the analysis. Student's *t*-test for the difference between the two means was performed on the $t_{1/2}$ values obtained from administration of indomethacin and its zinc salt in different subjects.

Results

Antiulcerogenic activity

As shown in Fig. 1, zinc-indomethacin complex, zinc sulphate and zinc sulphate-indomethacin mixture in a dose corresponding to 8 mg indomethacin and/or 0.672 mg zinc kg^{-1} showed a significant effect ($p < 0.05$) in reducing gastric ulcers as against indomethacin (8 mg kg^{-1}), however no significant difference among themselves was observed. Since a weak chelate of the complex would be strongly dissociated in solution, the mixture would of course partly associate in solution. The presence of Zn^{2+} in the molecule probably stabilizes the mucosal membrane.

Fig. 1 shows the gastric volume, and free and total gastric acid content in rats pretreated with either the complex, $ZnSO_4$ or zinc-indomethacin mixture at identical dose levels. The latter did not affect the acid output but the complex and $ZnSO_4$ progressively lowered acid secretions. These findings are in agreement with a report of the progressive reduction of these effects with increasing protective dose of $ZnSO_4$ (Cho et al., 1978).

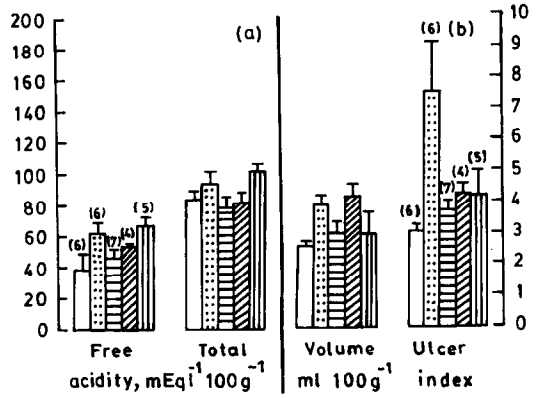

Fig. 1. Effect of various drugs on (a) free and total acidity, (b) gastric volume and ulcer index, in pylorus ligation-induced gastric ulceration in rats (each bar represents the average ± S.E. of number of animals used for each experiment denoted in parentheses. Bars (from left to right) indicate: control, indomethacin, zinc-indomethacin complex, zinc sulphate, zinc sulphate and indomethacin (physical mixture), respectively.

Bioavailability in human volunteers

The average urinary excretion rate curves of indomethacin after administration of a dose equivalent to 100 mg indomethacin of each test product are shown in Fig. 2. The mean values of peak urinary excretion rate (Table 1) for indomethacin capsules were 5.81 mg h^{-1} (range 4.67–6.81 mg h^{-1}) and 3.66 mg h^{-1} (range 1.28–5.07 mg h^{-1}) for the zinc complex. These parameters reflect the rate and extent of indomethacin absorption. The mean peak times of indomethacin capsules (1.7 h) and zinc complex (2.8 h) were significantly different ($p < 0.02$) and are in good agreement with the peak plasma concentrations that are reached 0.5–2 h after a dose of indomethacin (Reynolds, 1982). Similarly, a significant difference (Student's *t*-test, $p < 0.05$) in the mean biological half-life, as determined from urinary excretion rate, was observed between capsules containing the parent compound ($t_{1/2}$ = 4.49 h) and the zinc complex ($t_{1/2}$ = 5.24 h).

The average cumulative amounts of the drug excreted in urine at various time intervals are plotted in Fig. 3. The bioavailability of indomethacin (Table 1), as measured on the basis of

TABLE 1

Pharmacokinetic parameters of indomethacin (I) and zinc-indomethacin complex (II) (correlation coefficient, r in parentheses)

Group	Volunteer	Cumulative excreted unchanged drug in t_∞, $[X_u]_\infty$ (mg)		Peak urinary excretion rate, $(dx_u\,dt^{-1})_{max}$ (mg h^{-1})		Time at peak urinary excretion rate, t_{max} (h)		Elimination half-life, $t_{1/2}$ (h)		Elimination rate constant, k_e (h^{-1})		Absorption rate constant, k_a (h^{-1})	
		I	II	I	II	I	II	I	II	I	II	I	II
I	1	30.62	24.81	6.81	4.59	1.5	3.5	4.73	5.32	0.1464 (0.9680)	0.1302 (0.9725)	–	2.423 (0.9764)
	2	32.58	25.79	5.91	3.94	1.5	3.5	4.90	5.04	0.1413 (0.9909)	0.1375 (0.9741)	–	2.105 (1.000)
	3	21.41	18.83	6.17	3.66	0.5	2.5	5.10	5.58	0.1359 (0.9681)	0.1242 (0.9893)	–	1.065 (0.9995)
II	4	25.32	15.90	4.67	1.28	1.5	1.5	4.00	4.77	0.1732 (0.9954)	0.1454 (0.9561)	–	0.534 (0.9849)
	5	28.36	25.22	5.69	3.45	2.5	2.5	4.85	5.17	0.1429 (0.9857)	0.1341 (0.9975)	–	2.403 (0.9972)
	6	25.64	20.76	5.61	5.07	2.5	3.5	3.37	5.54	0.2059 (0.9980)	0.1252 (0.9793)	–	0.657 (0.9992)
Mean		27.32	21.88	5.81	3.66	1.7	2.8	4.49	5.24	0.1576	0.1328	–	1.531
S.D.		4.03	4.03	0.71	1.31	0.75	0.82	0.67	0.31	0.027	0.008	–	0.88

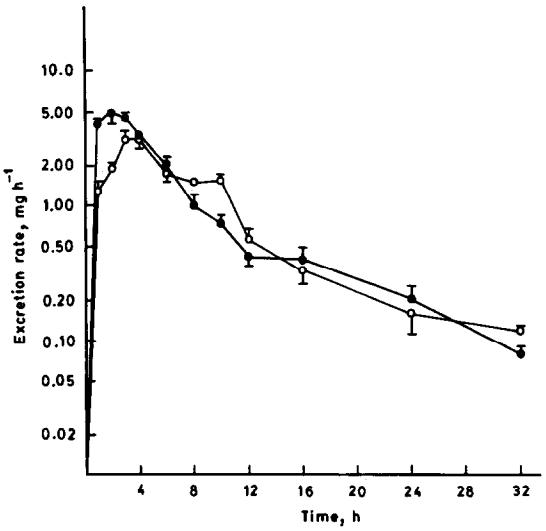

Fig. 2. Mean urinary excretion rate of indomethacin after administration of a dose corresponding to 100 mg indomethacin (averages of 6 subjects). (○) Zinc-indomethacin complex (●) indomethacin.

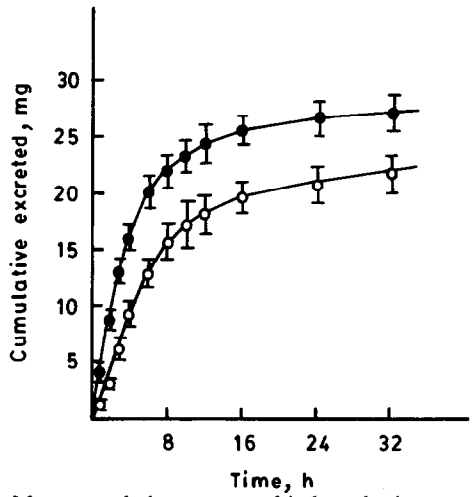

Fig. 3. Mean cumulative amount of indomethacin excreted in urine after oral administration of a dose corresponding to 100 mg indomethacin (averages of 6 subjects). (○) Zinc-indomethacin complex, (●) indomethacin.

urinary excretion after 32 h, from both types of capsule dosage forms is on average about 27.32% (range 21.41–32.58%) and 21.88% (range 15.9–25.79%), respectively. The observed standard deviations of the bioavailability parameters are of similar magnitudes in both cases. This correlates well with reports (Hucker et al., 1966; Duggan et al., 1972) that 40–60% of the administered dose of indomethacin is excreted in the urine within 24–48 h of which 50% occurs as indomethacin. ANOVA (Table 2) shows a significant difference ($p < 0.05$) due to subjects, subjects/groups and treatments. The source of variation in the cases of the groups and time periods, however, has no significant effect on the study.

Comparison of the elimination rate constants between two preparations (0.158 and 0.133 h^{-1} for the parent drug and its zinc salt, respectively) shows a statistically significant difference ($p < 0.05$). The absorption rate constant for indomethacin could not be determined due to absorption reaching completion within about 30 min except for volunteers 4 and 5 in which this occurred within 60 min. These data agree reasonably well with previously reported findings (Hucker et

TABLE 2

Analysis of variance for cumulative amount of drug excreted unchanged at infinite time

df, degree of freedom; ss, corrected sum of squares; NS, not significant; S, significant

Source of variance	df	ss	Mean square or variance	F	Significance level
Total	11	251.423			
Subjects	5	146.911	29.382	7.656	$0.01 < p < 0.05$
Groups	1	13.705	13.705	3.571	NS
Subject/group	4	133.206	33.302	8.677	$0.01 < p < 0.05$
Time periods	1	0.424	0.424	0.110	NS
Treatments	1	88.738	88.738	23.121	S
Residual	4	15.3504	3.838		

al., 1966: Duggan et al., 1972). However, for the zinc complex, the absorption rate constant was 1.531 h^{-1}.

Discussion

The present study was carried out in order to investigate the effect of zinc on the ulcerogenic activity and bioavailability of indomethacin from capsules.

Antiulcerogenic activity

The pyloric ligation-induced gastric ulceration technique was employed in the present study. Subcutaneous indomethacin exerts its action on the gastric mucosa over a period of 4 h and is a systemic effect. Bhargava et al. (1973) identified the release of catecholamines from the adrenal medulla after indomethacin and these are known to exert a regulatory effect on the action of prostaglandins. The presence of zinc not only decreases the ulcerogenic activity of indomethacin but also affects the gastric secretion. Although the exact cytoprotective effect of zinc is not fully understood, it is believed that zinc inhibits mast cell degranulation at least partially (Cho and Ogle, 1977). The deduction that acid secretion involves zinc itself is in accord with a previous report (Cho et al., 1976) where zinc chloride pretreatment was found to lower total acidity in gastric juice and to prevent ulcer formation in pylorus-occluded reserpine. The present results suggest that possibly the ability of zinc to reduce gastric acid secretion may be a factor contributing to its ulcer-healing action when used clinically.

Bioavailability of indomethacin

Although the rates of urinary recovery show standard deviations for the observed bioavailability parameters that are similar in magnitude for both cases, significant differences do occur between the rate and extent of bioavailability. This may be due to the fact that the rate and extent of bioavailability of the drug from capsules containing zinc-indomethacin complex is affected by various physiological factors in the gastrointestinal tract, which has a heterogeneous environment, since it is related to the entire process of dissolution and absorption of the drug in the tract. One of the variations in behaviour of this product might be attributed to complexation of the drug with zinc which further affects the solubility of the drug in the gastric juice. Thus, these differences and the larger area beneath the curve (Fig. 3) indicate the delaying effect of zinc on the rate of indomethacin absorption from capsules containing the complex. Various findings (Rothermich, 1966: Alvan et al., 1975: Rane et al., 1978) indicate that a rapidly releasing formulation is less desirable than a slowly dissolving formulation, since the increasingly frequent occurrence of side effects and decreasing of period therapeutic effectiveness may result from the steep rise and fall in blood drug levels.

Conclusion

These findings suggest that the use of zinc-indomethacin complex may be advantageous compared to indomethacin alone. The known antiulcerogenic and antiarthritic effects of Zn^{2+} may be beneficial when the zinc complex is used.

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