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The effect of polyvinylpyrrolidones on intestinal ulceration caused by indomethacin

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Summary

The absorption of indomethacin was studied in the rat following a single oral dose of indomethacin in the form of pure drug or indomethacin:polyvinylpyrrolidone (PVP) 17 or 90 dispersion systems. Each dosage form was administered as a suspension in water. The indomethacin serum levels produced after administration of the PVP90 systems were lower than those produced by the PVP17 systems and pure drug. The slower absorption rate of the drug from the PVP90 systems was due to an increase in viscosity of the diffusion layer around the dissolving particles which retarded drug diffusion to the absorption site. Gastrointestinal ulceration was assessed 72 h after dosing by measuring the tensile strength of the intestine and by counting the number of ulcers present. The PVP90 systems produced less severe ulceration than both the PVP17 systems and pure drug which suggests that the severity of ulceration could be related to the drug serum levels and therefore to a systemic rather than a local effect.

Introduction

Gastrointestinal ulceration in rats is a well known effect following doses of anti-inflammatory agents (Croft, 1966; Hitchens et al., 1967; Shriver et al., 1975), and indomethacin has been reported to produce intestinal ulcers in rats after a single toxic dose (Fang et al, 1977; Cioli et al., 1979). PVP has been shown to inhibit the absorption of paracetamol in rats when administered together (Sekikawa et al., 1979). Inhibition was thought to be due to the interaction between paracetamol and PVP.

Solid dispersion systems of indomethacin with polyvinylpyrrolidone (PVP) in high drug:PVP ratios have been prepared and exhibited slightly faster dissolution rates in water than the pure drug. The systems also exhibited similar dissolution rates compared to the pure drug in sodium cholate solution (Hilton and Summers, 1986). In this study, ulcerative doses of indomethacin in the form of solid dispersion systems and pure drug were administered as a single oral dose to rats to evaluate their effect on the incidence of ulceration.

Materials and Methods

Materials

Indomethacin (Nicholas Laboratories, Slough,

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U.K.), Polyvinylpyrrolidone (Kollidon-17 and Kollidon-90, B.A.S.F., supplied by Blagden Campbell Chemicals, Surrey, U.K.), flufenamic acid, (Richardson-Merrell, Slough, U.K.). Analar citric acid, di-sodium hydrogen orthophosphate dihydrate, acetonitrile for liquid chromatography, diethyl ether, 1 M acetic acid solution (B.D.H. Chemicals, Poole, U.K.), acacia B.P. (Macarthys, Essex, U.K.) and 0.24% w/v Evans Blue in saline (George T. Gurr, London) were used as received.

Methods

Preparation of solid dispersion systems

The systems were prepared as described previously (Hilton and Summers, 1986). Physical mixtures, coprecipitates and spray-dried products in the 80 : 20 drug:PVP ratio were used in this study.

Experimental procedure

Groups of 4 non-fasted Wistar rats (Bantin and Kingman, Hull) weighing 200–250 g were administered a single oral dose of either the solid dispersion system or pure drug freshly suspended in 1 ml of water. Oral administration was achieved using a syringe fitted with a round-ended needle which was inserted into the rat oesophagus. The dose, known to be ulcerative, was 15 mg/kg (Fang et al., 1977). Control groups were administered water or PVP solutions.

0.1 ml blood samples were taken from the tail vein at intervals for 3 h after dosing by making a small incision in the vein with a scalpel blade and collecting the blood using the syringe. The blood samples were extracted and assayed for unmetabolized drug using reverse-phase HPLC. The resulting drug serum levels were analysed by the Student's *t*-test.

The rats were sacrificed 72 h after dosing by placing them in a CO_2 chamber. Their intestines were removed and emptied of any intestinal content before evaluation of intestinal ulceration.

Extraction of indomethacin from whole blood

This technique is based on that of Rowe (1980). After centrifuging a blood sample for 15 min, 25 μl of serum was added to 150 μl of a 0.004% w/v solution of flufenamic acid in methanol (internal standard) and 100 μl of 0.5 M citrate buffer at pH 5 before mixing in a screw-capped glass container, 2 ml of diethyl ether was added and the glass container placed on a rotating wheel at 30 rpm for 15 min to mix the phases. The ether layer was transferred to a dreyers tube and evaporated to dryness under a stream of nitrogen. Reconstitution with 50 μl of acetonitrile took place prior to injection onto the HPLC column.

Reverse-phase HPLC

The apparatus used has previously been described (Hilton and Summers, 1986). The mobile phase was 0.1 M acetic acid : acetonitrile (55 : 45) and was pumped at a flow rate of 1.7 $ml \cdot min^{-1}$ at a pressure of 10.342×10^6 Pa. The retention times for flufenamic acid and indomethacin were 8.5 and 6.5 min, respectively, and the wavelength of analysis was 268 nm.

Evaluation of ulceration

Ulceration was evaluated by two methods. The first method determined ulceration by a tensile strength inflation technique (Ezer and Szporny, 1975) since ulcerogenesis leads to a weakening of the intestinal wall. The tensile strength value was determined as the pressure, in mmHg, required to rupture the intestinal wall. Animals having spontaneous perforations were scored zero tensile strength values. The results were analysed by the Student's *t*-test.

A disadvantage of this method is that it cannot differentiate between one serious perforation and a number of small ulcers producing the same reduction in tensile strength. Therefore, a second method involving viewing and counting the number of lesions present was also used. Several investigators have applied their own ulcer scoring systems depending upon the size and penetration in the mucosa (Sardal et al., 1972; Dekanski et al., 1975). In this study, the number of lesions in any condition was counted. Identification was made easier by injecting 1 ml of 0.24% w/v Evans Blue solution into a tail vein 30 min prior to the animal being sacrificed. The lesions were identified as blue areas where haemolysis had occurred (Cioli et al., 1979; Satoh et al., 1982). The intestine was opened along the antimesenteric side, opened and

observed under a dissecting microscope (Mag. × 16).

Results

Fig. 1 shows that the drug serum levels obtained after single doses of indomethacin:PVP90 systems were lower than those obtained after single doses of indomethacin:PVP17 systems and pure drug.

The indomethacin:PVP90 coprecipitate and physical mixture containing indomethacin form α exhibited the lowest drug serum levels half-an-hour after dosing compared to the spray-dried product and physical mixture containing indomethacin form γ.

The peak serum concentrations of indomethacin after administration of the PVP17 systems and pure drug were reached approximately 2 h after dosing. However, after dosing with the PVP90 systems, the peak serum concentrations were attained at or after 3 h.

The drug levels from the corresponding PVP17 and PVP90 systems were significantly different ($P < 0.05$) from each other at all sampling times. The exception being the PVP90 spray-dried product which was not significantly different from some of the PVP17 systems at 2 and 3 h after dosing. 2 h after dosing, each system was significantly different from the pure drug.

Ulceration occurred after dosing with all the indomethacin systems (Table 1). The ulcers generally consisted of small white nodules palpable from the serosal surface and were found mainly on the mesenteric side of the intestine, predominantly in the jejunal region. Microscopic examination of the gut section revealed lesions consisting of deep perforating ulcers accompanied by inflammatory reaction. The electron micrograph (Fig. 2) shows the dying mucosal cells. The absorption cells lose their microvilli and the mitochondria appear denser as they become inactive. These dying cells indicate the beginning of mucosal damage leading to ulceration.

On the third day after dosing, the animals which had received pure drug or PVP17 systems were lethargic and moribund, although none of

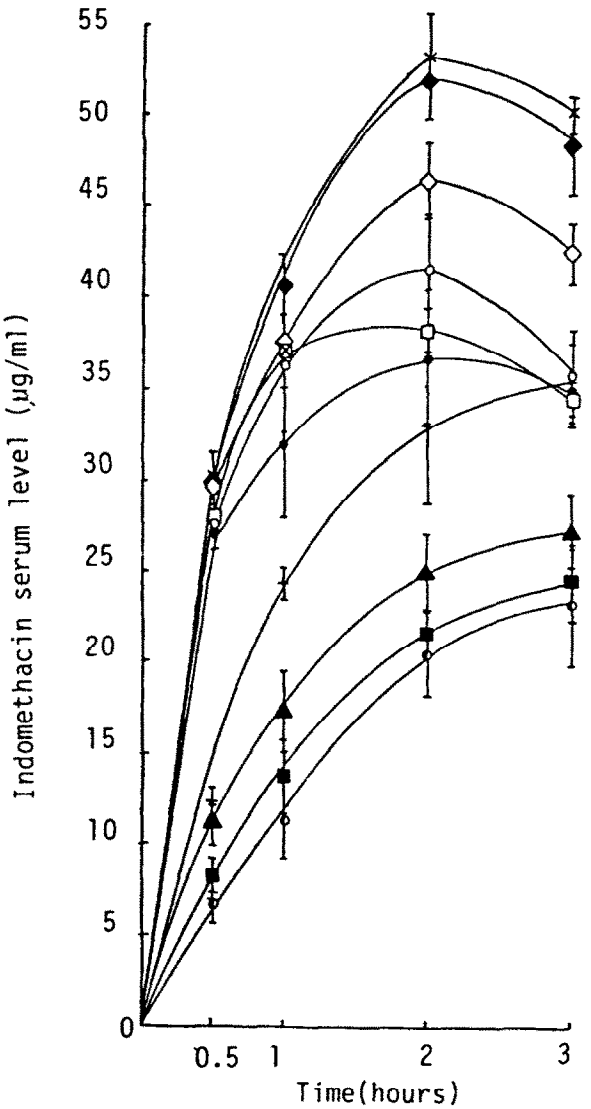

Fig. 1. Serum levels of indomethacin after oral administration of indomethacin:PVP solid dispersion systems.
Key:
× Indomethacin (Form γ)
◆ Indomethacin (Form α)
◇ PVP17 Spray-dried product (Form γ)
○ PVP17 Physical mixture (Form α)
● PVP17 Coprecipitate (Form α)
□ PVP17 Physical mixture (Form γ)
+ PVP90 Spray-dried product (Form γ)
■ PVP90 Physical mixture (Form α)
▲ PVP90 Physical mixture (Form γ)
◐ PVP90 Coprecipitate (Form α)

TABLE 1

EVALUATION OF ULCERATION IN THE RAT SMALL INTESTINE 72 h AFTER A SINGLE ORAL DOSE OF INDOMETHACIN OR INDOMETHACIN:PVP SOLID DISPERSION SYSTEM

Sample (Polymorphic form present)	Tensile strength after 72 h (mmHg ± S.E.M.)	Mean number of lesions and description	% weight loss
Control—water	222.0 ± 11.04	0	weight gain
Control—PVP solution	197.0 ± 6.50	0	
Indomethacin (γ)	38.4 ± 6.34	Many adhesions	−15%
Indomethacin (α)	37.0 ± 8.80	and perforations. Too numerous to count (> 400).	−16%
PVP17 Coprecipitate (α)	36.0 ± 4.9	Lesions, blood clots and	−13%
PVP17 Physical mixture (α)	32.0 ± 1.1	diarrhoea present.	−12%
PVP17 Physical mixture (γ)	32.0 ± 3.6		−14%
PVP17 Spray-dried product (γ)	39.3 ± 6.8		−12%
PVP90 Coprecipitate (α)	53.5 ± 1.7	125 ± 5.0. No adhesions,	−10%
PVP90 Physical mixture (α)	52.4 ± 7.1	few blood clots.	−11%
PVP90 Physical mixture (γ)	80.5 ± 11.9		−7%
PVP90 Spray-dried product (γ)	63.5 ± 5.7		−9%

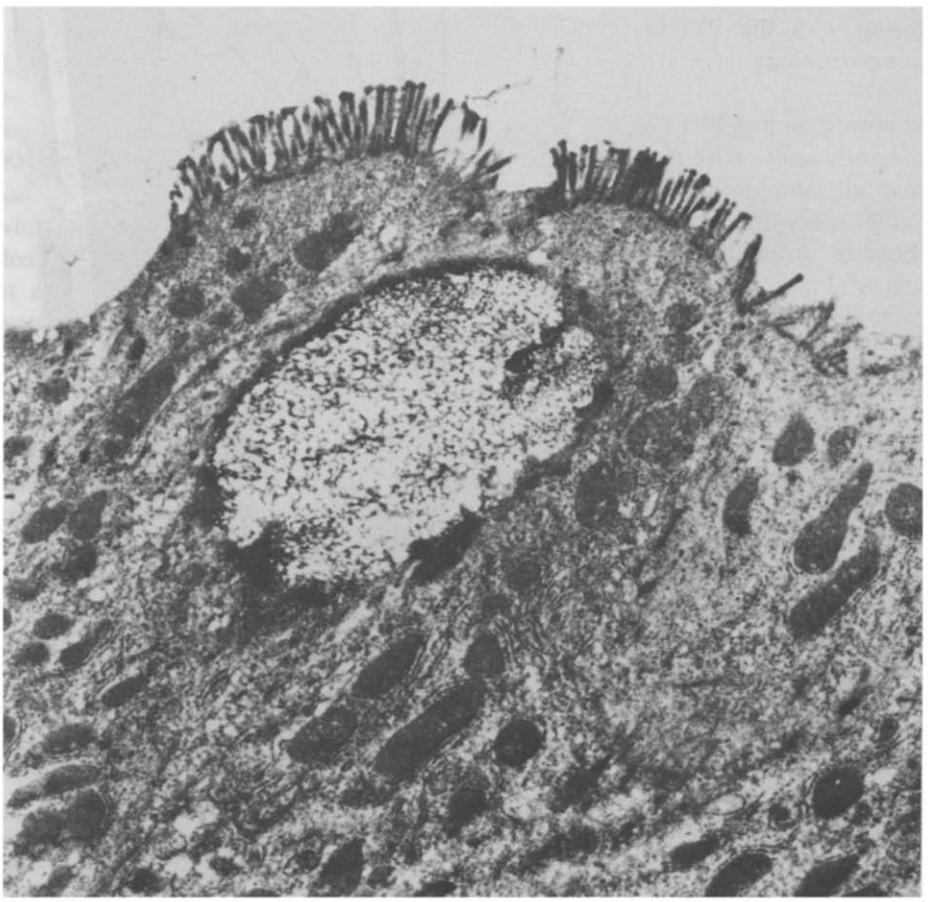

Fig. 2. Electron micrograph (×15,680) of dying mucosal cells after administration of indomethacin (15 mg/kg). Notice loss of microvilli and denser mitrochondria.

them died. They had pale eyes, and had lost weight, their hair follicles were piloerect and their faeces contained dried blood. These animals exhibited a high incidence of ulceration. However, animals which had received a single dose of PVP90 system were comparatively well. The incidence of ulceration was less severe as there were no adhesions between intestinal loops and less incidence of blood clots and diarrhoea. The reduction in ulceration may be due to the lower indomethacin serum levels obtained immediately after ingestion of the PVP90 systems.

Control rats administered water or aqueous PVP solutions did not exhibit ulceration 72 h after dosing and therefore, PVP and water were non-ulcerogenic.

Discussion

The incidence of ulceration after indomethacin administration appears to be related to the initial systemic drug concentration. The order of serum levels up to 3 h after drug dosing was pure drug > PVP17 systems > PVP90 systems and the order of intestinal ulceration was pure drug ≃ PVP17 systems > PVP90 systems. Whittle (1981) showed that the degree of intestinal ulceration caused by indomethacin probably depended upon primary intestinal damage due to an initial inhibition of cyclooxygenase activity. If this were true, the systemic drug concentration would determine the magnitude of enzyme inhibition which would in turn determine the incidence of ulceration. This hypothesis is consistent with the observed order of drug serum levels and ulceration noted in this study where PVP90 dispersion systems produced the lowest serum levels and the least ulceration. It is possible that the maximum observed serum levels were important since those after PVP90 systems were 60% of those after PVP17 systems and 50% of those after the pure drug. Maximum enzyme inhibition would have occurred at or just after maximal serum levels and hence determined the degree of intestinal ulceration. But it is not clear why the tensile strength values of the pure drug and PVP17 systems were not significantly different from each other when the maximal drug serum levels from the pure drug were significantly higher than those from the PVP17 systems.

Since PVP90 solutions are more viscous than PVP17 solutions (Lipman, 1982), the viscosity of the diffusion layer around the dissolving drug particles would be greatest from the PVP90 dispersion systems. Lower drug serum levels were therefore produced from the PVP90 systems because the diffusion of drug molecules to the intestinal mucosa was retarded. In addition, it is possible that the drug diffusion rate may be related to the degree of intestinal ulceration. If the previous hypothesis is true, the diffusion of drug to the intestinal mucosa and hence the concentration of drug in the mucosal cells during absorption will determine the inhibition of cyclooxygenase and hence the degree of ulceration.

When indomethacin (15 mg/kg) was administered in an acacia solution equiviscous to the PVP90 suspensions, the resultant drug serum levels and incidence of ulceration were similar (Table 2). As acacia and indomethacin do not interact (Fig. 3), this supports the hypothesis that the solution viscosity controlled the absorption rate and any effect due to PVP–indomethacin interaction (Hilton and Summers, 1986) is negligible.

When comparing the dissolution rates of the powdered indomethacin:PVP systems in sodium cholate solution (Hilton and Summers, 1986) with the in vivo results, there is a similarity. The PVP17 systems exhibited slightly faster dissolution rates than the PVP90 systems because the viscosity increase in the diffusion layer was greatest for the PVP90 systems. The dissolution rate order was PVP17 systems > PVP90 systems > pure drug. This order is not quite the same as that obtained in vivo because PVP17 systems and the pure drug gave similar results. It would appear that in the in vitro dissolution, the increase in viscosity of the diffusion layer was not great enough to retard diffusion to produce slower dissolution rates than the pure drug. The viscosity was greater in the diffusion layers in the in vivo studies probably because the dissolution volume in the gastrointestinal tract was considerably smaller and a higher PVP concentration resulted. The diffusion of the drug molecules from the PVP systems in the in vivo studies was therefore retarded to a greater

TABLE 2

DRUG SERUM LEVELS OBTAINED AND EVALUATION OF ULCERATION AFTER A SINGLE ORAL DOSE OF INDOMETHACIN IN 2.2% w/v AQUEOUS ACACIA SOLUTION (15 mg INDOMETHACIN/kg)

	Indomethacin in 2.2% w/v acacia	Indomethacin in water	Indomethacin:PVP90 coprecipitate in water
Drug serum levels × h after dose (μg/ml ± S.E.M.)			
x = 0.5 h	5.8 ± 0.6	30.2 ± 0.3	6.7 ± 0.9
= 1 h	12.8 ± 3.5	37.2 ± 1.6	11.6 ± 2.3
= 2 h	22.4 ± 2.9	53.3 ± 3.2	20.6 ± 2.3
= 3 h	27.7 ± 6.4	50.3 ± 0.7	23.3 ± 3.4
Tensile strength of small intestine after 72 h (mmHg ± S.E.M.)	57.7 ± 3.9	38.4 ± 6.34	53.5 ± 1.7
Ulcer number	115 ±8.5	too numerous to count (> 400)	125 ±5.0
% weight loss	−7%	−15%	−10%

extent resulting in slower dissolution rates from the systems than from the pure drug.

In the absence of PVP the pure drug exhibited a faster in vivo dissolution rate than the dispersion systems because drug diffusion was not retarded by an increase in viscosity in the gastrointestinal tract.

Miyazaki et al. (1980) have shown that bile plays an important role in the dissolution step of indomethacin absorption. They suggested that bile did not affect the absorption of the drug already in solution, but increased drug solubility of indomethacin solid in suspension by micellar solubilization and a wetting effect. However, this is not observed in vivo in this present study, since PVP greatly influenced drug dissolution rate more than the presence of bile salts.

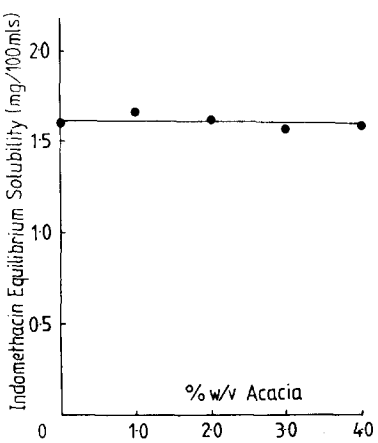

Fig. 3. Equilibrium solubility of indomethacin in aqueous acacia solutions after 48 h at 37 ± 0.5°C.

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