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Glycyrrhetinic acid, leucocytes and prostaglandins

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It has been reported that glycyrrhetinic acid, the aglycone of glycyrrhizinic acid, one of the constituents of *Glycyrrhiza glabra*, exerts anti-inflammatory activity on some models of inflammation such as formaldehyde oedema, cotton pellet granuloma, granuloma pouch and tuberculin reaction in BCG-sensitized guinea-pig (Finney & Somers 1958; Finney & Tarnoky 1960). Further investigations have also shown that this compound may be beneficial in rheumatoid arthritis and other inflammatory conditions (Trease & Evans 1972). However, despite extensive studies, the mode of action of glycyrrhetinic acid is still obscure.

Here we report the effects of glycyrrhetinic acid on carrageenan-induced oedema and dextran-induced pleurisy. We have also examined the effects of glycyrrhetinic acid in-vitro either on prostaglandin (PG)release by leucocytes phagocytosing bacteria or on PGE_2-induced contractions in the guinea-pig isolated ileum. In each experiment the effects of glycyrrhetinic acid were directly compared with the effects of hydrocortisone and indomethacin.

Some of the results reported in this paper were presented at XXI meeting of the Italian Pharmacology Society (Capasso et al 1982).

Material and methods

Male Wistar rats (Nossan, Correzzana, Milano, Italy), 110–120 g, and guinea-pigs, 250–280 g, were used.

Carrageenan oedema. Oedema of the rat right hind-paw was produced by injecting into the plantar surface 0·1 ml of a 1% carrageenan suspension (Winter et al 1962). The volume of the paw was determined immediately after this injection using a differential volume measuring instrument. Subsequent measurements of the same paw were made hourly for 5 h and compared with the initial value. Oedema was determined by difference in volume. Glycyrrhetinic acid (15, 30 and 60 mg kg^{-1}), indomethacin (2 and 8 mg kg^{-1}) and hydrocortisone (15 and 60 mg kg^{-1}) were administered by mouth 1 h before eliciting paw oedema.

Dextran pleurisy. This was induced as described by Hurley et al (1966). 1 ml of 6% dextran in 0·9% NaCl (saline) was injected into the pleural cavity while the animals were lightly anaesthetized with ether. At 4, 14 and 24 h groups of 6–8 animals were killed and 1 ml of saline containing 5 μ ml^{-1} heparin was injected into the pleural cavity. After the thorax had been massaged gently for 20–30 s the chest wall was opened and the effusion collected using a pasteur pipette. The total nucleated cell numbers were determined using a counting chamber. Differential white cell counts were made on the stained smears. Glycyrrhetinic acid (20, 40 and 80 mg kg^{-1}), indomethacin (5 mg kg^{-1}) and hydrocortisone (40 mg kg^{-1}) were administered as in paw oedema experiments.

Prostaglandin release by leucocytes. Rat peritoneal leucocytes were collected and incubated as described by Capasso et al (1981). Rats were killed by exposure to ether and bled. The peritoneal cavity was washed with 20 ml of heparinized Krebs solution. The harvested liquid was centrifuged (50 g) and the supernatant decanted. The cells pooled from 6–8 animals were washed twice by resuspension in Krebs solution enriched with bovine serum albumin (100 μg ml^{-1}). The final suspension contained $2–3 \times 10^6$ cells ml^{-1} (80% mononuclears and 20% polymorphonuclears). The cells were incubated for 2 h at 37 °C with killed bacteria (*B. pertussis*) in a ratio of 1000 bacteria per cell (Higgs et al 1975). Test compounds (glycyrrhetinic acid: 100, 200 and 400 μg ml^{-1}; indomethacin: 1 μg ml^{-1}; hydrocortisone: 5 μg ml^{-1}) were added separately to the incubation medium. After the incubation the cells were removed by centrifugation. Prostaglandins were extracted from the supernatant and bioassayed on the rat stomach strip (Capasso et al 1975) using synthetic PGE_2 as standard.

Interactions on guinea-pig ileum. Segments (4 cm) of guinea-pig ileum at least 10 cm from the caecum were suspended under a load of 0·5 g in Tyrode solution at 37 °C bubbled with 5% CO_2 in O_2. Contractions of the longitudinal muscle were recorded on a kymograph by an isotonic lever (×10 magnification). PGE_2, dissolved in 5 ml of Tyrode solution, was injected into the bath (muscle chamber) and allowed to remain in contact with the tissue until the maximal effect occurred (60 s) after injection, and was then washed out. The interval between injections was 12 min. After at least three control contractions, glycyrrhetinic acid, indomethacin and hydrocortisone were added to the bath 10 min before the next addition of agonist. Then both agonist and test compound were washed out and the ileum was again challenged at two consecutive intervals of 12 min with the agonist. Percentage inhibition was calculated on the preceding response in absence of drugs. Three to five observations were carried out for each drug concentration.

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Table 1. Effect of graded doses of glycyrrhetinic acid, indomethacin and hydrocortisone on carrageenan-induced oedema formation in the rat paw. Each value represents the mean oedema (ml ± s.e) induced in groups of 5–7 rats. The drugs were given by mouth 60 min before carrageenan $*P<0.05$**, $**P<0.01$.

Drugs	Dose mg kg^{-1}	Time (h) after carrageenan injection				
		1	2	3	4	5
None (controls)		0·55 ± 0·04	0·81 ± 0·07	1·36 ± 0·07	1·54 ± 0·09	1·47 ± 0·10
Glycyrrhetinic acid	15	0·61 ± 0·05	0·77 ± 0·07	1·09 ± 0·03**	1·28 ± 0·11*	1·28 ± 0·09*
	30	0·54 ± 0·07	0·73 ± 0·08	0·85 ± 0·07**	1·19 ± 0·05**	1·20 ± 0·07**
	60	0·50 ± 0·05	0·60 ± 0·08	0·75 ± 0·04**	1·00 ± 0·07**	1·06 ± 0·12**
Indomethacin	2	0·53 ± 0·04	0·70 ± 0·07	0·88 ± 0·05**	1·10 ± 0·04**	1·00 ± 0·07**
	8	0·49 ± 0·03	0·58 ± 0·10*	0·64 ± 0·05**	0·83 ± 0·08**	0·90 ± 0·05**
Hydrocortisone	15	0·50 ± 0·06	0·73 ± 0·05	0·97 ± 0·05**	1·31 ± 0·06**	1·37 ± 0·07
	60	0·46 ± 0·06	0·63 ± 0·05	0·60 ± 0·07**	0·93 ± 0·06**	1·06 ± 0·04**

Drugs. The drugs used were: carrageenan (Viscarin 402, by Marine Colloids), dextran (Pharmacia), *Bordetella pertussis* (Wellcome), PGE_2 (Upjohn), indomethacin and hydrocortisone (Sigma), glycyrrhetinic acid (Fluka). All other chemicals were analytical grade preparations obtained from usual commercial sources.

Results

Carrageenan oedema. Table 1 shows the results of the experiments in which oedema formation by carrageenan was induced in rats previously treated with glycyrrhetinic acid. The drug, when administered as a single oral dose 60 min before the subplantar injection of irritant, reduced the swelling induced in the paw. This action was closely related to the dose administered and occurred about 2 h after treatment with carrageenan. The ED25 of glycyrrhetinic acid, relative to the inhibition seen 3 h after carrageenan injection, was 30 mg kg^{-1}. Corresponding ED25 values for each of the substances tested were indomethacin 2·2 mg kg^{-1} and hydrocortisone 24 mg kg^{-1}.

Dextran pleurisy. Fig. 1 shows the total and differential leucocyte migration occurring after dextran injection into the pleural cavity. Treatment with glycyrrhetinic acid (80 mg kg^{-1}) resulted in a marked suppression of mononuclear migration which at 14 and 24 h was reduced by more than 60% of that of control rats. In contrast, polymorph migration was only slightly affected, the observed inhibition being about 10% at 4 and 24 h and about 18% at 14 h. Similar results were obtained with indomethacin (5 mg kg^{-1}) or hydrocortisone (40 mg kg^{-1}). The inhibitory effect exhibited by glycyrrhetinic acid on mononuclear migration was dose-dependent.

Prostaglandin release by leucocytes. Rat peritoneal leucocytes when incubated alone released about 3 ng PG per 1×10^6 cells in 2 h. When the cells took up killed bacteria this value rose to about 11 ng. Glycyrrhetinic acid, 100–400 μg ml^{-1} added separately to the medium produced a moderate inhibitory effect (Table 2). When indomethacin (1 μg ml^{-1}) and hydrocortisone (5 μg ml^{-1}) were present in the medium the PG release

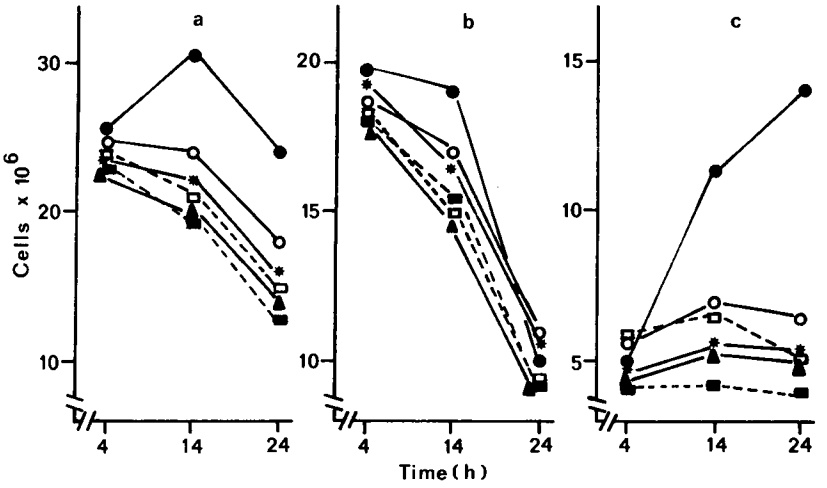

Fig. 1. Total leucocyte, polymorph and mononuclear counts in rat pleural cavity after intrapleural injection of 1 ml of 6% dextran in saline. Each point represents the mean value of 5–7 rats. In general, differences of at least 35% attained a level of $P<0.01$ (Student's *t*-test). Drugs were given by mouth 1 h before dextran injection. Controls ●——●; glycyrrhetinic acid 20 mg kg^{-1} ○——○, 40 mg kg^{-1} ⋆——⋆, 80 mg kg^{-1} ▲——▲; indomethacin 5 mg kg^{-1} ■ — — ■; hydrocortisone 40 mg kg^{-1} □— —□.

Table 2. Effect of glycyrrhetinic acid, indomethacin and hydrocortisone on PG production by phagocytosing leucocytes.

Drug $\mu g\ ml^{-1}$	ng PG/1 × 10^6 cells* mean ± s.e. (n)	% inhibition
None (controls)	11·4 ± 1·1 (4)	—
Glycyrrhetinic acid		
100	10·6 ± 0·7 (2)	7
200	9·7 ± 0·9 (3)	14
400	8·7 ± 1·2 (5)	23
Indomethacin		
1	2·6 ± 0·7** (3)	77
Hydrocortisone		
5	4·0 ± 0·8**	64

* Results expressed in terms of PGE_2 equivalents.
** $P < 0{\cdot}01$.

by phagocytosing leucocytes was strongly suppressed.

Interactions on guinea-pig ileum. PGE_2 10 ng ml^{-1} caused slow contractions with the latent period varying from 10 to 25 s. When glycyrrhetinic acid 10–50 $\mu g\ ml^{-1}$ was introduced into the bath the subsequent contractions elicited by the PGE_2 were not modified.

Discussion

Our results demonstrate that glycyrrhetinic acid reduces the inflammatory effects of carrageenan in the rat paw. The effect is closely related to the dose administered and is evident after 2 h. Although glycyrrhetinic acid was able to suppress carrageenan oedema, its activity was less potent when compared with other test compounds, particularly indomethacin. It has been suggested that the success of carrageenan oedema for the assay of anti-inflammatory drugs is due to the marked cellular migration which occurs in this type of acute inflammation. Hence, suppression of oedema formation is a rough measure of cell migration inhibition (Di Rosa & Willoughby 1971; Di Rosa 1972). Therefore, pleurisy experiments were designed to obtain precise quantitative data on the effects of glycyrrhetinic acid on cell migration. The drug was able to prevent total leucocyte migration into the pleural space induced by dextran. Differential leucocyte counts have shown that glycyrrhetinic acid exerts a selective effect in preventing mononuclear migration while polymorphs remain unaffected. This effect of glycyrrhetinic acid, although similar to, is weak compared with that exhibited by indomethacin and hydrocortisone. However, we found that glycyrrhetinic acid did not prevent the synthesis or release of prostaglandins. Glycyrrhetinic acid also did not antagonize the contractions induced by PGE_2 on guinea-pig isolated ileum.

In view of its anti-inflammatory activity, it is surprising that glycyrrhetinic acid showed relatively little ability to inhibit PG biosynthesis. The hypothesis that both steroidal and non-steroidal anti-inflammatory agents exert their various pharmacological actions via inhibition of prostaglandin biosynthesis (Vane 1971; Flower 1974; Gryglewski 1976: Bray & Gordon 1976; Parente et al 1978) has received wide support. However, it has also been suggested that anti-inflammatory compounds do not always inhibit PG biosynthesis (Brocklehurst & Dawson 1974; Bonta et al 1977; Kuehl et al 1977; Vinegar et al 1976). In fact, studies have shown that some drugs possess anti-inflammatory effects which appear to be independent of the prostaglandin system (Cashin et al 1977; Ford-Hutchinson et al 1977; Myles Gleen et al 1977; Capasso et al 1981). This suggests that not all anti-inflammatory drugs have a unique site of action, i.e. inhibition of PG biosynthesis, but they exert multiple interactions with various aspects of inflammatory responses. An interference with the migration of leucocytes into inflammatory sites could be another promising possibility (Walker et al 1976; Meacock & Kitchen 1979). Therefore it is possible that the anti-inflammatory effect of glycyrrhetinic acid on the inflammatory models considered could be the consequence of a inhibition of cell migration rather than suppression of PG biosynthesis.

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Hepatic clearance of indocyanine green during the course of glycerol-induced acute renal failure in the rat

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We have previously shown that in rats with glycerol-induced acute renal failure (ARF) there is a significant decrease in the plasma clearance of indocyanine green (ICG) and that this was probably due to decreased hepatic uptake of the dye (Bowmer et al 1982a). Similarly, the hepatic uptake of bromosulphophthalein is decreased in patients with chronic renal failure (Wernze & Spech 1971) and also in rats with glycerol-induced ARF (Bowmer et al 1982b). Furthermore, Tse et al (1976) found that the removal of rose bengal from plasma of rats with chronic renal failure was impaired. These studies provide evidence that both acute and chronic renal failure can affect the efficiency with which the liver removes these substances from plasma.

All these previous studies of hepatic function in renal failure have been done when either acute or chronic uraemia was well established. The purpose of this study was to determine how quickly the impairment of hepatic uptake of ICG occurs after the initiation of ARF and to see if this aspect of liver function is restored as renal function recovers. We have, therefore, studied the pharmacokinetics of ICG at various intervals after the induction of acute renal failure.

Materials and methods

Acute renal failure was produced by intramuscular injection of 50% v/v glycerol in 0·9% w/v sterile NaCl (saline), 10 ml kg^{-1} (Thiel et al 1967). Control rats were injected with saline, 10 ml kg^{-1}. Groups of rats were studied at 12, 24, 48 h and 7 days after injection of either glycerol or saline.

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.) and cannulae inserted into the trachea, left jugular vein and right carotid artery. Rectal temperature was maintained at 37 °C by means of a heating lamp. ICG (Hynson, Wescott and Dunning Ltd., Baltimore) was administered via the jugular vein as an aqueous solution (7·5 mg kg^{-1}, 10 mg ml^{-1}). Blood samples (0·1 ml) were taken from the carotid artery 1, 3, 5, 7, 10, 15, 20, 30, 40, 50 and 60 min after dosing. After each sample was collected, blood was replaced by an equal volume of saline.

The concentration of ICG in plasma was measured spectrophotometrically at 800 nm (Iga et al 1980). Plasma urea concentrations were measured by reaction with diacetyl monoxime using the reagents and procedure contained in Sigma Technical Bulletin No 535 (Sigma Chemical Co.). The packed cell volume (PCV) was determined for blood samples taken before the injection of ICG.

The kinetics of ICG can be explained on the basis of a two compartment model with elimination of the dye from the peripheral compartment (Bowmer et al 1982a). Plasma concentration-time data were fitted to a biexponential equation by non-linear least squares regression analysis (Snedecor & Cochran 1967). The apparent volume of the central compartment (Vc) was calculated as:

$$Vc = \frac{Dose}{A + B}$$

where A and B are the intercept values at zero time for the individual α- and β-phases. The apparent volume of distribution at steady-state (Vdss) was calculated from the equation:

$$Vdss = Vc \frac{k_{12} + k_{21} + k_{23}}{k_{21} + k_{23}}$$

where k_{12} and k_{21} are the apparent first-order intercompartmental rate constants, and k_{23} is the apparent first-order rate constant for elimination from the peripheral compartment. These rate constants were calculated using the equations given by Gibaldi & Perrier (1975). The plasma clearance (Clp) of ICG was calculated from the equation:

$$Clp = \frac{Dose}{AUC_{0\rightarrow\infty}}$$

where $AUC_{0\rightarrow\infty}$ is the area under the concentration-time curve from zero to infinity. $AUC_{0\rightarrow\infty}$ is given by the equation:

$$AUC_{0\rightarrow\infty} = \frac{A}{\alpha} + \frac{B}{\beta}$$

Results are expressed as mean ± s.d. and statistical comparison of data at 12, 24 and 48 h were made by the

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