REFERENCES

Bazzicalupo, P., Tocchini-Valentini, G. P. (1972) Proc. Nat. Acad. Sci. USA 69: 298–300

Bremer, K., Pinney, R. J., Smith, J. T. (1973) J. Pharm. Pharmacol. 25: Suppl. 131P–132P

Coetzee, J. N., Datta, N., Hedges, R. W. (1972) J. Gen. Microbiol. 72: 543–552

Crumplin, G. C., Smith, J. T. (1981) J. Antimicrob. Chemother. 7: 379–388

Davis, B. D., Mingioli, E. S. (1950) J. Bacteriol. 60: 17–28

Datta, N., Hedges, R. W. (1972) J. Gen. Microbiol. 72: 349–355

di Mauro, E., Synder, L., Marino, P., Lambert, A., Coppo, A., Tocchini-Valentini, G. P. (1969) Nature (London) 222: 533–536

Jensen, K. (1975) in: Williams, J. D., Geddes, A. M. (eds) Chemotherapy, Vol. 1, Clinical aspects of infections. Plenum Press, New York and London, pp 43–47

Johnson, J. H., Richmond, M. A. (1970) J. Gen. Microbiol. 60: 137–139

Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbour Laboratory, Cold Spring Harbour, New York

Mitsuhashi, S., Harada, K., Kamuza, M. (1961) Nature (London) 189: 947

Morrison-Smith, J. (1975) J. Antimicrob. Chemother. 1: 353–354

Nessi, R., Fowst, G. (1979) J. Int. Med. Res. 7: 179–186

Obaseiki-Ebor, E. E. (1983) J. Pharm. Pharmacol. 35: 130–131

Pinney, R. J., Smith, J. T. (1971) Genetic Res. 18: 173–177

Simpson, A. M., Breeze, A. S. (1981) J. Appl. Bacteriol. 50: 469–474

Tomoeda, M., Inuzuka, M., Kubo, N., Nakamura, S. (1968) J. Bacteriol. 95: 1078–1089

Watanabe, T., Fukasawa, T. (1961) ibid. 81: 679–683

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Effect of omeprazole and polyethylene glycol-400 on antipyrine elimination by the isolated perfused rat liver

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The effect of the substituted benzimidazole, omeprazole, a potent inhibitor of gastric acid secretion, on the hepatic elimination of antipyrine was studied in the rat isolated perfused liver. Bolus dosage (10 mg in 100 ml perfusate) and infusions (1 μg ml^{-1} perfusate concentrations) of omeprazole in its solvent, polyethylene glycol-400 (PEG-400), reduced antipyrine clearance by approximately one third ($P < 0.05$). PEG-400 alone caused a 15% decrease in antipyrine clearance ($P > 0.10$), indicating that the effect seen with omeprazole was at least partly due to the vehicle of dissolution. A significant but mild choleresis was noted in all preparations ($P < 0.01$) exposed to PEG-400. We conclude that the effect of omeprazole on hepatic drug elimination in patients warrants further study.

Omeprazole, a substituted benzimidazole, is a potent inhibitor of gastric acid secretion, acting by blocking the ($H^+ + K^+$) ATPase in the secretory canalicular membrane of the parietal cell (Olbe et al 1982). Benzimidazole derivatives have been shown to inhibit hepatic mixed function oxidases (MFO) (Murray et al 1982) and the potential inhibition of the metabolism of concurrently administered drugs by omeprazole may therefore be clinically important. In addition, the i.v. formulation for omeprazole may use polyethylene glycol (PEG-400) as the solvent, although the action of PEG-400 on the MFO enzyme system is unknown.

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We have previously employed the rat isolated perfused liver to examine the effects of H_2-receptor antagonists on MFO activity (Mihaly et al 1982). Antipyrine undergoes extensive multi-pathway metabolism by hepatic MFO enzymes, and its clearance is widely accepted as an index of hepatic drug metabolizing enzyme activity (Vuitton et al 1981). The isolated liver is particularly suitable for the short term study of hepatic drug elimination, since the volume of the system is held constant, liver blood flow is precisely controlled and other routes of elimination present in the intact animal are excluded. The present study compares the effect of single doses and infusions of PEG-400 and omeprazole in PEG-400 on antipyrine elimination by the rat isolated perfused liver.

Materials and methods

Livers of male Sprague-Dawley rats (190–240 g) under ether anaesthesia were isolated by standard techniques (Gollan et al 1981), and perfused in a constant flow (16 ml min^{-1}) recirculating system at 37 °C (Mihaly et al 1982). The main indices of liver viability were steady oxygen consumption (1·5–2·0 μmol g^{-1} liver min^{-1}), sustained bile production (0·4–1·0 ml h^{-1}), constant perfusion pressure (6–8 cm water), and normal appearance on light microscopy.

Omeprazole (H168/68, 5-methoxy-2- [[(4-methoxy-

3-, 5-dimethyl-2-pyridinyl)-methyl] sulphoxide]-1H-benzimidazole, Astra Pharmaceuticals, Sydney, Australia) has a low solubility in water and was therefore dissolved in 100% PEG-400 (polyethylene glycol, average molecular weight = 400). The effect of the vehicle alone on the disposition of antipyrine (phenazone BP, Prosana Laboratories Pty. Ltd, Sydney, Australia) was determined with doses identical to those used in the omeprazole experiments. The elimination of a 2·5 mg bolus dose of antipyrine (10 mg ml^{-1} in water) was studied over 4 h as follows (n = 6 livers per group): (i) control, with no added PEG-400 or omeprazole, (ii) after a 500 µl bolus dose of PEG-400, (iii) during a constant infusion of PEG-400, (iv) after a 10 mg bolus dose of omeprazole (delivered in 500 µl of PEG-400), and (v) during sustained 1 µg ml^{-1} perfusate concentrations of omeprazole. All doses were administered into the perfusate reservoir, thereby simulating systemic administration. All bolus doses consisted of 100% PEG-400. Group (iii) dosage included a 127 µl bolus dose of PEG-400, plus an infusion at 1 ml h^{-1} of 10% PEG-400 in carbonate buffer ($NaHCO_3$, 0·56 mg ml^{-1}, to stabilize omeprazole). A sustained omeprazole concentration of 1 µg ml^{-1} (Group (v)) was achieved with a bolus dose of 127 µg omeprazole in 127 µl of PEG-400 followed by an infusion at 1 ml h^{-1} of 750 µg ml^{-1} drug in 10% PEG-400 in carbonate buffer. This dosage regimen was determined (Gibaldi & Perrier 1982) using pharmacokinetic data obtained from previous single-dose omeprazole studies. The total amount of PEG-400 was similar (500 and 527 µl) in bolus and infusion experiments. Bile was collected on ice in preweighed vials and stored at −20 °C until assayed. Blood gases and liver function tests before and after each experiment ascertained the viability of the liver. Samples (1 ml) were taken from the perfusate reservoir for antipyrine estimations, predose and at 5, 10, 15, 20, 30, 60, 90, 120, 180 and 240 min; the red cells were removed from the perfusate, which was frozen at −20 °C until assayed. Extra samples were taken at various times to measure omeprazole concentrations. An equal volume of fresh perfusate was added to the reservoir to replace that removed by sampling. The total amount of antipyrine lost through sampling was less than 5% of the dose.

Antipyrine concentrations in perfusate and bile were measured by a specific and sensitive (450 ng ml^{-1}) high pressure liquid chromatographic (hplc) method (Shargel et al 1979). Omeprazole was quantified by a recently developed hplc assay (Mihaly et al 1983). The minimum detectable concentration of omeprazole in perfusate was 15 ng ml^{-1}.

Antipyrine pharmacokinetic parameters were calculated using standard model-independent pharmacokinetic formulae (Gibaldi & Perrier 1982). Statistical comparisons were made using one-way analysis of variance and Tukey's method of multiple comparisons (Miller 1966), accepting $P < 0.05$ as statistically significant. All data are represented as mean ± s.e.m.

Results and discussion

The mean perfusate concentration/time profiles of antipyrine for all five experimental groups demonstrate that antipyrine elimination was monoexponential (Fig. 1). The calculated pharmacokinetic parameters for antipyrine disposition are listed in Table 1. The volumes of distribution were not significantly different among any of the groups. After bolus dosage and infusion of omeprazole dissolved in PEG-400, antipyrine clearance was significantly reduced by 36 and 30%, respectively, when compared with the control ($P < 0.05$). Elimination half-life was increased by 33 and 20% in the respective groups. Murray et al (1982) have shown that many benzimidazole derivatives can inhibit hepatic MFO activity by reversible interactions with cytochrome P-450. The effect seen here is virtually immediate, suggesting a direct interaction between the inhibiting agent and drug-metabolizing enzymes, leading to a decrease in their activity. PEG-400 alone inhibited antipyrine clearance to an intermediate degree (15%, Table 1). Although this inhibition was not statistically significant, it does suggest that the effect seen with omeprazole was in part due to the vehicle of dissolution.

Omeprazole perfusate concentrations during infusion experiments ranged from 1·1 ± 0·1 µg ml^{-1} at 15 min, to 1·4 ± 0·2 µg ml^{-1} at 4 h. In contrast, after the 10 mg bolus dose, omeprazole concentrations fell from 52·3 ± 7·1 µg ml^{-1} at 5 min to 1·4 ± 0·5 µg ml^{-1} at 60 min, and

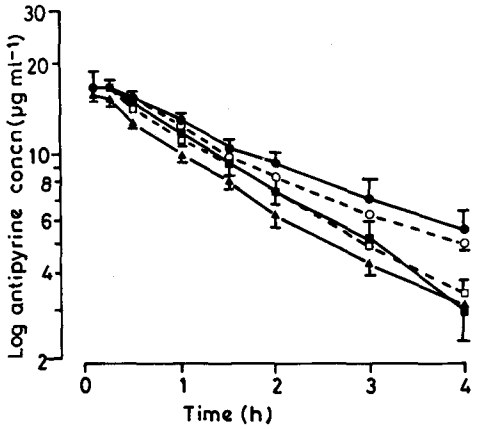

FIG. 1. Semilogarithmic plot of the elimination of a 2·5 mg bolus dose of antipyrine from male rat isolated perfused livers having a perfusate volume of 100 ml. Each point represents mean ± s.e.m. for 6 livers: (▲) Control, with no added PEG-400 or omeprazole; (■) after a 500 µl bolus of PEG-400; (□) during a constant infusion of PEG-400; (●) after a 10 mg bolus dose of omeprazole (in 500 µl PEG-400); (○) during a constant infusion of omeprazole in PEG-400 maintaining perfusate omeprazole concentrations of 1 µg ml^{-1}. Total PEG-400 delivered during infusion experiments was 527 µl. Clearance of antipyrine after both bolus and infusion of omeprazole in PEG-400 was significantly lower than the control ($P < 0.05$).

Table 1. Pharmacokinetic parameters for antipyrine disposition in male rat isolated perfused livers in the absence (control) and presence of PEG-400 or omeprazole in PEG-400. Values are mean ± s.e.m. for n = 6 livers.

	Clearance (ml h^{-1})	Half-life (h)	Volume of distribution (ml)
Control	70 ± 6	1·6 ± 0·1	155 ± 5
PEG bolus (500 µl)	59 ± 7	1·7 ± 0·2	141 ± 7
Omeprazole bolus (10 mg)[a]	45 ± 6[d]	2·4 ± 0·3[d]	143 ± 4
PEG infusion[b]	60 ± 2	1·6 ± 0·1	139 ± 7
Omeprazole infusion[c]	49 ± 2[d]	2·0 ± 0·1	137 ± 2

[a] In 500 µl PEG-400.
[b] Bolus 127 µl + infusion at 1 ml h^{-1} of 10% PEG-400.
[c] Bolus 127 µg (in 127 µl PEG-400) + infusion at 1 ml h^{-1} of 750 µg ml^{-1} in 10% PEG-400.
[d] $P < 0·05$ compared with control.

were undetectable by 90 min. Nevertheless, the inhibitory effect on antipyrine elimination was sustained throughout the 4 h after the bolus dose.

The average bile flow per 10 g liver in the control group was 2·7 ± 0·2 ml h^{-1}. This increased to 4·0 ± 0·5 ml h^{-1} in the livers exposed to PEG-400 ($P < 0·01$). A number of similar neutral compounds, e.g. PEG-1500 (Sperber 1965) and ferrioxamine derivatives (Meyer-Brunot & Keberle 1968), have been shown to increase bile flow in direct proportion to their own excretion into bile. It is likely that PEG-400 similarly produces an osmotic choleresis.

In control livers, cumulative biliary excretion of antipyrine was less than 1% of the dose. This increased proportionally with the increases in bile flow to 1–2% in the other experimental groups, and was greatest when overall hepatic clearance of antipyrine was inhibited. Changes in biliary excretion of antipyrine could not, therefore, account for the observed change in clearance.

It is not possible to predict whether the inhibition of MFO enzymes by omeprazole will be clinically important. After oral dosage (40 mg) in man, peak systemic plasma concentrations are less than on thirtieth of those found in the perfused liver system after a 10 mg bolus dose (unpublished observation). It therefore seems unlikely that oral omeprazole (without PEG-400) will inhibit the metabolism of other drugs to any extent. However, the possible inhibition of MFO enzymes by PEG-400 is of concern, especially as an intravenous formulation of omeprazole may use PEG-400 as the vehicle.

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REFERENCES

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics. 2nd edn, Marcel Dekker, Inc., New York and Basel, pp 271–287

Gollan, J., Hammaker, L., Licko, V., Schmid, R. (1981) J. Clin. Invest. 67: 1003–1015

Meyer-Brunot, H. G., Keberle, H. (1968) Am. J. Physiol. 214: 1193–1200

Mihaly, G. W., Prichard, P. J., Smallwood, R. A., Yeomans, N. D., Louis, W. J. (1983) J. Chromatogr. 278: 311–319

Mihaly, G. W., Smallwood, R. A., Anderson, J. D., Jones, D. B., Webster, L. K., Vajda, F. J. (1982) Hepatology 2: 828–831

Miller, R. G. (1966) Simultaneous Statistical Inference. McGraw-Hill

Murray, M., Ryan, A. J., Little, P. J. (1982) J. Med. Chem. 25: 887–892

Olbe, L., Haglund, U., Leth, R., Lind, T., Cederberg, C., Ekenved, G., Elander, B., Fellenius, E., Lundborg, P., Wallmark, B. (1982) Gastroenterology 83: 193–198

Shargel, L., Cheung, W., Yu, A. (1979) J. Pharm. Sci. 68: 1052–1053

Sperber, I. (1965) in: Taylor, We. (ed.) The Biliary System. Blackwell, Oxford, pp 457–467

Vuitton, D., Miguet, J. P., Camelot, G., Delafin, C., Joanne, C., Bechtel, P., Gillet, M., Carayon, P. (1981) Gastroenterology 80: 112–118