In conclusion, cat preparations may be useful in the evaluation of drugs enhancing acetylcholine release (or in the determination of this quality of effect of a drug which similarly to LF14 produces a mixed type of cholinergic stimulation).

The authors are greatly indebted to Professors Sandor Agoston (Department of Pharmacy, University of Groningen, The Netherlands) and Francis Foldes (Montefiore Hospital, Albert Einstein University, New York, USA) for helpful discussion and criticism.

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J. Pharm. Pharmacol. 1986, 38: 158–160
Communicated June 5, 1985

Lack of effect of omeprazole, a potent inhibitor of gastric (H^+ + K^+) ATPase, on hepatic lysosomal integrity and enzyme activity

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The substituted benzimidazole, omeprazole, is a potent inhibitor of the ATP-dependent proton pump of the parietal cell. Since there is accumulating evidence that hepatic lysosomes also possess an ATP-dependent proton pump system to maintain internal acidification, and since antibodies to the putative lysosomal proton pump protein are immunologically similar to the parietal cell (H^+ + K^+) ATPase, we studied the effects in rats of six days of omeprazole treatment on hepatic lysosomal function. Omeprazole, 5 mg kg^{-1}, a dose five times the ED50 for gastric acid secretion inhibition in rats, did not alter the activity of three representative lysosomal enzymes in liver (acid phosphatase, β-galactosidase and *N*-acetyl-β-glucosaminidase) nor did it alter lysosomal enzyme latency, a measure of the integrity of the lysosomal membrane. Furthermore, bile flow and the secretion of lysosomal enzymes into bile were also unaffected by omeprazole. These data indicate that in rats short-term treatment with omeprazole, in doses that markedly inhibit gastric acid secretion, has no major biological effect on liver lysosomal integrity and lysosomal enzyme activity.

Omeprazole, a substituted benzimidazole, is a potent new inhibitor of gastric acid secretion, which acts on the (H^+ + K^+) ATPase at the secretory canaliculus of the parietal cell (Fellenius et al 1981; Wallmark et al 1983). This ATP-dependent proton pump is responsible for the final step of gastric acid secretion (Rabon et al 1983).

The lysosome is an intracellular organelle which is responsible for the metabolism of a variety of endogenous and exogenous molecules, and which requires an acidic internal milieu for its degradative function (de Duve & Wattiaux 1966). There is strong evidence to indicate that an ATP-dependent proton pump is also present on the membrane of the lysosome (Dell 'Antone 1984; Ohkuma et al 1982; Schneider 1981), and furthermore, antibodies directed at the putative hepatic lysosomal proton pump protein, cross react specifically with the (H^+ + K^+) ATPase of the parietal cell, but not with other ATPase proteins (Reggio et al 1984). It is presently not known whether omeprazole interacts with the hepatocyte lysosomal proton pump, or whether it alters lysosomal function. Any impairment of lysosomal integrity by such a drug might constitute a serious side effect. For example, chloroquine, an antimalarial drug used in the treatment of rheumatoid arthritis is known

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to accumulate in lysosomes (Wibo & Poole 1974), to impair their acidification (Ohkuma & Poole 1978) and, in liver, to alter lysosomal function (Sewell et al 1983).

We were therefore prompted to investigate the effect of omeprazole on hepatic lysosomal function using the bile fistula rat model.

Methods

Liver lysosomal function. The biochemical function of liver lysosomes was assessed by measuring the activity of three representative lysosomal enzymes and the integrity of the lysosomal membrane by measuring lysosomal latency (de Duve et al 1955). Since we have recently provided evidence that a further function of hepatocyte lysosomes is to excrete their contents into bile (Sewell et al 1984), we also measured biliary lysosomal enzyme excretion as a marker of this pathway.

Experimental procedure. Female, Sprague-Dawley rats (230–300 g) were treated with six daily intraperitoneal injections of omeprazole (H168/68, 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridyl)-methyl]sulphinyl]-benzimidazole, Astra Pharmaceuticals, Sydney, Australia), dissolved in polyethylene glycol 400 (PEG 400), 5 mg kg^{-1} (n = 8). Controls were given injections of PEG 400 alone (n = 9) or saline (0·15 M, n = 9). The volume of omeprazole in PEG, PEG alone or saline given daily to each rat was 0·12–0·14 ml. The rats were provided with a bile fistula under sodium pentobarbitone anaesthesia (30 mg kg^{-1}). The anaesthetized rats were placed in a constant temperature-humidity apparatus and bile was collected into preweighed tubes in hourly samples for 5 h. The rats were then killed by exsanguination and their livers removed and quickly immersed in preweighed beakers containing an ice-cold solution of 250 mM sucrose, 3 mM imidazole, and 0·1% ethanol at pH 7·4. Liver tissue (5 g) was homogenized and centrifuged to separate nuclei, unbroken cells and tissue debris (pellet or N-fraction) from other organelles and cell sap (supernatant or E-fraction), as previously described (Sewell et al 1984). Analytical studies were performed on aliquots of bile or liver E- and N-fractions suitably diluted in the sucrose solution. Values for total liver were calculated as the sum of the activities found in the E- and N-fractions. Latent hydrolase activity in E-fractions of liver was used as an index of lysosomal membrane integrity (de Duve et al 1955) and was determined for acid phosphatase by subtracting free activity (activity in the absence of 0·1% Triton X-100 in the assay mix) from total activity (activity in the presence of 0·1% Triton X-100) and dividing this number by total activity.

Analytical procedures. The lysosomal glycosidases, β-galactosidase (EC 3.2.1.23) and *N*-acetyl-β-glucosaminidase (EC 2.3.1.30), were assayed fluorometrically using 4-methyl-umbelliferyl substrates (Koch-Light Laboratories, Ltd, Colnbrook, Buckinghamshire, UK) (Sewell et al 1984). The assay conditions employed for liver and bile have previously been established (La Russo & Fowler 1979). Suitable enzyme and substrate blanks were carried out for all assays. For these enzymes, 1 unit of activity corresponded to the hydrolysis of 1 μmol of substrate min^{-1}. Acid phosphatase activity was determined as the amount of inorganic phosphate liberated from hydrolysis of organic phosphate ester (Barrett & Heath 1972). Protein content was measured by the method of Lowry et al (1951). Statistical analyses used Student's unpaired *t*-test for between-group comparisons. *P* values of less than 0·05 were considered statistically significant.

Results and discussion

The indices of hepatic lysosomal function assessed in this study were first the activities of three lysosomal enzymes, *N*-acetyl-β-glucosaminidase (β-NAG), β-galactosidase (β-GAL), and acid phosphatase in liver cell homogenates, and second, the 5 h cumulative secretion of β-NAG and β-GAL in bile. These indices were chosen since it has been shown by our group (Sewell et al 1984) and others (Lafont et al 1983) that they are affected by substances which interact with or accumulate in lysosomes. Chloroquine, for example, which has been shown to accumulate in lysosomes (Wibo & Poole 1974), alters the activities of the above enzymes profoundly (Sewell et al 1983).

Table 1 summarizes the results of the present study.

Table 1. Lysosomal enzyme activities in liver and outputs into bile.

	Hepatic enzyme activities			Biliary cumulative 5 h enzyme excretion	
	N-Acetyl-β-glucosaminidase (u g^{-1} liver)	β-Galactosidase (u g^{-1} liver)	Acid phosphatase (nmol $Pimin^{-1}$ (mg protein)$^{-1}$)	*N*-Acetyl-B-glucosaminidase (mu g^{-1} liver)	β-Galactosidase (mu g^{-1} liver)
Saline (n = 9)	1·52 (± 0·09)	0·31 (± 0·03)	84·4 (± 6·9)	10·2 (± 2·7)	1·5 (± 0·4)
Polyethylene glycol 400 (n = 9)	1·40 (± 0·10)	0·27 (± 0·01)	79·4 (± 3·2)	15·8 (± 3·3)	2·4 (± 0·5)
Omeprazole (n = 8)	1·62 (± 0·20)	0·29 (± 0·03)	82·6 (± 4·5)	11·4 (± 1·9)	1·7 (± 0·3)

* Mean values ± s.e.m.

No change was observed in the activities of any of the lysosomal enzymes in liver or in bile following pretreatment with omeprazole dissolved in PEG or PEG alone when compared with saline controls. All three groups of animals were comparable with respect to body weight, liver weight, liver protein content, bile volume and bile protein secretion. The latency of acid phosphatase in the E fractions of livers for the omeprazole-treated rats was 93 ± 7% of control values (*P* NS). These results indicate that omeprazole has no observable effect on hepatic lysosomal integrity, lysosomal enzyme activity or biliary lysosomal enzyme secretion at doses which are five times the ED50 for gastric acid inhibition in rats (Larsson et al 1983). It remains to be determined whether omeprazole can interfere with other in-vivo functions of lysosomes such as degradation of macromolecules (de Duve et al 1966).

Omeprazole is believed to bind specifically to the ($H^+ + K^+$) ATPase of the parietal cell and current evidence suggests that it does not act on ATP-dependent pumps in other organ systems (Smolka et al 1983; Sachs 1984). Although lysosomal acidification by the purported lysosomal membrane ATP-dependent proton pump may well be analagous to that found in the canalicular membrane of the parietal cell and while cross-reactivity to antibodies against purified gastric mucosal ($H^+ + K^+$) ATPase has been reported for lysosomes (Smolka et al 1983; Reggio et al 1984), the lack of effect of omeprazole on hepatic lysosomal enzyme activities suggests that there is no major biological effect on the lysosome at a dose several-fold greater than that which profoundly inhibits gastric secretion (Larsson et al 1983). These experiments cannot be regarded as completely ruling out an effect of omeprazole on the lysosomal proton pump system, since H^+ transport has not been measured directly. One can calculate a theoretical partition coefficient for omeprazole due to ion trapping between the interior of the lysosome and the cell sap, knowing that the drug has one pK_a of 8·8, and that the pH of the lysosome is approximately 4·75. This comes to 400 : 1, which suggests that sufficient omeprazole should be available to affect the lysosomal proton pump were it identical to that of the parietal cell. However, the efficient hepatic metabolism of omeprazole (Webster et al 1985) might limit the amount of drug available to lysosomes in the liver cell, thus preventing any biologically significant effect of omeprazole on hepatic lysosomal function.

The excellent technical assistance of Adrienne Osborne and manuscript preparation by Jane Bell are greatly appreciated. Omeprazole was donated by Astra Pharmaceuticals, Sydney, Australia. R. B. Sewell was an Australian NH MRC Postdoctoral Fellow. This work was supported by a grant from the Australian National Health and Medical Research Council.

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