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# Intracellular binding is an important determinant of the avid hepatic uptake of the high clearance drug omeprazole

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**Abstract**—The contribution of intracellular storage to hepatic uptake of the high clearance drug, omeprazole, was examined in the recirculating isolated perfused rat liver preparation. Following injection of [$^3$H]omeprazole (7.5 $\mu$Ci, 5 mg) into the portal vein over 1 min, livers were perfused for 5 min (N = 3) or 30 min (N = 3) and then homogenized at 4° and fractionated by differential centrifugation. Radiolabelled omeprazole and metabolites were determined by scintillation counting of fractions of eluant from HPLC. Seventy per cent of drug had been taken up by the liver at 5 min and 85% at 30 min, with unchanged drug representing 43 and 7.4%, respectively, of drug taken up. At both times, 70–75% of intracellular unchanged drug and the major metabolites were located in the cytosol, and the cytosol : perfusate concentration ratio was approximately 10 : 1. Mitochondrial, lysosomal and microsomal fractions contained relatively little drug. Extensive cytosolic binding of omeprazole therefore contributes substantially to the initial avid hepatic first-pass uptake of this drug.

*Key words:* liver; drug distribution; pharmacokinetics; drug binding; cell components

Most studies of the effect of hepatic drug disposition on systemic bioavailability of drugs have focused on the importance of the efficiency of the final eliminating mechanism within the liver cell, i.e. intrinsic clearance [1, 2]. Events within the liver cell prior to the final elimination step have received little attention, but there is evidence to suggest that these events require more careful appraisal. Evans *et al.* [3] found that tissue uptake appeared to be an important determinant of hepatic extraction of the high clearance drug propranolol, independent of the activity of the eliminating enzymes. Our own data [4] and those of Anderson *et al.* [5] on propranolol uptake by hypoxic livers also suggested that, with single, pre-systemic doses, first-pass hepatic uptake might not depend primarily on the metabolizing enzyme system, but rather on avid tissue uptake and "storage". Tissue homogenates prepared from isolated hepatocytes or perfused livers have demonstrated higher native drug concentrations in the homogenate than in the surrounding medium (or perfusate) for a number of drugs, the concentration ratios varying from 3 to 100 [6–12]. It has also been shown that hepatic intracellular binding can be an important determinant of systemic plasma drug concentrations following pre-systemic dosage [13–15].

Omeprazole, a gastric antisecretory compound, is avidly extracted by the rat liver [16]. We hypothesized that the high first-pass extraction after an initial dose of the drug might partly reflect avid tissue uptake with storage of drug in one or other compartment of the liver cell. The aim of this study was to examine, in the isolated perfused rat liver preparation, the intracellular uptake and localization of omeprazole following pre-systemic, i.e. portal vein, dosage.

## *Materials and Methods*

*Experimental preparation.* Livers were obtained from male Sprague–Dawley rats (180–230 g) under sodium pentobarbitone (40 mg/kg) anaesthesia. Livers were isolated and perfused as described previously [17] using a recirculating perfusion circuit and a reservoir volume of 100 mL. Liver viability was demonstrated by oxygen consumption greater than 2.0 $\mu$mol/min/g liver, bile production greater than 0.4 mL/hr, stable perfusate transaminase levels and a normal macroscopic appearance of the preparation. A 15 min equilibration period was allowed prior to the commencement of each experiment.

*Hepatic fractionation.* The procedure was based on that described by de Duve *et al.* [18] and modified by Lardeux *et al.* [19]. The liver was flushed with ice-cold sucrose-phosphate buffer (250 mM sucrose, 10 mM $KH_2PO_4$, pH 7.4) then immediately immersed in a pre-weighed beaker containing an ice-cold sucrose-phosphate buffer. All subsequent steps were carried out at 4°. Liver (5 g) was minced and homogenized in buffer with one stroke of a Potter–Elvejheim homogenizer and centrifuged at 385 *g* for 10 min. The pellet was then resuspended and centrifuged at 265 *g* twice more to separate nuclei, unbroken cells and tissue debris (pellet or N-fraction) from other organelles and cell sap [20]. The supernatant was centrifuged at 30,000 *g* for 5 min and the supernatant was removed; the pellet was then resuspended in a small volume of buffer and homogenized in a Dounce homogenizer with 5 strokes of a loose manually driven type A pestle, resuspended in buffer and recentrifuged at 30,000 *g* for 5 min. The final pooled supernatants were diluted with buffer to a set volume and constituted the microsomal and cell sap fraction. This was centrifuged at 160,000 *g* for 60 min in a Damon IEL ultracentrifuge and the supernatant removed. The pellet was resuspended in buffer, homogenized and re-centrifuged. The combined supernatants constituted the cell-sap (S-fraction*) and the re-homogenized pellet constituted the microsomal-enriched fraction (P-fraction).

*Isolation of mitochondrial and lysosomal rich fractions.* Percoll separation of the mitochondrial-lysosomal (ML) fraction followed the method of Pertoft and co-workers [21, 22]. The ML preparation (80–100 mg protein) (4 mL) was applied to 36 mL of Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) suspension (starting density 1.09 g/L) and centrifuged at 43,000 *g* for 60 min. The mitochondria were concentrated at the less dense top of the tube and lysosomes at the lower end.

Lysosomal latency (mean ± SD) for *N*-acetyl glucosaminidase was 92.1 ± 1.98% in the initial supernatant (i.e. following separation of the N-fraction, N = 6); 91.0 ± 2.71% in the ML fraction (N = 6) and 90.3 ± 4.21% in the L fraction (following Percoll separation).

The fractionation procedures were validated by measure-

* Abbreviations: N-fraction, nuclei fraction, S-fraction, cytosol-fraction; P-fraction, microsomal-enriched fraction; ML-fraction, mitochondrial-lyosomal fraction.

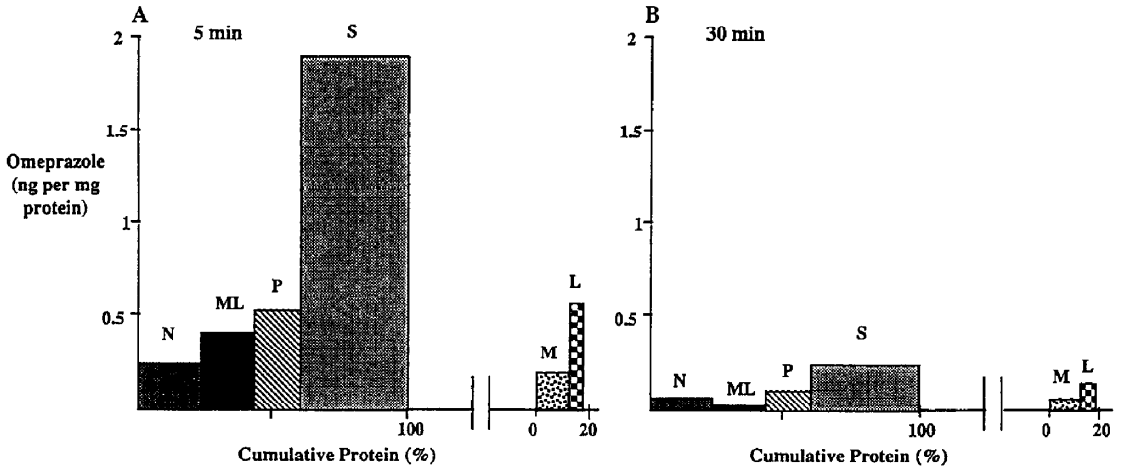


Fig. 1. Concentration of unchanged omeprazole in each hepatic subcellular fraction at 5 min (N = 3) (A) and at 30 min (N = 6) (B). The amount of protein in each subfraction is shown on the abscissa as a percentage of total protein content.

Table 1. Radiolabel recovery of omeprazole and metabolites in subcellular fractions of liver, in perfusate and bile expressed as a percentage of administered dose (means ± SD) at 5 and 30 min after dosage

| | Polar metabolites | Unchanged omeprazole | Non-polar metabolite |
|---|---|---|---|
| 5 min (N = 3) | | | |
| Liver subfraction | | | |
| N | 1.14 ± 0.47 | 1.71 ± 0.35 | 0.73 ± 0.37 |
| ML | 0.99 ± 0.05 | 2.11 ± 0.47 | 0.50 ± 0.09 |
| P | 0.86 ± 0.25 | 1.69 ± 0.36 | 0.47 ± 0.07 |
| S | 12.0 ± 2.63 | 20.8 ± 5.16 | 4.90 ± 1.36 |
| Perfusate | 9.77 ± 2.03 | 30.0 ± 3.05 | 3.84 ± 0.69 |
| 30 min (N = 3) | | | |
| Liver subfraction | | | |
| N | 1.08 ± 0.35 | 0.96 ± 0.53 | 0.44 ± 0.20 |
| ML | 0.90 ± 0.30 | 0.89 ± 0.13 | 0.47 ± 0.20 |
| P | 0.83 ± 0.18 | 1.03 ± 0.27 | 0.55 ± 0.15 |
| S | 11.1 ± 4.96 | 9.26 ± 3.98 | 2.64 ± 1.15 |
| Perfusate | 30.2 ± 5.23 | 12.1 ± 2.30 | 3.79 ± 1.27 |
| Bile | 14.0 ± 5.20 | 5.67 ± 2.41 | 1.72 ± 0.05 |

ments of enzymes localized to specific subcellular fractions as follows: mitochondria-succinate dehydrogenase [23]; endoplasmic reticulum-microsomal esterase [24]; lysosomes-*N*-acetyl-β-glucosamidase [25]; cytosol-lactate dehydrogenase [26]; cell plasma membranes-alkaline phosphodiesterase-I[24]. Protein was measured by the Lowry method [27].

*Experimental design.* In six experiments [$^3$H]omeprazole (7.5 μCi, 5 mg) was injected over 60 sec just proximal to the portal vein inflow cannula. Perfusion was continued for 5 min (N = 3) or 30 min (N = 3). When perfusion was stopped the liver was flushed rapidly with ice-cold sucrose-phosphate buffer and fractionation was performed at 4° as described above. In three further experiments unlabelled omeprazole (5 mg) only was injected and the livers fractionated after 30 min of perfusion. Cumulative bile was collected during the 30 min period.

Unchanged unlabelled omeprazole in perfusate and subcellular fractions (1 mL suspended in 0.25 mL isotonic phosphate buffer) was determined by HPLC by adding the internal standard (H.168.24, Astra Pharmaceuticals, Sydney, Australia) to the sample followed by extraction with dichloromethane (10 mL). The organic extract was evaporated at 37° in a clear tube under a gentle stream of nitrogen and reconstituted with mobile phase. A 100 μL aliquot was injected into the HPLC, the details of which have been described previously [28]. Recovery of omeprazole from each spiked tissue fraction was 102 ± 1.5%. The content of unchanged unlabelled omeprazole in each subcellular compartment was converted to a concentration using estimates of liver volume and the volumes of the various intracellular compartments [29].

For the determination of [$^3$H]omeprazole and metabolites, perfusate, bile and subcellular fractions were extracted with dichloromethane and chromatographed as described above. The HPLC eluate was collected in 60 sec

aliquots over a period of 16 min. The resulting fractions (2 mL) were dissolved in Aquasol (10 mL, New England Nuclear, Boston, MA, U.S.A.) and counted in a liquid scintillation spectrometer (Packard Model 3330) using external standardization. Recovery of counts from the HPLC column for each of the tissue fractions ranged from 81.6 to 96.6% (mean 87.3 ± 2.7%). The fractions corresponding to a retention time range of 4–6 min corresponded to unchanged [$^3$H]omeprazole and a small amount (<5%) of sulphone metabolite. $^3$H-label that eluted before the unchanged drug (retention time 1–3 min) was designated as representing polar metabolites of omeprazole on this reverse phase system and $^3$H-label that eluted after the unchanged drug (retention time 11–13 min) was designated as representing nonpolar metabolites of omeprazole.

*Results and Discussion*

The intrahepatic distribution of unchanged unlabelled omeprazole, as determined by HPLC, is shown in Fig. 1. After 5 min, only 30% of the injected dose remained in the perfusate. Unchanged omeprazole could be detected in all subcellular fractions, amounting in all to a further 30% of the dose. Thus, at this point, approximately 43% of drug taken up by the liver had not been metabolized. Most of the unchanged drug within the cell (70%) was present in the cytosol and there was no evidence of concentration of drug in microsomes or in lysosomes, relative to perfusate.

After 30 min, when at least 85% of the dose had disappeared from perfusate, 7.4% of the dose still remained unchanged within the cell. Again the cytosol was the principal site of drug storage (75%) (Fig. 1).

The distribution of unchanged [$^3$H]omeprazole and of polar and non-polar metabolites of $^3$H-labelled drug in perfusate and liver fractions, as determined by scintillation counting of HPLC fractions, is shown in Table 1. These experiments confirmed the relatively high concentrations of unchanged drug in cytosol (cytosol : perfusate concentration ratios of 10:1 after 5 min and 9:1 after 30 min.). In perfusate at 5 min, the majority of radiolabel was present as unchanged drug, although nearly 14% of the radiolabel had already appeared in perfusate as metabolites, and by 30 min 34% of the radiolabel was drug metabolite (both polar and non-polar), indicating significant release of metabolite from the liver. After 30 min, 21.5% of total radiolabel had appeared in bile, the majority as polar metabolites, but with some unchanged drug present. Polar metabolites predominated and these were localized primarily within the cytosol. The total recovery of radiolabel in these experiments was 85–95%.

Homogenization and ultracentrifugation of liver tissue will inevitably cause some damage to intracellular organelles, which might lead to an artefactual "leakage" of drug from one or other organelle to cytosol. Our data showed a high lysosomal latency, suggesting good preservation of this organelle which is the most fragile of those we examined. Furthermore, the validity of our data are supported by the reproducibility (Table 1) of the data among the different livers. A second line of evidence supporting the validity of our experimental approach is that two experiments done under identical conditions showed that propranolol localized primarily in microsomes (data not shown), as has been shown previously [7].

This study indicates that net hepatic uptake of omeprazole is due in the first instance to elements other than the metabolic "sink" created by irreversible elimination of drug by the microsomal mixed-function oxidase system. It is probable that binding to cytosolic proteins was primarily responsible for maintaining the blood-intracellular concentration gradient during movement of drug into the liver. Localization in the cytosol of omeprazole, a lipophilic weak base [30], its polar metabolites and its non-polar metabolites (Table 1) is not consistent with the proposition of Dale and Nilsen [31] that the more lipophilic drugs associate with intracellular membrane components whereas the more polar drugs associate with cytosolic protein constituents.

Only a limited number of studies have examined either the subcellular localization of drugs and metabolites in detail or the time course of this localization. Localization primarily to membrane constituents, including microsomes, has been shown for native propranolol [7], methadone [8] diphenylhydantoin [9] and chloramphenicol [32] while their more polar metabolites tend to partition to a variable degree into the cytosol. Morphine [8], ouabain and tetracycline [32] have been shown predominantly in cytosol, while lysosomal concentration of drug has been shown for D-tubocurarine [33] and for primaquine [34].

Reversible intrahepatic binding of drug should not theoretically affect bioavailability of pre-systemically administered drug [14, 35]. However, the shape of the systemic plasma drug concentration–time curve may be affected considerably, particularly if hepatic binding is avid [13–15]. The extensive cytosolic binding of omeprazole is therefore likely to exert a profound influence on systemic plasma omeprazole concentrations following oral dosage.

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