Disposition of Glycosidase Inhibitors in the Isolated Perfused Rat Liver: Hepatobiliary and Subcellular Concentration Gradients of 1-Deoxymannojirimycin and *N*-Methyl-1-Deoxynojirimycin

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The hepatic disposition of two glycosidase inhibitors was studied in the isolated perfused rat liver and after subcellular fractionation. The mannosidase inhibitor 1-deoxymannojirimycin (dMM) and the glucosidase inhibitor *N*-methyl-1-deoxynojirimycin (MedNM) exhibited minimal binding to albumin and reached liver concentrations that approximately equaled their medium concentrations, after 30 min (MedNM) or 90 min (dMM). Within 2 hr 0.5% of the dose of MedNM and 2.9% of dMM were excreted in bile. No metabolites were found for MedNM, whereas minor (bio)degradation was inferred for dMM. After subcellular fractionation, dMM and MedNM were found predominantly in the cytosolic fraction. Compared to the other particulate fractions, MedNM was elevated in the microsomal fraction, and both compounds were slightly enriched in the lysosomal fraction. We conclude that dMM and MedNM will likely inhibit liver enzymes when sufficiently high plasma levels are reached.

KEY WORDS: 1-deoxymannojirimycin; *N*-methyl-1-deoxynojirimycin; glycosidase inhibitors; rat liver perfusion; subcellular distribution; biliary excretion.

INTRODUCTION

Glycosidase inhibitors interfere with glycoprotein synthesis by inhibiting glucosidases or mannosidases responsible for oligosaccharide trimming within the endoplasmic reticulum (ER) and/or Golgi apparatus (1). Further, members of this group of compounds display antihyperglycemic, anticancer, or antiviral activity (see Ref. 2 for a review).

Effects of the glucosidase inhibitor 1-deoxynojirimycin (dNM) and derivatives on the biosynthesis of secretory glycoproteins were reported in cultures of rat hepatocytes (3) and rat liver slices (4), containing a large pool of glycosidases. In addition, dNM and some of its derivatives inhibited glycogenolysis in rat liver *in vivo* (5,6) and in isolated hepatocytes (7), which can result in specifically localized glycogen storage (6). We therefore studied the hepatic disposition of glycosidase inhibitors and extended previous work on their transport (7,8).

Experiments were performed in the isolated perfused rat liver (IPRL) (9). In addition, we investigated the subcellular distribution of glycosidase inhibitors in organellar fractions, including microsomal (containing ER and Golgi) and lysosomal fractions. The compounds investigated are *N*-methyl-1-deoxynojirimycin (MedNM), a specific inhibitor of ER trimming glucosidase I, and 1-deoxymannojirimycin (dMM), a specific inhibitor of the trimming enzyme Golgi mannosidase I (1,8).

MATERIALS AND METHODS

Chemicals. 1-Deoxymannojirimycin (dMM; 1,5-dideoxy-1,5-imino-D-mannitol) and *N*-methyl-1-deoxynojirimycin (MedNM; *N*-methyl-1,5-dideoxy-1,5-imino-D-glucitol) and the corresponding radioactive compounds were obtained from the Netherlands Cancer Institute (Amsterdam), in cooperation with Organon International (Oss, The Netherlands). They were prepared as described previously (8,10). Specific activities were 15 mCi/mmol for [^{14}C]MedNM, and 25 Ci/mmol for [^{3}H]dMM. The high specific activity of the latter compound made it susceptible to autoradiolysis, and therefore it was purified before experiments by thin-layer chromatography (TLC) as described (8).

Animals. Male Wistar rats (outbred strain, CDL, Groningen, The Netherlands) were maintained on tap water and rat chow (Hope Farms) ad libitum in a temperature-controlled chamber at 24°C with a 12-hr light/dark cycle. Rats were fasted for 16 hr prior to experiments. Rat weights after starvation ranged from 250 to 280 g.

Isolated Liver Perfusions. IPRL experiments were performed as described by Meijer *et al.* (9), with slight modifications. Rats were anesthetized with pentobarbital (60 mg/kg, i.p.). Bile duct and portal vein were cannulated with polyethylene (PE) tubing and the liver was perfused for a few seconds to wash away blood. An outflow canula was inserted in the superior vena cava and the inferior vena cava was ligated just above the renal vein. The liver was excised and placed in the perfusion apparatus. Recirculating medium (ca. 100 mL) consisted of a Krebs–bicarbonate buffer, containing 25 m*M* $NaHCO_3$, 10 m*M* glucose, and 1% bovine serum albumin (BSA), and was gassed constantly with 95% O_2 and 5% CO_2. pH was monitored on line and ranged between 7.36 and 7.42. Temperature was kept at 38°C. Sodium taurocholate (Fluka Chemie, Buchs, Switzerland) was infused (15 μmol/hr) to replace bile salts. Thirty minutes after the surgical procedure, 4 or 40 μmol of the compound was added to the perfusion medium, in 0.2 mL of 0.15 *M* NaCl solution (1.2–2.4 μCi [^{3}H]dMM; 0.4–0.6 μCi [^{14}C]MedNM). To ensure linear pharmacokinetics, other doses were also tested: 40 and 200 pmol (n = 1; results not shown). Medium samples were taken at 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 90, and 120 min. Bile was collected in 5- or 10-min fractions. After 120 min the liver was removed, weighed, and homogenized in a fourfold volume of saline and the drug concentration was determined. At various other time points the drug concentration in liver was calculated from medium and bile data.

Subcellular Fractionation. Glycosidase inhibitors were administered to the rat *in vivo*, as described previously (10), with slight modifications. Briefly, rats were anesthetized as described above and the jugular vein was cannulated for ad-

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ministration of compound. The renal vessels were ligated to prevent the reported excessive loss of compound through the urine (10). After 30 min of stabilization, dMM or MedNM was given as a bolus dose of 4 μmol. The liver was given 30 min to accumulate the compound, and was then perfused for a few seconds with ice-cold sucrose solution to remove blood cells, dissected out, and placed in a (tared) beaker with 35 mL of ice-cold sucrose solution (250 m*M* sucrose, 1 m*M* EDTA, pH 7.0, at 4°C). All subsequent steps were performed at 4°C. The liver was minced with scissors and homogenized by 12 strokes with a Teflon pestle (1000 rpm) in a Potter–Elvehjem tube (clearance, 0.150 mm). Differential pelleting of the homogenate was carried out according to De Duve *et al.* (11), with slight modifications (12). To obtain the nuclear fraction (N) the homogenate was centrifuged for 10 min at 600*g* in a Beckman J2-21 centrifuge with a JA-20.1 rotor. The pellet was resuspended in sucrose solution, followed by centrifugation at 600*g* for 7 min. This was repeated twice. The supernatants of the N fraction were combined, giving a mitochondrial fraction (Mi) after centrifugation at 6780*g* for 8.5 min and a lysosomal fraction (L) after centrifugation at 35,000*g* for 9 min. The microsomal fraction (Ms) was prepared in a Beckman L8-55 ultracentrifuge equipped with a SW28 rotor and the Ω^2t integrator set at 2.88×10^{10} rad^2/sec (96,000*g*). The final supernatant represents the cytosol fraction (S). Control studies, in which the compounds were added to blank homogenate and fractioned as described, ascertained that the observed localization of the test compound was not caused by redistribution during the fractionation period. The various sediments were suspended in 10 mL of sucrose solution containing 0.25% of the nonionic detergent Triton-X 100. Aliquots of homogenate and subcellular fractions were taken for determination of radioactivity, protein content [according to Lowry *et al.* (13) using BSA as standard], and enzyme activity, using acid phosphatase as marker for lysosomes (14), with enzymatically liberated phosphate determined according to Chen *et al.* (15).

Protein Binding. Binding of the glycosidase inhibitors to albumin was determined by ultrafiltration in a 1% BSA–Krebs solution (pH 7.4) after incubation for 1.5 hr at 37°C, using the Amicon Micropartition I System (Amicon B.V., Rotterdam, The Netherlands) with 30-kDa filters.

Detection of Metabolites. Separate liver perfusion experiments were performed to obtain bile samples for TLC. Furthermore, the stability of [^{3}H]dMM and [^{14}C]MedNM was examined after incubation with bile at 20 and −20°C for 1 day to 4 weeks. The stationary phase consisted of Kieselgel$_{60}$ plates (Merck; 20 × 20 × 0.25 cm); the mobile phase was 2-propanol:H_2O:ammonia (25%), 80:20:0.5 (v/v/v). Detection was performed by scanning of radioactivity on an Isomess IN-3016 Radio-TLC-Analyser.

Radiochemical Analysis. The ^{14}C- and ^{3}H-containing compounds were determined by liquid scintillation counting in medium, bile, liver homogenate, and subcellular fractions. The collected samples were mixed with 4 mL of scintillator (Opti Fluor, Packard, Groningen, The Netherlands). Aliquots of liver homogenate and subcellular fractions (0.3 g) were counted in triplicate. All samples were counted for 5 min in a Beckman LS 1701 liquid scintillation counter. Counting was corrected for quenching by external standardization.

Statistical Analysis. All data are given as mean ± SD, unless stated otherwise. Statistical comparisons were made with the unpaired Student's *t* test. $P < 0.05$ was the minimum level of statistical significance.

Pharmacokinetic Analysis. Medium concentration–time curves were analyzed with the computer program MultiFit, as described previously (10). The program derives the best-fitting curve and corresponding pharmacokinetic parameters. Clearance was calculated by dividing the dose by the area under the curve extrapolated to infinity.

RESULTS

Protein Binding. After 1.5 hr of incubation at 37°C, protein binding of MedNM was negligible, whereas 1% of dMM was protein bound.

Metabolite Studies. [^{14}C]MedNM (R_f value, 0.28) was recovered unchanged from the collected bile. Further, it was stable for at least 4 weeks when incubated with bile and stored, at both 20 and −20°C. This is consistent with earlier findings (10). [^{3}H]dMM (R_f value, 0.23) was stable in bile when stored for 24 hr at 20°C. However, after 3 weeks of incubation at this temperature, its R_f value had shifted to 0.15, pointing to slow decomposition. dMM may be partially metabolized or decomposed in rat liver as, in bile samples of two separate liver perfusion experiments, apart from the dMM peak, an additional small peak was found, with an R_f value of 0.35. However, considering the fraction of dMM excreted in bile (2.9% of dose), the contribution of this (bio)-degradation product to the total dose of dMM is negligible.

Isolated Liver Perfusions. The results of IPRL experiments are shown in Figs. 1 and 2, and Tables I and II. The dose of 4 μmol was chosen to ensure medium concentrations in the reported therapeutic dose range and to be able to compare the results with previous studies performed in the rat *in vivo* (10). At the concentration range used, no saturation of hepatic uptake or excretion was observed: both for MedNM and for dMM, the kinetics of tracer doses (40 and 20 pmol; results not shown) was identical to that of 4 and/or 40 μmol (see Fig. 1 and Table I, where 4 and 40 μmol MedNM are compared). Therefore, linear pharmacokinetic analysis can be applied to the observations found. Mean medium decay curves were fitted with MultiFit and pharmacokinetic parameters were calculated (Table I).

For both compounds it was found that radioactivity was eliminated very slowly from the medium, exhibiting a low hepatic clearance. Medium disappearance curves could be fitted best by a monoexponential function (Figs. 1 and 2, left). For [^{14}C]MedNM half-lives in medium after administration of 4 and 40 μmol varied from 20 to 30 hr, and for [^{3}H]dMM a half-life of 11.3 hr was found (4 μmol dose). For both compounds half-lives reflect the biliary elimination process.

The liver concentration–time curves of both compounds (Figs. 1 and 2, middle) showed similar shapes, but the "buildup" of hepatic concentration occurred slower for dMM. Plateau values in liver concentration were reached after about 30 min for MedNM and after approximately 90 min for dMM. The latter compound reached a slightly higher liver concentration after 120 min (31 μ*M*, compared to 24 μ*M* for MedNM; 4-μmol doses; $P < 0.05$).

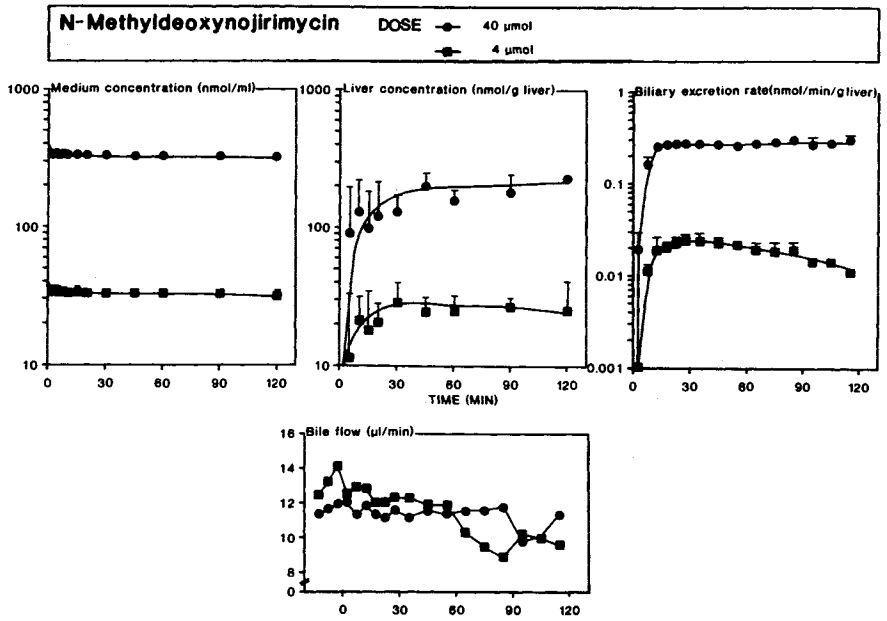

Fig. 1. Kinetics of MedNM in the IPRL after a bolus dose of 4 μmol (squares; $n = 4$) or 40 μmol (circles; $n = 3$). Concentrations in medium (left) and liver (middle) and biliary excretion rate (right) are indicated. The amounts excreted into bile are plotted at the midpoint of the sampling interval (5 or 10 min). Data are the mean ± SD; SD is shown only when it exceeds the size of the symbol. Bile flow (middle) is shown independently for each dose.

Biliary excretion rate–time curves showed similar profiles for both compounds: after 15 min for dMM, and after 30 min for MedNM, maximal values were reached. However, the absolute amount of dMM excreted per minute per gram of liver was higher than for MedNM by a factor of 5–7 (Figs. 1 and 2, right; Table I). For both compounds bile flow was important for excretion from the liver: changes in biliary excretion rate correlated well with changes in bile flow (Figs. 1 and 2, right and bottom). Table II shows concentration ratios among plasma, bile, and liver to see if the compounds concentrate in bile and liver.

Subcellular Distribution Studies. Thirty minutes after administration of the compound *in vivo*, livers were subjected to cell fractionation, containing 6.1 ± 0.3% ($n = 3$) of the administered dose of dMM and 3.5 ± 0.3% ($n = 4$) of the administered dose of MedNM. These results are in agreement with earlier *in vivo* experiments (10), where after 120 min the liver content of dMM and MedNM in rats with li-

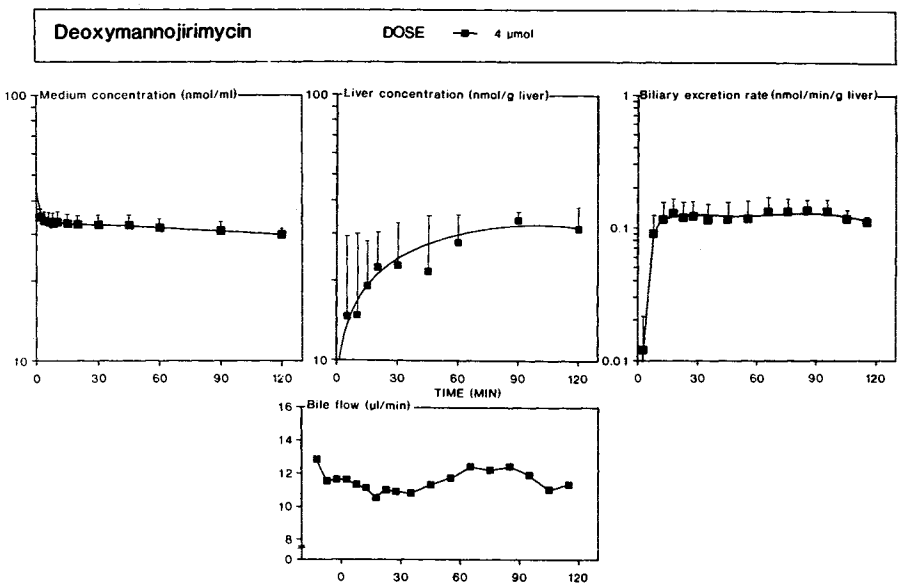

Fig. 2. Kinetics of dMM in the IPRL after a bolus dose of 4 μmol (squares; $n = 4$). Details are as indicated in the legend to Fig. 1.

Table I. Distribution Characteristics of dMM and MedNM in Isolated Perfused Liver 120 min After Addition of a Bolus Dose of 4 and/or 40 μmol, and Pharmacokinetic Parameters Clearance, (Cl), and Volume of Distribution of the Central Compartment (V_1)[a]

	dMM	MedNM	
	4 μmol	4 μmol	40 μmol
Rat weight (g)	261 ± 10	275 ± 4	260 ± 0
Liver weight (g)	7.90 ± 0.16	8.99 ± 1.0	8.79 ± 0.10
% liver	6.2 ± 0.6	5.3 ± 1.1	5.0 ± 0.3
% bile	2.9 ± 0.5	0.5 ± 0.06	0.5 ± 0.04
% perfusate	79.1 ± 1.9	81.4 ± 1.6	87.3 ± 2.2
% total recovery[b]	92.4 ± 2.6	92.3 ± 2.0	97.7 ± 2.1
$Cl_{0-\infty}$ (mL/min)	0.12 ± 0.01	0.07 ± 0.01	0.05 ± 0.01
V_1 (mL)	118.8 ± 0.6	118.3 ± 0.7	120.4 ± 0.4

[a] The percentages in liver, bile, and medium and total recovery are percentages of the administered dose. Values are means ± SD of 3 or 4 experiments.
[b] In "total recovery" is included the amount of drug removed from the medium by sampling.

gated renal vessels was 7.5 ± 2.3% for dMM (n = 3) and 4.4% for MedNM (n = 2). These values are also comparable to the percentage of dMM (4.5 ± 1.9%) and MedNM (6.1 ± 0.2%) calculated to be present in the IPRL after 30 min (values derived from Figs. 1 and 2).

Recoveries of radioactivity, protein, and acid phosphatase after homogenation and fractionation procedures were 99 ± 5, 100 ± 6, and 93 ± 6%, respectively (n = 14). The distribution patterns of acid phosphatase, dMM, and MedNM among the subcellular fractions are presented in Figs. 3A, B, and C, respectively, where the relative specific (radio)activity (y axis) is plotted against the relative protein content of the fraction (x axis), in a cumulative way. In such representations relative content of marker enzyme or radioactive label is proportional to relative area of each fraction. Acid phosphatase showed a distribution comparable to literature values (11,12).

The distribution after the addition of drug to cold liver homogenates (*in vitro*) showed significantly lower amounts of dMM and MedNM in all organelle fractions compared with the *in vivo* distribution (level of significance marked with circles in Fig. 3). This implies that the slight accumulation of compound in particular subfractions, observed after uptake *in vivo*, is unlikely to be caused by redistribution during liver homogenation and fractionation. dMM and MedNM, were predominantly present in the cytosol fraction, after both uptake *in vivo* and addition *in vitro*. After *in vivo* uptake, dMM was also present in the organellar fractions, with a slightly higher content in the lysosomal fraction, but not clearly in favor of any particulate fraction (Fig. 3B). Of MedNM, significant amounts accumulated in the lysosomal and in the microsomal fractions after administration *in vivo* (Fig. 3C).

DISCUSSION

Liver Perfusion Experiments. The present experiments do not indicate dose-dependent kinetics in the hepatic disposition of dMM and MedNM, consistent with previous findings *in vivo* (10). Both compounds equilibrate between medium and liver, and medium disappearance could be described best by a one-compartment model, leading to a central volume of distribution V_1 that approximately equals the sum of liver volume and recirculating medium. The clearance was slightly higher for dMM (0.12 mL/min) than for MedNM (0.07 mL/min), but the difference was not significant when individual curves were compared.

Metabolism did not occur for MedNM but might play a minor role in the disposition of dMM. However, it remains to be established whether the small additional peak (with an R_f value of 0.35) represents a metabolic product or is simply a decomposition product. Evidence is in favor of the latter possibility, because in aqueous solutions with high specific activities of [^{3}H]dMM, after prolonged storage, a peak with an R_f value of 0.35 was formed also.

The observation that dMM is excreted at a higher rate in bile of the IPRL than MedNM agrees with *in vivo* studies in rats with both compounds (10). Under comparable excretion conditions (ligated renal vessels), percentages of the dose recovered in bile were also comparable: 2.9% (IPRL) and

Table II. Ratios of Concentrations in Perfusion Medium (P), Liver (L), and Bile (B) at Various Time Points During the Perfusion Experiments After a Bolus Dose of 4 μmol[a]

Compound	Time (min)	L/P	B/L	B/P
dMM	5	0.4	4.3	1.8
	10	0.4	5.5	2.4
	20	0.7	3.8	2.6
	30	0.7	3.6	2.5
	60	0.9	3.0	2.7
	120	1.1	2.5	2.6
MedNM	5	0.3	0.7	0.2
	10	0.6	0.6	0.4
	20	0.6	0.8	0.5
	30	0.9	0.6	0.5
	60	0.8	0.7	0.5
	120	0.8	0.6	0.5

[a] Mean values of four experiments.

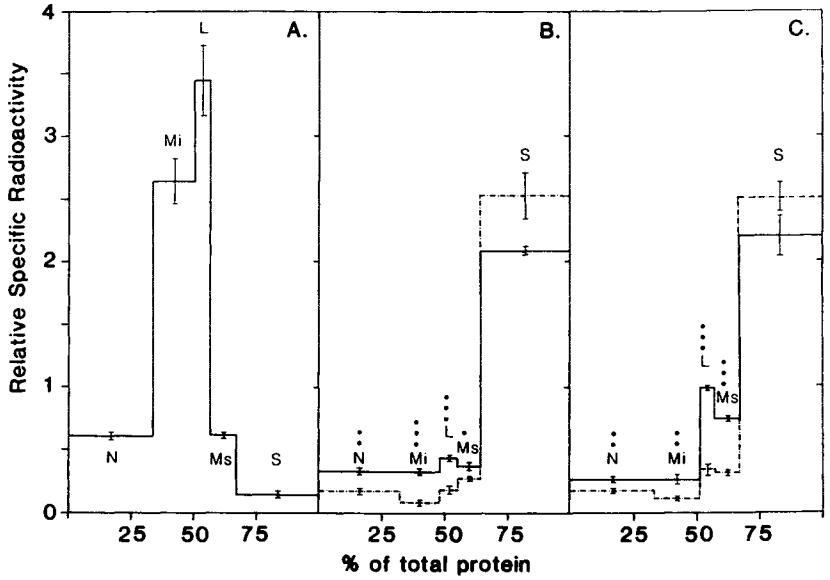

Fig. 3. Subcellular distribution patterns of (A) acid phosphatase, (B) dMM, and (C) MedNM, after differential centrifugation of rat liver homogenate. The relative specific activity (percentage of total recovered enzyme activity or radioactivity divided by percentage of total recovered protein) is plotted against the relative protein content of the fractions, displayed in a cumulative way. Blocks from left to right represent fractions in the order in which they were isolated: nuclear (N), mitochondrial (Mi), lysosomal (L), microsomal (Ms), and cytosol (S). Data are the mean ± SE; $n = 12$ for A, $n = 3$ for both lines in B, and $n = 4$ for both lines in C. Dashed lines indicate the subcellular distribution of dMM (B) and MedNM (C) after addition to cold liver homogenates; solid lines, after uptake of drug by the rat *in vivo*. Significant differences are marked with circles: $P < 0.05$, •; $P < 0.01$, ••; $P < 0.001$, •••.

3.2% (*in vivo*) for dMM and 0.5% for MedNM both *in vitro* and *in vivo*.

Although the liver concentrations of dMM and MedNM were similar, their biliary output differed, possibly as a result of different physicochemical properties of dMM and MedNM. dMM can exist in two conformations, whereas only one conformation is possible for MedNM because of the substituent on the ring nitrogen atom. Their lipophilicity also differs, with *n*-octanol/Krebs partition coefficients of 0.016 for dMM and 0.004 for MedNM (10). Furthermore, at physiological pH their protonation is 40 and 10%, respectively, with pK_a values of 7.2 for dMM and 6.4 for MedNM (10). Consequently, dMM and MedNM may be differentially transported by carriers in the canalicular membrane, e.g., the excretory systems for cationic drugs (16,17). Alternatively, liver cytosol concentration may not be the only driving force for biliary excretion. Direct transport of compounds from medium into bile (termed "permselective transport") via tight junctions or negatively charged pores has been suggested for small cations (with similar partition coefficients as MedNM and dMM) (17) and for small neutral compounds that are inert, lipid insoluble, and highly diffusible (18,19). Table II demonstrates the differences in disposition between dMM and MedNM. The latter equilibrates faster between liver and medium, in agreement with the observation that its parent compound dNM equilibrates rapidly across plasma membranes of isolated hepatocytes (7). The experimentally derived liver-to-medium concentration ratios (L/P) at steady state can also be predicted by assuming that the monobasic amines dMM and MedNM are passively distributed and that liver plasma membranes are impermeable to protonated amines, by using Eq. (1) (from Ref. 20):

$$K_{liver} = L/P = (K_A + H_i)/(K_A + H_o) \quad (1)$$

where K_A is the dissociation constant of the amine, and H_i and H_o are liver and medium proton concentrations. Assuming a liver pH_i of 7.1 (medium pH_o is 7.4), the expected distribution ratio at steady state (120 min) would be 1.4 for dMM and 1.1 for MedNM. The measured L/P ratios gave close to the expected values, 1.1 for dMM, and 0.8 for MedNM, respectively, especially when accounting for ≈20% liver wet weight as particulate material.

If, on the other hand, a protonated tertiary amine was taken up via an electroneutral cation/proton exchanger with a stoichiometry of 1:1, then the steady-state accumulation ratio would be expressed by Eq. (2):

$$K_{liver} = L/P = H_i/H_o \quad (2)$$

leading to a L/P ratio of 2 for both compounds. Comparison with the values of Table II suggest that liver uptake of dMM and MedNM from perfusate is due to a passive process.

The same calculations were made for the bile-to-liver concentration ratio (B/L). Assuming a biliary pH of 7.31 (21), ratios of 0.8 for dMM and 0.9 for MedNM can be predicted using Eq. (1), and a ratio of 0.6 for both using Eq. (2). For MedNM, these calculated values are in the range of the experimental value, indicating that the distribution of this

compound between liver and bile might be governed by the pH gradient solely and is passive in nature.

For dMM an additional (active) process should be involved to explain the large B/L ratio, e.g., concentrative cation transport from liver to bile and/or direct transport from medium to bile as proposed above. In favor of the former possibility are the observed similarities in biliary excretion between MedNM and dMM compared to small organic cations of similar molecular weight and lipophilicity (17). In favor of the latter possibility is the observation that for dMM the maximum biliary excretion rate is reached much faster than the maximum liver concentration, suggesting bypass of the liver.

The B/P ratios as calculated from medium and bile pH with Eqs. (1) and (2) lead to values of 1.1 and 1.2, respectively. These values are lower than the experimental value for dMM (2.5) and somewhat higher than the value for MedNM (0.6). Both compounds might be translocated through tight junctions or negative pores; however, dMM might be a more favorable substrate because a larger portion is in the cationic form (19). On the other hand, reabsorption of water, known to occur down the biliary tract (19), combined with the observed slow penetration of dMM into the liver might have caused the higher B/P ratio for dMM compared to MedNM. More studies will be necessary to discriminate between the excretion mechanisms for dMM.

Subcellular Distributions. The observed concentrations very likely underestimate the *in situ* concentration in the particular organelles since, during the time needed to perform the homogenization procedure (2.5–3 hr), partial loss to the supernatant cannot be prevented. In these experiments liver concentrations of approximately 27 μ*M* for dMM and 16 μ*M* for MedNM were achieved (assuming 1 g = 1 mL). As expected for relatively hydrophilic agents, the major part of dMM and MedNM was present in the supernatant. This fraction likely contains drug originating from the cytosol of parenchymal cells and other cell types, as well as extracellular fluid, which together make up 56% of liver wet weight (12) and 34% of liver protein (Fig. 3). The contribution of drug originating from bile in the supernatant was negligible for MedNM and 0.1% for dMM [calculated as described (12)]. dMM was found not to inhibit α-D-mannosidases in the cytosol (22), and to our knowledge, no data have been reported on cytosolic glucosidases.

In lysosomes, dMM was slightly and MedNM was considerably enriched, compared to the other particulate fractions (Figs. 3B and C). dMM is known to have no effect on rat liver lysosomal α-D-mannosidase (22). However, lysosomal α-glucosidases are shown to have a high affinity for dNM-derivatives, resulting in inhibitory concentrations of 0.2 μ*M* for *N*-hydroxyethyl-dNM and *N*-[β-(4-ethoxycarbonylphenoxy)ethyl]-dNM (6). The apparent accumulation in lysosomes might be caused by the fact that both dMM and MedNM are weak bases. Swainsonine, a Golgi mannosidase inhibitor and weak base (pK_a, 7.4), has been suggested to accumulate in the lysosomes of cultured fibroblasts (23). If one assumes an average pH of 5.5 in these compartments and that membranes would be impermeable to protonated amines, we calculate (20) that passive equilibration with the cytosol (pH 7.1) and protonation within the organelles would result in a 10- to 20-fold accumulation for dMM and MedNM, with 90–98% of the compounds present in the protonated form. Furthermore, ATP-dependent proton antiport of the cationic forms of glycosidase inhibitors leading to accumulation in acidified compartments may occur, as has been demonstrated by us for some quaternary ammonium compounds (20). However, no direct comparison of these accumulation factors and the data depicted in Fig. 3 can be made because we are dealing with subcellular fractions and not with pure lysosomes.

MedNM was found to be somewhat enriched in the microsomal fraction also (Fig. 3C). Consequently, inhibitory concentrations of MedNM are likely to be obtained in the ER after dosage of 4 μmol. For MedNM, 50% inhibition of glucosidase I of calf liver microsomes was found to occur at concentrations of 0.07–0.3 μ*M* (24,25). While dMM was not enriched in the microsomal fraction compared to homogenate, Golgi α-mannosidase I activity is sensitive to dMM, with 50% inhibition occurring at 1–2 μ*M* (26).

In conclusion, disposition of the glycosidase inhibitors dMM and MedNM is found to be comparable *in vivo* and in the IPRL, and therefore it is likely that inhibition of glycosidases will occur in the liver *in vivo* when plasma levels of about 30 μ*M* dMM and MedNM are attained. Preliminary studies (E. D. Faber, manuscript in preparation) indeed indicate an inhibitory effect of MedNM on glycogenolysis in perfused livers of fed rats at the doses used here.

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