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CLEARANCE OF *N*-NITROSODIMETHYLAMINE AND *N*-NITROSODIETHYLAMINE BY THE PERFUSED RAT LIVER

RELATIONSHIP TO THE K_m AND V_{max} FOR NITROSAMINE METABOLISM

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Abstract—The first-pass clearance of dietary *N*-nitrosodimethylamine (NDMA) by the liver is the most important factor in the pharmacokinetics of this carcinogen in the rat, but is less important in the pharmacokinetics of *N*-nitrosodiethylamine (NDEA). The reason for the difference in clearance of these two nitrosamines is not known. These experiments were carried out to see whether the general characteristics of the clearance of these two carcinogens *in vivo* could be reproduced in the perfused liver, and whether the clearance could be correlated with the Michaelis–Menten parameters K_m and V_{max} for their metabolism. If this could be done one would be able to predict the possible extent of first-pass clearance of nitrosamines in man from measurement of K_m and V_{max} for nitrosamine metabolism by the human liver. The K_m (22 μM) and V_{max} (10.2 and 13.4 nmol/g liver/min) for the metabolism of NDMA by slices from two human livers, the inhibition of that metabolism by ethanol (K_i 0.5 μM), and the rate of N-7 methylation of DNA when slices are incubated with NDMA, were measured. These results are similar to those reported previously with rat liver. The K_m (27 μM) for the metabolism of NDEA by rat liver slices and the inhibition of that metabolism by ethanol (K_i 1 μM) were estimated from the rate of ethylation of the DNA of the slices. The clearance of both these nitrosamines by the perfused rat liver was measured, and the results appeared to parallel those *in vivo* with a striking difference between the clearance of NDMA and NDEA. The maximal rate of clearance of NDMA was 11.2 nmol/g liver/min and of NDEA 8.9 nmol/g liver/min, similar to the V_{max} for metabolism of NDMA by liver slices and to the estimated maximal rate of liver metabolism of both nitrosamines in the living rat. However, although the K_m for metabolism of these two nitrosamines by liver slices is similar (about 25 μM), the logarithmic mean sinusoidal concentration [see Bass and Keiding, *Biochem Pharmacol* **37**: 1425–1431, 1988] giving half maximal clearance during perfusion (the equivalent to K_m) was 2.3 μM for NDMA and 10.6 μM for NDEA. The almost 5-fold difference between these two values is the basis for the difference between the clearance of the two nitrosamines. These values could not be correlated with the K_m for metabolism of these nitrosamines by liver slices or microsomes, suggesting that it will be impossible to predict the clearance of nitrosamines in man from measurements of the characteristics of the metabolizing enzymes *in vitro*.

Despite the difficulty of measuring human exposure to *N*-nitroso compounds, there is growing evidence that they may be important in the induction of certain human cancers [1]. *N*-Nitrosodimethylamine (NDMA‡), a potent carcinogen in rats [2], has been detected in human blood [3–9] and the major part of human exposure may come from endogenous formation in the gastro-intestinal tract from dietary precursors [10].

Nitrosamines require metabolic activation for their carcinogenic action and produce tumours only in those organs which have the appropriate cytochrome P450 to carry out this metabolic activation. Thus the consequence of exposure to nitrosamines is an interplay between the distribution of the nitrosamine in the animal, the ability of each organ to activate it metabolically and the inherent susceptibility of each organ to the carcinogen [11, 12]. For NDMA in the rat, the most important of these factors is the effect on distribution of first-pass clearance by the liver (the removal of nitrosamine in the portal blood as it passes through the liver), which prevents the nitrosamine absorbed from the gut from entering the general bloodstream and reaching extrahepatic organs [13]. This tends to concentrate the nitrosamine in the liver, which is relatively resistant to the nitrosamine, and thus protect more sensitive extrahepatic organs [14, 15].

Before we can assess the potential danger to man of small amounts of nitrosamine in the diet or synthesized in the gastrointestinal tract, we need to know, or to be able to predict, the extent of first-pass clearance of nitrosamines in man. This cannot be done directly, but the question is whether it can be predicted from measurements *in vitro*, in particular from knowledge of the Michaelis–Menten parameters for the nitrosamine-metabolizing system

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‡ Abbreviations: NDMA, *N*-nitrosodimethylamine; NDEA, *N*-nitrosodiethylamine; TCA, trichloroacetic acid.

as these can be measured with human liver samples. This question is particularly important because studies in pigs [16] and dogs [17], although not directly addressing the question, have suggested that in these species there is only an imperfect degree of first-pass clearance of NDMA and have raised the possibility that essentially total first-pass clearance may be peculiar to the rat.

First-pass clearance of NDMA in the rat was first inferred from measurement of the relative alkylation of DNA of the liver and extrahepatic organs [13, 14] and has since been confirmed by direct measurement [18]. The clearance depends upon metabolism but the exact mechanism of clearance has not been examined, and the only previous experiment with perfused rat liver showed maximum clearance of only 60% of the NDMA in the portal blood [19], compared with 90% clearance seen in the living rat [18]. It is not known why there is this divergence between the situation *in vivo* and the results *in vitro*. Furthermore, measurement of the relative alkylation of DNA of the liver and extrahepatic organs by the homologous nitrosamine *N*-nitrosodiethylamine (NDEA) suggests that it is not subjected to the same degree of first-pass clearance as NDMA [20]. This difference between the clearance of NDMA and NDEA has never been confirmed directly and no reason for it has previously been advanced.

The object of this work was to find whether the very different characteristics of first-pass clearance of NDMA and NDEA in the rat could be reproduced in the perfused liver, and whether the clearance of NDMA and NDEA could be predicted from the Michaelis–Menten parameters (K_m and V_{max}) for their metabolism. The results show that it is possible to reproduce in the perfused liver the general characteristics of first-pass clearance of NDMA and NDEA in the living rat, but that the extent of clearance cannot be predicted from the Michaelis–Menten parameters for their metabolism by liver slices or microsomes. The Michaelis–Menten parameters for the metabolism of NDMA by human liver slices were also measured, and the K_i for inhibition of NDEA metabolism by ethanol measured.

MATERIALS AND METHODS

Chemicals. [^{14}C]NDMA was synthesized [21] from [^{14}C]dimethylamine (58 mCi/mmol, Amersham) and the purity checked using a Zorbax ODS (DuPont) C18 HPLC column (1 mL/min, 20% methanol:water): 95% of the radioactivity eluted as a single peak with unlabelled marker NDMA (Eastman Kodak). [^{3}H]-NDEA was prepared [20] from sodium [^{3}H]acetate (Amersham, 3.15 Ci/mmol) and the purity (97%) was determined by HPLC.

Metabolism of NDMA to CO_2 by rat liver slices. Metabolism of NDMA to CO_2 by rat liver slices was measured in a Warburg apparatus as described previously [20]. Female Wistar rats (150 g) were allowed food and water until being killed by cervical dislocation. Livers were removed into cold Krebs–Ringer phosphate buffer, and 0.2 mm thick slices cut from the median lobe. Each flask side arm contained [^{14}C]NDMA (180,000 dpm) to give a final concentration in the incubation medium of between 5 and 100 μM. The concentrations were measured spectrophotometrically taking the molar extinction coefficient for NDMA at 230 nm to be 7240.

Metabolism of NDMA by human liver slices: inhibition by ethanol. Liver samples (about 1 g each) from two women aged 60 and 70, and a man aged 72 were sliced and incubated with [^{14}C]NDMA alone, or with sufficient ethanol to give a final concentration of 1 mM. In two experiments, the amount of NDMA metabolized was determined by removing 1 mL of incubation medium into a capped centrifuge tube containing 2 mL CH_2Cl_2. After mixing and centrifuging for 2 min, 1 mL of the organic layer was removed and the radioactivity measured. Approximately 75% of the NDMA was recovered using this technique. The total amount of NDMA metabolized was estimated from the difference in radioactivity measured between CH_2Cl_2 extracts from the incubated flasks and from the stock NDMA solutions.

DNA methylation in human and rat liver slices incubated with NDMA or NDEA. Slices from a liver sample of a 63-year-old male or a sample of rat liver were incubated for 10 min in Krebs–Ringer phosphate buffer containing 100 μM [^{14}C]NDMA (2.4 μCi/flask). Following incubation, the contents of the flask were centrifuged for 5 min at 1000 rpm, the tissue washed once with fresh buffer and DNA purified as described previously [20]. The DNA (typical yield 2 mg/g liver) was heated for 30 min at 100° in 2 mL 0.5 N HCl and the hydrolysate chromatographed on a cation exchange resin (HC-x12, 10–15 μ, 4.6 × 150 mm; Hamilton, Reno, NV, U.S.A.) with ammonium formate as eluant (0.4 M, pH 4, 1 mL/min, 60°). Fractions of 1.5 mL were collected, 0.1 mL 4 N HCl was added to the fractions containing guanine, and both the absorbance at 260 nm and the radioactivity were measured. The amount of guanine and adenine was calculated by taking E_{260nm} for adenine in neutral solution to be 13,000, and E_{260nm} for guanine in acid to be 8000. The amount of N7-methylguanine was calculated from the specific activity of the [^{14}C]NDMA. DNA ethylation in rat liver slices was measured for NDEA concentrations between 10 and 100 μM (6 μCi [^{3}H]-NDEA/flask) with or without ethanol (1 mM).

Rat liver perfusion. The perfusion technique was that of Hems *et al.* [22] and Brunengraber *et al.* [23] with minor modifications. Livers from 150 g female Wistar rats were perfused with Krebs–Ringer phosphate buffer containing either washed rat or human erythrocytes, giving a haemoglobin concentration of approximately 2.5 g/100 mL. Although this is less than the physiological concentration (10–15 g/100 mL), Hems *et al.* [22] found that this concentration of haemoglobin was sufficient for full oxygenation at flow rates as low as 10 mL/min/rat liver. The oxygenation of the perfusion fluid was maintained by passing it through a gas exchanger and equilibrating with 95% O_2, 5% CO_2. The liver was not removed from the rat before perfusion. The portal vein and vena cava were cannulated *in situ* and perfusion was carried out in a temperature-controlled cabinet maintained at 37°. After the residual blood had been washed out of the

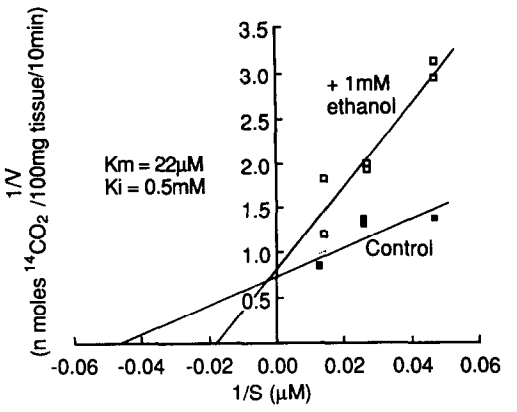

Fig. 1. Lineweaver–Burk plot for the metabolism of NDMA to CO_2 by human liver slices and the inhibition of that metabolism by 1 mM ethanol. The data points represent individual measurements.

liver with cold Krebs–Ringer phosphate buffer, the livers were perfused for 15 min with buffer containing washed erythrocytes. During this period the buffer was recycled and passed repeatedly through the liver. Perfusion with buffer and erythrocytes containing concentrations of NDMA or NDEA between 0.05 and 40 μM (5000–20,000 dpm [^{14}C]-nitrosamine/mL) then began. This solution was passed only once through the liver and only one nitrosamine concentration was used for each liver. During the first 2 min of perfusion in open circuit the flow increased to a steady state of 10–15 mL/min/rat liver. Once this steady state had been achieved 10 consecutive 1 min samples were collected and placed on ice before analysis.

Duplicate 0.4 mL samples from each fraction were mixed well with 0.4 mL cold 10% aqueous trichloroacetic acid (TCA). After 2 min at room temperature and centrifugation for 2 min the liquid was removed. This was extracted three times with CH_2Cl_2 (0.4 mL). The combined CH_2Cl_2 extract was dried over Na_2SO_4. The CH_2Cl_2 was then removed and the Na_2SO_4 washed with 0.5 mL CH_2Cl_2. The combined CH_2Cl_2 extract was evaporated under nitrogen in a fume hood to leave a white crystalline deposit of TCA. This was redissolved and the radioactivity determined. The vapour pressure of NDMA is markedly diminished in the presence of TCA and the nitrosamine is trapped in the TCA [24]. This permits near quantitative recovery of the nitrosamine. Approximately 85% of the NDMA and 90% of the NDEA were recovered. All the recovered radioactivity was co-eluted with marker NDMA or NDEA when analysed by HPLC.

RESULTS

Metabolism of NDMA by human and rat liver slices: the effect of ethanol

There is an unavoidable delay between the removal of human liver samples and the time at which measurements can be made. To check that this delay did not affect the ability to metabolize nitrosamines, preliminary experiments were carried out with rat liver. These showed that there was no significant reduction in NDMA metabolism when the liver samples were stored in cold buffer for up to 6 hr, and that the results obtained after 6 hr were indistinguishable on a Lineweaver–Burk plot from those obtained with fresh liver. Therefore, there was unlikely to be loss of metabolic activity between collection of the human liver samples from the operating theatre and the time the measurements were made (usually less than 2 hr). The K_m and V_{max} for [^{14}C]NDMA metabolism to $^{14}CO_2$ by human liver slices were calculated from Lineweaver–Burk plots (Fig. 1). $^{14}CO_2$ is not a primary product of [^{14}C]-NDMA metabolism but previous experiments give ample justification for its use as a measure of NDMA metabolism [25]. The K_m for NDMA metabolism in human liver slices (Table 1) was similar to the K_m (25 μM) obtained with rat liver slices. The respiration rate of the human samples was less than that of rat liver slices and showed considerable individual variation. In the human, as in the rat, ethanol was a competitive inhibitor of NDMA metabolism, and the calculated K_i (0.5 mM) is identical to the K_i measured in rat liver slices [20]. One millimolar ethanol did not affect the proportion of NDMA metabolized to CO_2 by human liver slices.

With two of the human samples the disappearance of NDMA during metabolism was measured and compared with the amount of $^{14}CO_2$ produced from the [^{14}C]NDMA. In one case 10% of the NDMA

Table 1. K_m and V_{max} for NDMA metabolism by human liver slices: effect of ethanol, and comparison with metabolism in rat liver slices

Sample	K_m (μM)	Apparent K_m in 1 mM ethanol	K_i (mM)	V_{max} (nmol CO_2/g tissue/min)	V_{max} in 1 mM ethanol
1	21.5 (10.2–40.4)	55.5 (36.5–89.9)	0.5	1.34 (1.08–1.78)	1.21 (0.94–1.69)
2	24.5 (14.0–45.5)	73.4 (31.0–324.7)	0.5	2.03 (1.52–3.05)	2.19 (1.28–7.60)
3	19.9 (16.8–23.6)	73.1 (57.1–98.3)	0.5	3.07 (2.81–3.39)	3.43 (2.81–4.42)
Rat*	24.5 (19.7–31.1)	ND	ND	3.31 (2.87–3.90)	ND
Rat†	25.1	100	0.5	4.13	5.26

* This paper; † [20].
ND, Not determined.

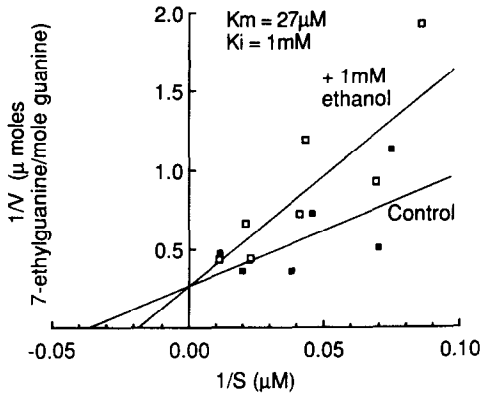

Fig. 2. Lineweaver–Burk plot for formation of N7-ethylguanine in the DNA of rat liver slices incubated with NDEA and the inhibition of that alkylation by 1 mM ethanol. The data points represent individual measurements.

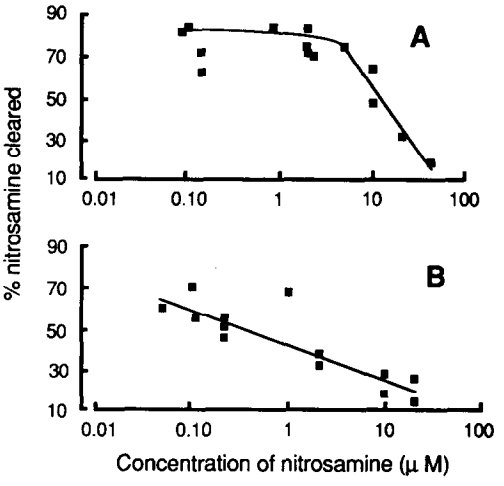

Fig. 3. Clearance of NDMA (A) and NDEA (B) from the portal blood on passage through the perfused rat liver. The *Y*-axis shows the percentage of the nitrosamine in the input, portal blood cleared during passage through the liver; the *X*-axis shows the concentration in the portal blood. The data points represent individual measurements.

metabolized was converted to CO_2 and in the other 30% was converted to CO_2, compared to rat liver slices which convert 29% of [^{14}C]NDMA to $^{14}CO_2$ [24]. Therefore, the V_{max} for CO_2 production in these experiments can be converted to a true V_{max} for total nitrosamine metabolized. Although the small number of samples prevents an accurate determination, the values of 13.4 nmol NDMA/g tissue/min and 10.2 nmol are similar to the V_{max} of 14 nmol NDMA in rat liver [20].

The fourth human liver sample was used to compare DNA methylation in rat and human liver slices when incubated with 100 μM NDMA. The amount of N7-methylguanine produced in human liver DNA (220 μmol N7-methylguanine/mol guanine/10 min) was similar to that in rat liver DNA (161 μmol N7-methylguanine/mol guanine/10 min).

The effect of ethanol on NDEA metabolism

Although ethanol inhibits the metabolism of [^{14}C]NDEA to $^{14}CO_2$ it also inhibits the metabolic steps occurring between metabolism of the nitrosamine and the eventual production of CO_2 [20], and therefore the K_i has not been measured previously. However, as the production of N7-alkylguanine depends only upon the initial cytochrome P450-mediated hydroxylation of the nitrosamine and since the formation of N7-alkylguanine parallels nitrosamine metabolism [26], it was used as a measure of NDEA metabolism and to study the effect of ethanol upon that metabolism. Rat liver slices were incubated with [^{3}H]NDEA, the DNA purified and hydrolysed, and the purine bases separated by HPLC. The amounts of guanine and adenine were assessed from their absorbance at 260 nm, and the amount of [^{3}H]N7-ethylguanine from measurement of radioactivity. A Lineweaver–Burk plot gives a V_{max} of 3.73 (2.62–6.47) μmol N7-ethylguanine/mol guanine/10 min and a K_m of 26.8 (12.4–62.3) μM NDEA, comparable with the K_m of 20 μM obtained previously [20]. Inhibition by ethanol was competitive, with a K_i of 1 mM (Fig. 2).

Perfusion experiments

Rat livers were perfused with NDMA or NDEA and the difference in nitrosamine concentration in blood entering (C_{in}) and leaving (C_{out}) the liver determined. The results were analysed using the sinusoidal perfusion model of Bass *et al.* [27] in which the liver is modelled as a collection of sinusoids through which blood has unidirectional flow. Elimination of substrate at every point along the sinusoidal path is assumed to obey Michaelis–Menten kinetics. The mathematical treatment substitutes a logarithmic mean sinusoidal blood concentration (C) into the standard Michaelis–Menten equation, i.e. $V = V_{max} \cdot C/(K_m + C)$, where $C = (C_{in} - C_{out})/[\ln(C_{in}/C_{out})]$; C_{in} = inlet blood concentration (μM); C_{out} = outlet blood concentration (μM); F = flow (mL/min); V = rate of uptake (nmol/min/g liver) i.e. $V = F(C_{in} - C_{out})$.

Figure 3 shows the concentration of NDMA or NDEA passing out of the liver, expressed as a percentage of the input concentration, plotted against the input concentration. Figure 4 shows the rate of uptake of NDMA or NDEA plotted against the mean sinusoidal concentration C. The points are the actual data, and the lines are the computer-generated Michaelis–Menten best fit to these points (Multifit programme, Day Computing, Cambridge, U.K.). The computer programme fits the data to a Hanes plot so that data at all concentrations are given appropriate weight. NDMA was cleared from blood much more effectively than NDEA by the perfused liver. When the inlet concentration was 5 μM NDMA or less, about 80% of the NDMA was removed in a single passage, but the maximum clearance of NDEA was only 50%. Furthermore, there was a rapid increase in clearance as the concentration of NDMA was decreased from 20 μM, which was not seen in the clearance of NDEA. From

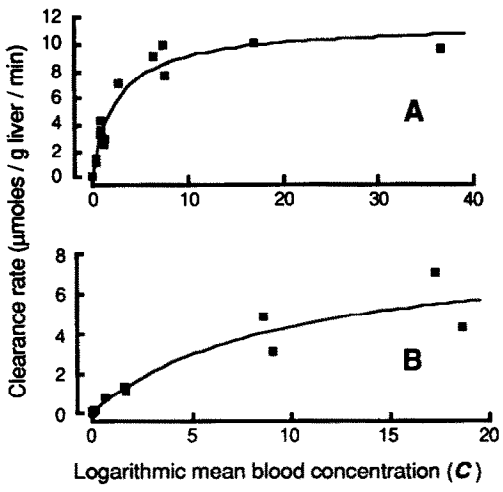

Fig. 4. The rate of clearance plotted against the logarithmic mean sinusoidal concentration (C) of nitrosamine (see text) showing the computer-generated Michaelis–Menten fit to the results. NDMA (A), NDEA (B). The data points represent individual measurements.

the computer-generated curves, the K_m for NDMA clearance was calculated as 2.3 μM and the maximum rate of uptake (V_{max}) was 11.2 nmol NDMA/min/g liver. For NDEA the calculated K_m was 10.6 μM and the V_{max} 8.9 nmol NDEA/min/g liver.

Since the uptake of NDMA or NDEA is due to metabolism of the nitrosamine, inhibition of metabolism by ethanol prevented clearance [20]. With an input concentration of 2 μM NDMA about 80% was cleared, but if the perfusate contained 5 mM ethanol there was no measurable clearance.

DISCUSSION

The perfusion experiments were carried out to explain why there is a difference in the extent of first-pass clearance in the rat, and whether this difference could be correlated with the K_m and V_{max} for metabolism of the two nitrosamines by the liver. The perfusion results are broadly parallel to the *in vivo* results. In particular, there was a very sharp increase in the clearance of NDMA as the input blood concentration was reduced from 20 to 2 μM. At 20 μM NDMA there was insignificant clearance, but at 2 μM about 85% was cleared. This mimics the sharp change in clearance in the rat, where the clearance of oral doses greater than 300 μg NDMA/kg body weight is insignificant, but doses of less than 30 μg NDMA/kg body weight are about 90–95% cleared [13, 14]. This sharp change in clearance was not seen with NDEA either *in vivo* [20] or in the perfused liver. With NDEA there was a slow increase in clearance in the perfused liver as the input concentration was lowered. However, even at an input concentration of 0.05 μM NDEA only 60% was cleared.

Both the perfusion results and the results *in vivo* indicate an upper limit of 90–95% for the proportion of NDMA which is removed from the blood during first-passage through the organ. It is possible that a part avoids clearance because part of the blood flow bypasses the sinusoids containing cells which actively metabolize NDMA. Such "porto-systemic shunting" is thought to occur physiologically when blood bypasses liver sinusoids via the vascular supply of bile ducts, tissue in the portal tract and vessels close to the capsule [28]. This process may be increased significantly in the diseased and cirrhotic liver [29].

Several mathematical approaches have been used to model and analyse the clearance of drugs by the liver and there are numerous papers on this subject [reviewed in 30]. None of these has been entirely accepted but probably the most successful has been the sinusoidal perfusion model proposed by Bass and his colleagues [27, reviewed in 31]. In this the liver is seen as a set of parallel tubes through which the blood flows. There is an obvious analogy between these tubes and the liver sinusoids. The mathematical treatment (see Results for a definition of terms) substitutes the logarithmic mean sinusoidal blood concentration (C) into the standard Michaelis–Menten equation to give $V = V_{max} \cdot C/(K_m + C)$. The V_{max} obtained should be exactly the same as the V_{max} obtained when metabolism is carried out by liver slices, but as there is a concentration gradient along the sinusoids, the concentration of substrate is measured as the logarithmic mean concentration (C). For this reason the K_m in this equation, the value of C at which there is half-maximal clearance, has a different value to the K_m of the underlying metabolizing enzyme measured using, for example, microsomes, although the two numbers are of the same order of magnitude. One test of the validity of this approach is that for any fixed value of C_{in} the value of C is independent of the flow rate [31]. This was not tested systematically but does seem to be the case for these experiments.

As would be expected the V_{max} for NDMA (11.2 nmol/g liver/min) calculated from the perfusion results is similar to the V_{max} (14.4 nmol/g liver/min) for NDMA metabolism by rat liver slices. However, the K_m values obtained from the perfusion (NDMA = 2.3 μM; NDEA = 10.6 μM) were much lower than the K_m obtained for metabolism by microsomes (15–35 μM) [32] or slices (25 μM) [20], but for NDEA the value of 27 μM (Fig. 2) or 20 μM [20] obtained with slices was slightly lower than the value of 40 μM obtained with microsomes [32]. Furthermore, whilst the K_m values for *metabolism* of NDMA and NDEA are similar, the K_m for NDMA from the *perfusion* is almost five times lower than that for NDEA. The apparent K_m for the metabolism of NDEA by liver slices was calculated from the Lineweaver–Burk plot shown in Fig. 2. Although there is a relatively large scatter in the data, the apparent K_m (27 μM) is close to the value of 20 μM obtained previously [20] and is sufficiently accurate for the purposes of this paper.

Bass and Keiding's analysis [31] gives an interesting insight into the effect that the difference in the values for K_m for the perfusion will have on the relationship between concentration and clearance. The clearance ratio $C_{in}/C_{out} = e^{(V_{max} - V)/FK_m}$. The expansion of e^x is $1 + x + x^2/2! + x^3/3! \ldots$ This will rapidly converge if $(V_{max} - V)/F \cdot K_m$ is <1 either

because $V_{max}/F \cdot K_m < 1$, or because V approaches V_{max}. In this case F is approximately 10^{-3} L/g/min, V_{max} is approximately 10^{-8} mol/g/min and the K_m either 2×10^{-6} for NDMA or 10^{-5} for NDEA. The maximum clearance will occur at very low input concentration when V is negligible in relation to V_{max} [i.e. $(V_{max} - V)/F \cdot K_m = V_{max}/F \cdot K_m$]. From the figures above $V_{max}/F \cdot K_m$ is approximately 5 in the case of NDMA, and 1 in the case of NDEA. If these figures applied *in vivo* the maximum clearance ratio (C_{in}/C_{out}) for NDMA would be e^5 (i.e. more than 99% would be cleared) but only e^1 in the case of NDEA (i.e. the maximum clearance would be only 63%). These figures illustrate that the dramatic difference which is seen *in vivo* and which has such an important consequence is the result of the fortuitous fact that for these nitrosamines $V_{max}/F \cdot K_m$ is approximately unity. Slight changes in V_{max}, K_m or F which changed $V_{max}/F \cdot K_m$ to a number greater than one will result in a high maximal clearance; any change which reduced this to below one will result in a low maximal clearance. An interesting example of this is the results of the previous study of NDMA clearance by the perfused liver [19]. These did not show the high clearance seen in our experiments and *in vivo*. The reason may be that the flow used then was about three times greater than the flow we used.

The difference in clearance of NDMA and NDEA therefore reflects the difference in the K_m for perfusion. In part this may reflect differences in diffusion and, in part, differences in distribution within the liver lobule of cells containing cytochromes P450 capable of metabolizing these nitrosamines. Expression of cytochrome P450 IIE1 which metabolizes NDMA [33–37] occurs almost exclusively in the perivenous zone in the rat, with a sharp transition towards the midzone [38, 39], in contrast to the enzymes metabolizing phenobarbital and 3-methylcholanthrene which are more evenly distributed between the perivenous and midzone [40]. NDEA is partly metabolized by P450 IIE1 and partly by other uncharacterized P450s. Heath [41] was the first to suggest that more than one enzyme is involved in NDEA metabolism after he had observed that although NDEA is a simple competitive inhibitor of NDMA metabolism in the rat, NDMA does not act as a simple competitive inhibitor of NDEA metabolism. Studies [32] on rat liver microsomes which show that cytochrome P450 IIE1 is responsible for more than 80% of the metabolism of micromolar concentrations of NDMA, but for only 50% of the metabolism of similar concentrations of NDEA, confirm Heath's view. Similar studies with human liver also show that cytochrome P450 IIE1 is not the only enzyme involved in NDEA metabolism [42]. The lobular distribution of the other P450s involved in NDEA metabolism is not known.

How do these calculations affect our confidence that we could predict the extent of first-pass clearance of a nitrosamine by human liver from knowledge of the K_m and V_{max} for their metabolism? If the V_{max} for metabolism of a nitrosamine by human liver was very much greater than those of NDMA and NDEA metabolism in the rat and/or the K_m measured on slices or microsomes much lower, one would be fairly confident that there would be a high degree of first-pass clearance. In fact, the K_m for human liver microsomes [42] and slices (20–24.5 μM) and the V_{max} for slices (10.2–13.4 nmol NDMA/g slices/min) are similar to those reported for rat liver slices (K_m = 25 μM, V_{max} = 14.4 nmol/g liver slices/min). The difference is insufficient to allow any reasonable prediction to be made from these numbers alone. The K_m and V_{max} for the human metabolism of other nitrosamines is unknown. It is possible that when the enzymes metabolizing nitrosamines have been better characterized and their distribution in the human liver is known, we will be able to predict the extent of clearance of nitrosamines by human liver. However, the experiments reported here show that at the present time it is not possible to do this.

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