Pharmacokinetic Model for N-Nitrosodimethylamine Based on Michaelis-Menten Constants Determined with the Isolated Perfused Rat Liver

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ABSTRACT

A pharmacokinetic model was constructed to describe the absorption, distribution, and metabolic clearance of N-nitrosodimethylamine. The model is composed of two compartments, total body water and the liver, which are linked by blood flow. Metabolic clearance is presumed to occur only in the liver. Liver clearance kinetics was determined with isolated perfused livers. Clearance appeared to obey Michaelis-Menten kinetics with \( K_m = 8.3 \pm 4.8 \mu M \) and \( V_{\max} = 0.15 \pm 0.02 \mu mol/min-liver. \) The observed value for \( K_m \) is about 1 order of magnitude lower than any observed when clearance is determined using liver microsome preparations. The model is used to calculate whole-body clearance of N-nitrosodimethylamine and relative tissue exposure as a function of the route of administration. The calculations are compared with previously published experimental data, and it is shown that the accuracy of the model for low doses is a result of the novel value observed for \( K_m \) in the perfused liver.

RESULTS

Rates of NDMA Clearance in Perfused Liver. In experiments using recirculating perfusion, the concentration of NDMA in the perfusate reservoir was determined at timed intervals. It is assumed that the entire system, which is composed of liver, reservoir, and perfusate circulating in the connecting tubing...
behave as a well-mixed homogenous system, that is, that no significant concentration gradients are established anywhere. This is certainly true in the perfusate reservoir, which is stirred continuously with a magnetic stirrer. As for the liver, data from open-circuit perfusions (discussed below), in which the actual concentration gradients across the liver were measured, support this assumption. It can be seen from Table 1 that, at concentrations greater than 10 to 20 μM, there no longer is a dramatic change as the perfusate passes through the liver. The initial concentrations used in the recirculating perfusions ranged from 9.5 to 690 μM, with all but 4 being greater than 20 μM. A total of 27 experiments were performed.

At high concentrations (>50 μM initial), the clearance of NDMA from the system appeared to obey zero-th-order kinetics; i.e., a linear plot of concentration versus time gave the best fit and directly provided the initial rate. At low concentrations (<20 μM initial), plotting log concentration versus time gave a better fit, in which case a first-order rate constant was determined and used to calculate the initial rate. Intermediate initial concentrations yielded data which showed a transition from zero-th- to first-order kinetics as NDMA was cleared from the system (Chart 1). In such cases, only the initial points were used to determine the rate.

The open-circuit perfusions require a different analytical approach, since the experimental arrangement does not include a perfusate reservoir. However, at the low concentrations of NDMA used in these experiments, a measurable and sometimes quite large decrease of NDMA concentration is observable between influent and effluent perfusate. From this decrease the rate of NDMA clearance can be calculated; it is simply the concentration difference, \( \Delta C \), multiplied by the volumetric flow rate, \( Q \), of the perfusate.

Eleven open-circuit perfusions were performed using influent concentrations of NDMA which ranged from 1.4 to 23 μM. In 4 experiments, several influent concentrations were used with the same liver, thereby generating a total of 20 rate determinations from the 11 perfusions. The influent and effluent concentrations and calculated rates from all the experiments are collected in Table 1.

**Michaelis-Menten Analysis of NDMA Clearance.** In order to derive a functional relationship between rates of disappearance of NDMA and concentration of NDMA, it is necessary to make some simplifying assumptions about the processes occurring in the liver which lead to clearance of NDMA. The first is that it is an enzymatic process and that the enzyme(s) obey Michaelis-Menten kinetics. A further assumption, which has already been made in order to obtain rates from the recirculating perfusions, is that, at any instant, all the metabolizing enzymes, regardless of location in the organ, are exposed to the same concentration of NDMA. These properties are characteristic of the CSTR familiar to chemical engineers, and it is this model of an organ as a reactor which has been most widely applied in pharmacokinetic modeling.

It can be argued that the CSTR is not the best model when there are large concentration differences between influent and effluent, such as we observe at very low NDMA concentrations. In this case, a plug-flow model, i.e., one in which equilibration of substrate concentrations occurs only in directions perpendicular to the flow through the organ, might be more appropriate. With this model, a concentration gradient is established from the inlet to the outlet of the reactor. A full discussion of the merits and applicability of these 2 models is, unfortunately, beyond the scope of this paper. We have examined each thoroughly, with respect to both kinetic analysis and pharmacokinetic modeling. Similar results are obtained from the 2 models. However, since the CSTR is simpler, both conceptually and computationally, and because it has been used far more extensively in pharmacokinetic modeling, we have chosen to adopt it here.

Given this model for the liver, it is clear that each measured rate obtained from open-circuit perfusions corresponds to the rate of clearance at a concentration equal to the effluent concen-
tation. Similarly, from the recirculating perfusions, we have a series of rate versus concentration data in which the concentration in the liver is well represented by that in the perfusate reservoir. The data from both types of perfusions may be combined for kinetic analysis, since the same model of the liver is applied to each.

Using the regression analysis of Wilkinson (25) for enzyme kinetics, we fitted the 47 pairs of rate versus concentration data to the function, \( V = V_{\text{max}} \cdot C/(K_m + C) \). The experimental and calculated values of clearance as a function of NDMA concentration are illustrated in Chart 2. \( V_{\text{max}} \) and \( K_m \) were found to be 0.15 ± 0.02 µmol/min-liver and 8.3 ± 4.8 µM, respectively. The errors correspond to 1 S.D.

Pharmacokinetics. In choosing how to model the pharmacokinetics of NDMA clearance, 3 factors appear to be of primary importance. These include the observations (11) that NDMA is distributed rapidly and uniformly throughout total body water. Thus, there need be no division of the body into distinguishable compartments save those which are responsible for metabolism. Second, the liver is clearly the predominant organ of metabolism. Although it is known that the kidneys and lungs, as well as some other tissues, also have the capacity to metabolize NDMA (2, 6, 7, 19), we can estimate this capacity as a small percentage of the total from the following observations. The level of alkylation of DNA (µmol 7-MeG per mol DNA) in the kidney is typically 10% of that in the liver after complete clearance of a dose of NDMA (7, 19). Since the weight of the liver is 4 times that of the combined weight of the kidneys, it might be expected that the rate of metabolism by the kidneys is only one-fourth of the rate by the liver. In the case of the lungs, \( V_{\text{max}} \) for lung microsomes has been shown to be only 10–3 of \( V_{\text{max}} \) for liver microsomes (6), and again the liver is approximately 4-fold larger in mass. Thus, although the lungs receive the total cardiac output, which is about 8 times the blood flow to the liver, their contribution to total body clearance remains small. We recognize the capacity of other organs to metabolize NDMA. However, we are initially concerned with relating measurements of NDMA to administered or fortuitous exposure. Thus, although extrahepatic organs are capable of clearing NDMA through metabolism, since they have little significant impact on total body clearance they are not included as separate compartments in our model. Finally, since urinary excretion of NDMA is less than 1% of the dose for any dose less than 30 mg/kg (400 µmol/kg) (13, 15), this pathway is unimportant. Biliary excretion is likewise negligible. These routes of removal of unchanged NDMA are thus omitted, leaving the focus entirely on metabolic clearance, which is dominated by the liver. Thus, in order to model total body water concentrations of NDMA, it is necessary only to use a single depot, total body water, connected to the liver by the blood flow between them.

The mathematical description of the mass balance of NDMA in this model following the absorption of a dose consists of 2 simultaneous differential equations:

\[
\frac{d C_{\text{bw}}}{dt} = Q \cdot C_L - Q \cdot C_{\text{bw}}
\]

\[
\frac{d C_L}{dt} = Q \cdot C_B - Q \cdot C_L - \frac{V_{\text{max}} \cdot C_L}{K_m + C_L}
\]

The volumes of the 2 compartments, total body water and the liver, are represented by \( C_{\text{bw}} \) and \( C_L \), and the concentrations of NDMA in these compartments by \( C_{\text{bw}} \) and \( C_L \), respectively. The blood flow rate between compartments is \( Q \). The term \( C_B \) represents the concentration of NDMA in the portal vein and is identical to \( C_{\text{bw}} \) during the postabsorption phase as well as during the absorption phase if NDMA is being introduced into the body at any site other than the gastrointestinal tract. However, if NDMA is absorbed from the gastrointestinal tract, the concentration entering the liver will be greater than \( C_{\text{bw}} \), since portal blood will contain NDMA absorbed from the gut. In this case, the combined flux of NDMA from total body water and from the gut are combined to yield \( C_B \), as follows.

\[
C_B = (C_{\text{bw}} \cdot Q + M \cdot g(t))/Q
\]

In Equation C, \( M \) represents the dose of NDMA, and \( g(t) \) is a function which describes the time course of absorption. If instead the input of NDMA is parenteral, \( C_B \) will be replaced with \( C_{\text{bw}} \) in Equation B, and the time-dependent term for NDMA input will be added to Equation A. For i.v. administration, a short pulse is appropriate; for absorption, an exponential function such as the following would be used (5).

\[
M \cdot g(t) = M \cdot \frac{1}{\tau} e^{-t/\tau}
\]

in which \( \tau \) is a constant with the units of time. This same function is applicable in Equation C. Note that \( t_B \) and \( \tau \) are not the same. The relationship between the 2 is \( t_B = -\tau \cdot \ln(1/2) \) and must be derived from integration of Equation D.

Total body water NDMA concentration curves were generated from Equations A and B using a Hewlett-Packard HP-85 computer and appropriate programs written for that purpose. The values used for \( K_m \) and \( V_{\text{max}} \) were those determined in this study, namely, \( K_m = 8.3 \) µM and \( V_{\text{max}} = 0.15 \) µmol/hr-liver. Blood flow to the liver was 6.5 ml/min based on reported cardiac output of 50 ml/min for a 200-g rat (3) and the fraction (13%) of that which the liver receives (21). For i.g. administration, a value of \( \tau = 5 \) min was chosen based on the reported half-life of 3 to 3.5 min for absorption of NDMA from small intestine (19).

Chart 3 illustrates the time dependency of NDMA concentration in TBW for both i.g. and i.v. administration of 10 mg/kg and 1000, 100, and 1 µg/kg. At doses in excess of about 1 mg/kg,
there is little difference between the 2 routes of administration. Also, the clearance is linear following the absorption phase until a concentration of about 100 $\mu$M is achieved. When doses below 1 mg/kg are administered, there is increasing difference between the maximum concentrations attained by the 2 different routes of administration, and the postabsorption phase is marked by nonlinear clearance.

The predicted total body water concentrations may be compared to measured blood or plasma clearance, since, according to this model, blood or plasma concentrations accurately reflect total body water concentrations. The model predicts linear clearance at concentrations greater than about 100 $\mu$M. Heath (10) observed a constant rate of decrease of NDMA concentration in the blood of rats given 49 mg/kg over 95 to 20% of the maximum concentration attained. Observations at lower concentrations were not reported. The value of 20% corresponds to approximately 200 $\mu$M. Similarly, Somogyi et al. (22) observed linear clearance over a plasma concentration range of 95 to 10 $\mu$g/ml (1300 to 140 $\mu$g/ml). The rates observed by these investigators are greater than those predicted by the present model, but each used animals different from those used as liver donors in the present work (female Porton, 165 to 185 g, and female Sprague-Dawley, 80 to 100 g, respectively). Rates at lower concentrations have not been accurately measured.

An alternative approach to evaluating the accuracy of the model is to calculate the ratios of extrahepatic tissue exposure resulting from i.v. and i.g. administration of NDMA, and compare the calculated to measured ratios. In this model, the total exposure of any tissue to NDMA at time $t$ is proportional to the integral of the total body water concentration versus time curve from 0 to $t$ (AUC). Experimental evidence of this type exists for kidney exposure (7, 19) in the form of kidney DNA methylation by NDMA. In Table 2, reported values have been collected for the ratio of 7-MeG in kidney DNA following i.v. administration to 7-MeG after i.g. administration. It was demonstrated in these studies that the amount of 7-MeG is proportional to the dose given i.v. and that it is stable over the time course of the experiments. Hence, 7-MeG is a reliable measure of kidney exposure. The increasing ratio at lower doses is an indication of the increasing efficiency of the liver to metabolize an i.g. dose.

The effect becomes pronounced at doses less than 500 $\mu$g/kg, eventually reducing exposure of extrahepatic tissues from an i.g. dose to less than 10% of that resulting from an i.v. dose. Also in Table 2 are values for the ratio of AUC$_{iv}$ to AUC$_{ig}$. These have been calculated using the mean values determined by us for $V_{max}$ and $K_m$ as well as the mean values ± S.D. It may be noted from a comparison of these calculated ratios to those observed in experimental animals that, within the experimental error, the perfused liver kinetic constants combined with a simple pharmacokinetic model do a creditable job of predicting extrahepatic tissue exposure. What error there is seems to result from overestimating $K_m$. Alternatively, it may be that the CSTR model for the liver is too conservative.

There is also experimental evidence concerning liver exposure which is based on measurement of 7-MeG levels following administration of NDMA (19). In this case, the data consist of 7-MeG levels as a function of time, and it was found that the level at 15 min postdosing (i.g.) was already at least 85% of the final value for doses of 5 to 100 $\mu$g/kg. For comparison, we have calculated the AUCs for doses of 1, 10, and 100 $\mu$g/kg at 15 min and at 4 hr. The ratio of the AUCs at the 2 different time points is the result to be used for comparison. The AUCs were calculated using $C_t$, not $C_{bw}$, since $C_t$ is the concentration to which the liver is exposed.

The ratio of the AUC for the liver at the 2 different times is highly dependent on the value of $K_m$. For any particular $K_m$ and $V_{max}$, the ratios are the same at all 3 doses. When $K_m = 8.3$ $\mu$M, the ratio of the AUCs for the liver at 15 min and 4 hr ranges from 0.75 to 0.80. If $K_m = 13.1$ $\mu$M (mean + 1 S.D.), then the ratios are between 0.67 and 0.72. However, if $K_m = 3.5$ $\mu$M (mean − 1 S.D.), then the ratios become 0.86 to 0.88. This last range of values is indeed greater than 0.85 as is observed experimentally. Again, it is suggested that either the perfused liver kinetic constants overestimate $K_m$ or that we are applying a too conservative model of the liver.

**DISCUSSION**

It is experimentally well established that the clearance of NDMA in the rat is dominated by metabolism in the liver (18). Other organs contribute to metabolic clearance, but to a much lesser extent. Excretion of unaltered NDMA is nominal (13, 15). Thus, the accuracy of any pharmacokinetic model is highly dependent on the correctness of assumptions about rates of metabolism in the liver.

The present work began with attempts to construct a phar-
macokinetic model for clearance based on reported kinetic data for the P-450 systems isolated from rat liver. The microsomal oxidation systems have repeatedly displayed the characteristic of having 2 kinetic entities, one with \( K_m = 50 \) to 400 \( \mu M \) and the other with \( K_m = 50 \) to 100 nm (1, 23). The maximum velocities have been expressed variously but appear to correspond to approximately 15 nmol/min-g whole liver. Application of these values in the present model fails to predict accurately the behavior of doses of NDMA below 5 mg/kg, although at higher doses they are satisfactory. The perfused liver clearance kinetics, in contrast, may be used to make predictions which agree well with the available experimental evidence.

The origin of the difference observed for \( K_m \) in the perfused-liver and in microsomes is unclear. One possibility is that a structural or functional perturbation of the cytochrome occurs during the preparation of microsomes which alters the kinetics of metabolism. On the other hand, it is possible that a cytosolic enzyme exists which has a very low \( K_m \). Such an enzyme would during the preparation of microsomes which alters the kinetics of metabolism. On the other hand, it is possible that a cytosolic enzyme exists which has a very low \( K_m \). Such an enzyme would be overlooked in studies on metabolism by S-9 fractions, since these have rarely explored the low \( \mu M \) concentration range. Indeed, there is one report strongly suggesting that there is such an enzyme with \( K_m = 5 \mu M \) (14).

Finally, we wish to emphasize particularly the value of accurate liver clearance kinetics because of their importance to the understanding of the disposition of low doses, i.e., those to which humans might be exposed, as a function of the route of administration. Exposure via the gastrointestinal tract leads to, for all practical purposes, liver exposure only. Administration i.v. exposes all tissues. (Other parenteral routes will also expose all tissues if there is no significant first-pass effect at the site of administration.) This cannot be predicted using microsomal kinetics. Furthermore, it may be that the liver is better equipped than other organs with repair mechanisms and that therefore the tremendous "first-pass effect" constitutes an efficient protective mechanism. However, such protection may easily be vitiated by the simultaneous consumption of a potent enzyme inhibitor such as ethanol (20). If metabolism in the liver is blocked, then p.o. administration as well as administration by other routes leads to the same uniform distribution of NDMA before significant clearance occurs. All tissues which are metabolically competent to activate NDMA are then potentially susceptible to its carcinogenic action. The experimental findings that concurrent p.o. administration of NDMA and ethanol leads to oafactory neuro-epithelium in C57BL mice (9) may well be a manifestation of this phenomenon.

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