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KINETICS OF CONJUGATION AND OXIDATION OF NITROBENZYL ALCOHOLS BY RAT HEPATIC ENZYMES

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Abstract—Previous work has suggested that quantitative differences in the *in vitro* and *in vivo* metabolism of mononitrotoluene isomers are a result of differences in the hepatic conjugation and oxidation of the first metabolic intermediates, the mononitrobenzyl alcohols. We have determined the steady-state kinetic parameters, V_{max}, K_m and V/K, for the metabolism of the nitrobenzyl alcohols by rat hepatic alcohol dehydrogenase, glucuronyltransferase, and sulfotransferase. 3-Nitrobenzyl alcohol was the best substrate for cytosolic alcohol dehydrogenase (V_{max} = 1.48 nmoles/min/mg protein, V/K = 3.15×10^{-3} nmoles/min/mg protein/μM, K_m = 503 μM). V_{max} and K_m values for 4-nitrobenzyl alcohol were similar, but V/K was about 60% of that for 3-nitrobenzyl alcohol. 2-Nitrobenzyl alcohol was not metabolized by the alcohol dehydrogenase preparation used here, but it was metabolized to 2-nitrobenzoic acid by a rat liver mitochondrial preparation. 2-Nitrobenzyl alcohol was the best substrate for microsomal glucuronyltransferase (V_{max} = 3.59 nmoles/min/mg protein, $V/K = 11.28 \times 10^{-3}$ nmoles/min/mg protein/μM, K_m = 373 μM). The V_{max} for 3-nitrobenzyl alcohol was similar, but the V/K was about half and the K_m was about twice that for 2-nitrobenzyl alcohol. The V_{max} for 4-nitrobenzyl alcohol was about 40% and the V/K was about half that for 2-nitrobenzyl alcohol. The best substrate for cytosolic sulfotransferase was 4-nitrobenzyl alcohol (V_{max} = 1.69 nmoles/min/mg protein, $V/K = 37.21 \times 10^{-3}$ nmoles/min/mg protein/μM, K_m = 48 μM). The V_{max} values for the other two benzyl alcohols were similar, but the V/K and K_m values were about 11 and 400%, respectively, of those for 4-nitrobenzyl alcohol. These data are in qualitative agreement with results obtained when the nitrobenzyl alcohols were incubated with isolated hepatocytes, but they do not allow quantitative modeling of the data from hepatocytes.

The mononitrotoluenes are used in the synthesis of azo dyes, sulfur dyes, rubber chemicals and agricultural chemicals [1]. 2-Nitrotoluene is active in the *in vivo–in vitro* rat hepatocyte unscheduled DNA repair assay for genotoxicity, while 3- and 4-nitrotoluene are not [2]. When the mononitrotoluenes are incubated with rat isolated hepatocytes, each is oxidized to the corresponding nitrobenzyl alcohol in a cytochrome P-450-dependent process. The nitrobenzyl alcohols are then further metabolized in an isomer-specific fashion. 2-Nitrobenzyl alcohol is primarily conjugated with glucuronic acid. 3-Nitrobenzyl alcohol is primarily oxidized further to 3-nitrobenzoic acid, and 4-nitrobenzyl alcohol is converted to *S*-(4-nitrobenzyl)glutathione through the intermediate, 4-nitrobenzyl sulfate [3, 4].

The various pathways followed by the isomeric nitrobenzyl alcohols may have toxicological consequences, since 2-nitrotoluene, but not 3- or 4-nitrotoluene, has been shown to bind to rat hepatic DNA [5]. Previous work has suggested that both 2-nitrotoluene and the hepatocarcinogen 2,6-dinitrotoluene are activated via similar pathways. The methyl group is oxidized to an alcohol [3, 6], and the alcohol is conjugated with glucuronic acid and excreted in the bile [7, 8]. Intestinal microflora hydrolyze the glucuronide and reduce a nitro group to yield an aminobenzyl or an aminonitrobenzyl alcohol which is reabsorbed and N-hydroxylated. Presumably, the N-hydroxy group is conjugated with sulfate to yield an unstable sulfate ester which decomposes to an electrophilic nitrenium (or carbonium) ion capable of covalently binding to DNA ([5, 9, 10]; Kedderis and Rickert, manuscript submitted for publication). Thus, in the context of binding to hepatic macromolecules, conjugation of the benzyl alcohol with glucuronic acid can be considered to lead to toxic products, while oxidation to benzoic acid or conjugation with sulfate can be considered detoxication pathways. To better understand the structural requirements for each of these pathways, the kinetic parameters for the metabolism of the isomeric nitrobenzyl alcohols by rat hepatic UDP-glucuronyltransferase (EC 2.4.1.17), sulfotransferase (EC 2.8.2.1), and alcohol dehydrogenase (EC 1.1.1.1) were determined. The results are in qualitative agreement with, but do not quantitatively reflect, results in rat isolated hepatocytes.

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MATERIALS AND METHODS

Animals. Male Fischer 344 rats [CDF(F-344)/CrlBR] weighing 200–250 g were purchased from Charles River Breeding Laboratories (Kingston, NY) and fed pelleted NIH-07 rodent chow (Zeigler Bros., Gardener, PA) and purified water *ad lib.* Animals were housed in mass air displacement temperature- and humidity-controlled rooms (22 ± 1° and 60 ± 15% respectively). The lighting was controlled so that the lights were on from 7:00 a.m. to 7:00 p.m. daily. Animals were allowed to acclimate for at least 2 weeks prior to use in experiments. Sentinel animals were screened weekly for viral infection (Standard Rat Screen, Microbiological Associates Inc., Bethesda, MD) and were negative throughout the studies described here.

Chemicals. [U-ring ^{14}C]-2-, 3- and 4-Nitrotoluene (sp. act. 52.5, 55.8 and 52.5 mCi/mmole, respectively) were purchased from the Midwest Research Institute (Kansas City, MO). High performance liquid chromatographic (HPLC) analysis showed the mononitrotoluenes to be >99% radiochemically pure. $Na_2{}^{35}SO_4$ was purchased from the New England Nuclear Corp. (Boston, MA). The isomeric nitrobenzyl alcohols were purchased from the Aldrich Chemical Co. (Milwaukee, WI). UDPGA, ATP, and NAD were from the Sigma Chemical Co. (St. Louis, MO).

Preparation of ^{14}C-nitrobenzyl alcohols. Radiolabeled 2-, 3- or 4-nitrotoluene was incubated with rat hepatic microsomes and an NADPH-generating system (containing 2.5 μM glucose-6-phosphate, 1.0 unit/ml glucose-6-phosphate dehydrogenase, and 2.5 μM each NADP and NADPH) for 4–6 hr; a fresh portion of cofactors was then added and the incubation was allowed to proceed overnight. Diethyl ether extracts of these incubations were evaporated to dryness; the residue was redissolved in methanol and applied to two reverse phase HPLC columns (30 cm Hibar II LiChrosorb RP-18, E. Merck, Darmstadt, FRG) connected in series. The alcohols were eluted using a 20-min linear gradient from 20 to 90% methanol in 0.005 M phosphate buffer, pH 7.4. Fractions containing the alcohols were pooled and extracted with diethyl ether. The extracts were evaporated to dryness, and the purified alcohols were redissolved in sufficient dimethylformamide to yield approximately 200,000 dpm/5 μl.

Enzyme preparation. Rats were anesthetized with methoxyflurane (Pittman-Moore, Inc., Washington Crossing, NJ). The liver was exposed via a midline incision and a catheter was placed in the portal vein. The vena cava was cut, and the liver was perfused with isotonic KCl, removed, minced and homogenized in Tris-buffered (0.005 M, pH 7.4) isotonic KCl. Cytosol and washed microsomes were prepared by differential centrifugation [11, 12] and stored at −80° until used for sulfotransferase and UDP-glucuronyltransferase assays respectively. Cytosol for use in alcohol dehydrogenase assays was prepared as above except that the livers were minced and homogenized in 0.05 M Tris buffer, pH 8.5, containing 330 μM dithiotheitol [13].

Rat liver mitochondria were isolated by differential centrifugation [14] and resuspended in 2 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer, pH 7.4, containing 222 mM mannitol, 70 mM sucrose and 1 mM Na EDTA [15] to yield mitochondria from 0.25 g liver in 1.0 ml.

Metabolism of nitrobenzyl alcohols by rat isolated hepatocytes. Hepatocytes were isolated as previously described [3] and incubated with each nitrobenzyl alcohol at a concentration of 50 μM for 15 min at 37°. The incubation mixtures contained 4.38×10^6 hepatocytes in a total volume of 2.5 ml Krebs bicarbonate buffer, pH 7.4. At the end of the incubation period 2.5 ml methanol was added, the samples were centrifuged, and an aliquot was analyzed by HPLC. Metabolites were separated on two LiChrosorb RP-18 columns connected in series. The flow rate was 2 ml/min, and a 20-min linear gradient from 20 to 90% methanol in 0.005 M phosphate buffer was used. Fractions (1 ml) were collected, and radioactivity was determined by liquid scintillation spectrophotometry.

Assay for alcohol dehydrogenase activity. The radiolabeled nitrobenzyl alcohols were incubated with 5 mg of rat hepatic cytosolic protein in 0.3 M Tris buffer, pH 7.8, containing NAD (0.8 mM), magnesium chloride (24 mM) and dithiothreitol (0.15 mM) for 20 min at 37°. The final incubation volume was 1.25 ml. Preliminary experiments demonstrated that these conditions were optimal for alcohol dehydrogenase activity toward 3-nitrobenzyl alcohol. The intermediate aldehydes were not detected in incubations with any of the nitrobenzyl alcohols, so the appearance of the nitrobenzoic acids was taken as an accurate reflection of the oxidation of the nitrobenzyl alcohols. At the end of the incubation period 1.25 ml methanol was added, the samples were centrifuged, and 250 μl of the supernatant fraction was applied to two reverse phase HPLC columns (see above) connected in series. The nitrobenzoic acids were eluted with 70% methanol in 0.005 M phosphate buffer, pH 7.4, at a flow rate of 2 ml/min. Fractions (0.4 ml) were collected and assayed for radioactivity by liquid scintillation spectrophotometry. When mitochondria were used as the source for alcohol dehydrogenase activity, the buffer used for incubations was the same as that used for the isolation procedure described above, and a single substrate concentration of 500 μM was investigated.

Assay for UDP-glucuronyltransferase activity. Radiolabeled nitrobenzyl alcohols were incubated with 4 mg of rat hepatic microsomal protein in 66 mM Tris buffer, pH 7.8, containing magnesium chloride (4.5 mM), UDPGA (3.0 mM) and a final concentration of 0.1% Brij 58 for 5 min at 37°. Total volume was 550 μl. Preliminary experiments indicated that these conditions were optimal for UDP-glucuronyltransferase activity toward 2-nitrobenzyl alcohol. The reactions were stopped by the addition of 500 μl methanol. The samples were centrifuged, and 250 μl of the supernatant fraction was applied to two reverse phase HPLC columns (see above) connected in series. The nitrobenzyl glucuronides were eluted with 50% methanol in 0.005 M phosphate buffer, pH 7.4, at a flow rate of 2.00 ml/min. One-ml fractions were collected and assayed for radioactivity by liquid scintillation spectrophotom-

etry. The identity of the nitrobenzyl glucuronides was confirmed in preliminary experiments by hydrolysis of the putative glucuronide with β-glucuronidase to regenerate the nitrobenzyl alcohol.

Assay for sulfotransferase activity. Radiolabeled adenosine 3′-phosphate 5′-phosphosulfate (PAPS) was generated by preincubating 5.0 mg of rat hepatic cytosolic protein with 10.0 μmoles ATP, 6.0 μmoles magnesium chloride, and 10 μmoles $Na_2{}^{35}SO_4$ in 66 mM Tris buffer, pH 7.4 (total volume, 1.89 ml), for 30 min at 37°. At the end of the preincubation, EDTA (20 μmoles) was added followed by unlabeled nitrobenzyl alcohol. The incubation was carried out for 15 min and was stopped by addition of 2.0 ml methanol. Samples were centrifuged, and 250 μl of the supernatant fraction was applied to two reverse phase HPLC columns connected in series (see above). The nitrobenzyl sulfates were eluted by a 10-min linear gradient from 10 to 90% methanol in 0.005 M phosphate buffer, pH 7.4, at a flow rate of 2.0 ml/min. One-ml fractions were collected and assayed for radioactivity by liquid scintillation spectrophotometry. Preliminary experiments indicated that these conditions were optimal for sulfotransferase activity toward 4-nitrobenzyl alcohol.

Determination of kinetic parameters. The above assays were done with substrate concentrations ranging from 25 to 2000 μM for alcohol dehydrogenase, from 25 to 1000 μM for sulfotransferase, and from 10 to 1500 μM for glucuronyltransferase. The data were plotted as v (nmoles product formed/min/mg protein) versus substrate concentration $[S]$ (μM) and fit by computer to the following equations:

$$v = \frac{V_{max} \cdot (V/K) \cdot [S]}{V_{max} + \{(V/K) \cdot [S]\}} \quad (1)$$

$$v = \frac{V_{max} \cdot [S]}{K_m + [S]} \quad (2)$$

where V_{max} is the velocity of the reaction at infinite substrate concentration and V/K is the second-order rate constant for binding and catalysis [16, 17].

RESULTS

Metabolism by rat hepatocytes. Experiments in which the radiolabeled nitrobenzyl alcohols (50 μM) were incubated with freshly isolated rat hepatocytes yielded 2.5 times as much 2-nitrobenzyl glucuronide as 2-nitrobenzoic acid from 2-nitrobenzyl alcohol (Table 1). 3-Nitrobenzyl alcohol was also metabolized to a glucuronide and a nitrobenzoic acid, but nearly 7 times as much acid as glucuronide was formed. 4-Nitrobenzyl alcohol was metabolized to four products, 4-nitrobenzyl glucuronide, 4-nitrobenzyl sulfate, *S*-(4-nitrobenzyl)glutathione and 4-nitrobenzoic acid, in a ratio of 6:3:2:0.1. These are the same metabolites which were observed when each of the mononitrotoluene isomers was metabolized by isolated hepatocytes [3].

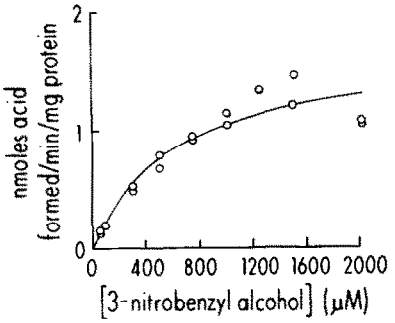

Fig. 1. Typical v versus $[S]$ plot for alcohol dehydrogenase activity when 3-nitrobenzyl alcohol was used as substrate. Open circles represent data points. The computer fit of Equation 1 (see Materials and Methods) is given by the solid line.

Metabolism by alcohol dehydrogenase. Results from a typical experiment using 3-nitrobenzyl alcohol as a substrate for rat hepatic alcohol dehydrogenase are shown in Fig. 1. The V_{max} for alcohol dehydrogenase with 3-nitrobenzyl alcohol as substrate was somewhat higher than that of 4-nitrobenzyl alcohol (Table 2), while the V/K was nearly twice that for 4-nitrobenzyl alcohol. The K_m values for the two substrates were similar. 2-Nitrobenzoic acid was not detected when 2-nitrobenzyl alcohol was incubated with this preparation of rat hepatic alcohol dehydrogenase. To determine whether 2-nitrobenzyl alcohol was capable of binding to the active site of alcohol dehydrogenase, the metabolism of 3-nitrobenzyl alcohol was studied in the presence of 2-nitrobenzyl alcohol. No inhibition of 3-nitrobenzyl alcohol metabolism by 2-nitrobenzyl alcohol was observed even at a concentration twice that of 3-nitrobenzyl alcohol (Fig. 2). To determine whether non-cytosolic alcohol dehydrogenase activity was responsible for the formation of 2-nitrobenzoic acid from 2-nitrobenzyl alcohol in isolated hepatocytes, the nitrobenzyl alcohols were incubated with rat liver micro-

Table 1. Metabolism of nitrobenzyl alcohols by rat hepatocytes

	Metabolite formed			
Substrate	Nitrobenzoic acid	Nitrobenzyl glucuronide	Nitrobenzyl sulfate	Nitrobenzyl glutathione
2-NBAlc	1.4	3.5	ND*	ND
3-NBAlc	18.8	2.8	ND	ND
4-NBAlc	0.3	14.1	6.7	4.5

Values are nmoles formed/30 min/4.38 × 10^6 cells for a typical experiment.
* ND = none detected.

Table 2. Metabolism of nitrobenzyl alcohols by rat hepatic alcohol dehydrogenase

Substrate	V_{max} (nmoles/min/mg)	V/K ($10^3 \cdot$ nmoles/min/mg/μM)	K_m (μM)
2-NBAlc	NM*	NM	NM
3-NBAlc	1.48 ± 0.26	3.15 ± 0.38	503 ± 130
4-NBAlc	1.09 ± 0.13	1.87 ± 0.54	695 ± 138

Values are means (±S.E.) of four to six experiments.
* NM = no metabolism observed.

somes or mitochondria. Oxidation of the nitrobenzyl alcohols by microsomes was not observed in the presence of NAD, NADP, NADH, or NADPH. With rat hepatic mitochondria more 2- and 4-nitrobenzoic acid (14.2 ± 4.3 and 14.2 ± 2.9 nmoles/20 min/g liver respectively) than 3-nitrobenzoic acid (4.6 ± 1.3 nmoles/20 min/g liver) were formed.

Metabolism by UDP-glucuronyltransferase. All three nitrobenzyl alcohols were conjugated with glucuronic acid by rat hepatic microsomes. Figure 3 shows results of a typical experiment with 2-nitrobenzyl alcohol as the substrate. V_{max} values for 2- and 3-nitrobenzyl alcohol were similar and about 2.5 times that for 4-nitrobenzyl alcohol (Table 3). V/K values for 3- and 4-nitrobenzyl alcohol were about half that for 2-nitrobenzyl alcohol. K_m was the smallest for 4-nitrobenzyl alcohol and the largest for 3-nitrobenzyl alcohol.

Metabolism by sulfotransferase. Figure 4 shows the results of a typical experiment with 4-nitrobenzyl alcohol as substrate for sulfotransferase. There were no large differences between V_{max} values for the three nitrobenzyl alcohols, but those for 2- and 4-nitrobenzyl alcohol were somewhat higher than that for 3-nitrobenzyl alcohol (Table 4). V/K was nearly 10 times higher for 4-nitrobenzyl alcohol than for the other two alcohols. The K_m values for 2- and 3-nitrobenzyl alcohol were 5–6 times higher than that for 4-nitrobenzyl alcohol.

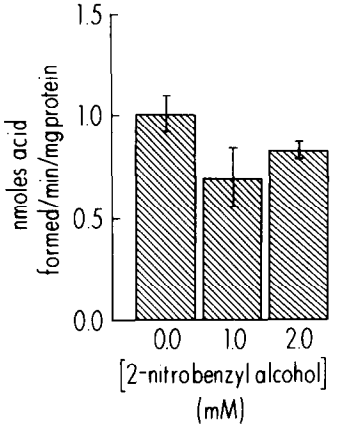

Fig. 2. Effect of addition of 2-nitrobenzyl alcohol to incubations of 3-nitrobenzyl alcohol with cytosolic alcohol dehydrogenase. The height of each bar represents the mean (±S.E.M.) for three determinations.

DISCUSSION

Previous work has demonstrated that isolated rat hepatocytes transform the mononitrotoluenes to the nitrobenzyl alcohols, and that further metabolism of the alcohols follows different pathways depending on the position of the nitro group. The results presented here on the metabolism of the nitrobenzyl alcohols are in substantial agreement with those earlier studies, but some quantitative differences appear to exist. Whereas the ratio of 2-nitrobenzyl glucuronide to 2-nitrobenzoic acid was 2 to 1 in this study, nearly 10 times as much glucuronide as acid was formed when 2-nitrotoluene was incubated with hepatocytes [3]. Similarly, the ratio of 3-nitrobenzoic acid to 3-nitrobenzyl glucuronide was 7 to 1 in this study but only about 4 to 1 when 3-nitrotoluene was incubated with isolated hepatocytes [3]. Additionally, 58% of the 4-nitrobenzyl alcohol metabolized by isolated hepatocytes was converted to 4-nitrobenzyl glucuronide, but only about 2% of the 4-nitrotoluene metabolized by isolated hepatocytes was converted to the glucuronide. The reasons for these quantitative differences in the metabolism of nitrotoluenes and the nitrobenzyl alcohols are not known, but it is likely that different concentrations of nitrobenzyl alcohol are present in hepatocytes depending on whether the alcohol is added or formed from a nitrotoluene precursor.

The kinetic parameters determined here are consistent with the different pathways followed by each nitrobenzyl alcohol. The best substrate for alcohol dehydrogenase was 3-nitrobenzyl alcohol. 2-Nitrobenzyl alcohol was the best substrate for glu-

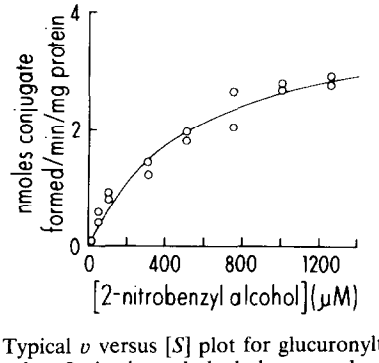

Fig. 3. Typical v versus $[S]$ plot for glucuronyltransferase activity when 2-nitrobenzyl alcohol was used as substrate. Open circles represent data points. The computer fit of Equation 1 (see Materials and Methods) is given by the solid line.

Table 3. Metabolism of nitrobenzyl alcohols by rat hepatic glucuronyltransferase

Substrate	V_{max} (nmoles/min/mg)	V/K ($10^3 \cdot$ nmoles/min/mg/μM)	K_m (μM)
2-NBAlc	3.59 ± 0.30	11.28 ± 3.08	373 ± 102
3-NBAlc	3.79 ± 0.62	5.25 ± 0.23	732 ± 137
4-NBAlc	1.56 ± 0.20	6.00 ± 0.76	262 ± 22

Values are means (±S.E.) of four to six experiments.

Table 4. Metabolism of nitrobenzyl alcohols by rat hepatic sulfotransferase

Substrate	V_{max} (nmoles/min/mg)	V/K ($10^3 \cdot$ nmoles/min/mg/μM)	K_m (μM)
2-NBAlc	1.66 ± 0.07	4.87 ± 0.10	340 ± 13
3-NBAlc	1.14 ± 0.14	4.23 ± 0.13	273 ± 40
4-NBAlc	1.69 ± 0.09	37.21 ± 6.09	48 ± 8

Values are means (±S.E.) of four to six experiments.

curonyltransferase, and 4-nitrobenzyl alcohol was the best substrate for sulfotransferase. In an attempt to better characterize the isomer specificity of each of the enzymes studied, we have utilized the kinetic analysis of structure–activity relationships between alternative substrates developed by Williams and Northrop [18] for gentamicin acetyltransferase I. The analysis requires a knowledge of the kinetic mechanism of the enzyme and uses the kinetically independent [16, 17] rate constants V_{max} and V/K to distinguish between changes in binding or catalysis. UDP-glucuronyltransferase [19] and sulfotransferase [20] both exhibit random kinetic mechanisms. Derivation of expressions for V_{max} and V/K in terms of rate constants according to the net rate constant method [21] and inspection of these expressions for a random kinetic mechanism revealed that V_{max} is a function of the rates of catalysis and product release, while V/K is a function of the rates of catalysis, product release and substrate binding. Thus, decreases in both V_{max} and V/K reflect a decrease in the rate of catalysis (or product release); a decrease in V/K with an increase in V_{max} reflects a decrease in substrate binding [18].

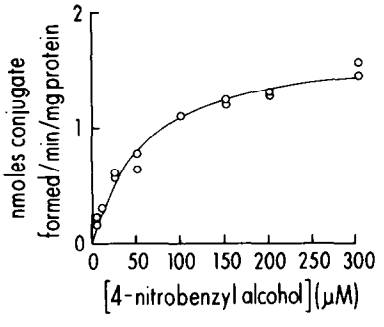

Fig. 4. Typical v verus [S] plot for sulfotransferase activity when 4-nitrobenzyl alcohol was used as substrate. Open circles represent data points. The computer fit of Equation 1 (see Materials and Methods) is given by the solid line.

Compared to 2-nitrobenzyl alcohol, the best substrate for UDP-glucuronyltransferase, 3-nitrobenzyl alcohol exhibited the same V_{max} but a lower V/K. 4-Nitrobenzyl alcohol exhibited decreases in both parameters. Following the reasoning outlined above, decreased binding of 3-nitrobenzyl alcohol is responsible for its decreased metabolism, while decreased catalysis is responsible for the decreased metabolism of 4-nitrobenzyl alcohol. Not enough is known about the active site of UDP-glucuronyltransferase to allow conclusions regarding the chemical reasons for these results. It is possible, however, that a nitro group *ortho* to the benzyl alcohol group assists in anchoring the substrate in the active site; a *meta* nitro group may sterically hinder substrate binding. The *para* nitro group may not hinder binding but may allow the substrate to "wobble" in the active site, leading to less efficient catalysis.

4-Nitrobenzyl alcohol was the best substrate for sulfotransferase. In comparison, 2-nitrobenzyl alcohol exhibited the same V_{max} but a markedly decreased V/K. 3-Nitrobenzyl alcohol exhibited decreases in both parameters. These observations suggest that the decreased metabolism of 2-nitrobenzyl alcohol is a consequence of decreased substrate binding. According to the analysis outlined above, the decreased metabolism of 3-nitrobenzyl alcohol by sulfotransferase should be due to decreased catalysis. However, the K_m values for both 2- and 3-nitrobenzyl alcohol are about 7 times greater than the K_m for 4-nitrobenzyl alcohol. If the mechanism for sulfotransferase metabolism of these substrates is truly rapid equilibrium random, then K_m is simply a binding constant. Thus, the similar decreases in V/K and increases in K_m for 2- and 3-nitrobenzyl alcohol suggest that the decreased metabolism of 3-nitrobenzyl alcohol is due to decreased substrate binding.

The alcohol dehydrogenases have an ordered bi-bi kinetic mechanism, with the nucleotide substrate adding first [22, 23]. Derivation of expressions for the kinetic parameters of this mechanism using the net rate constant method reveals that V_{max} is a func-

tion of catalysis and product release (both products), while V/K reflects catalysis, produce release (first product only) and substrate binding. Thus, a simple analysis of isomer-dependent changes in V_{max} and V/K is not possible for alcohol dehydrogenase.

Both 2-nitrotoluene and 2-nitrobenzyl alcohol are metabolized to 2-nitrobenzoic acid in isolated hepatocytes, but 2-nitrobenzyl alcohol was not a substrate for cytosolic alcohol dehydrogenase and did not inhibit 3-nitrobenzyl alcohol metabolism by that enzyme. Therefore, we investigated the metabolism of 2-nitrobenzyl alcohol by rat liver microsomes and mitochondria. Microsomal preparations failed to convert any of the nitrobenzyl alcohols to the corresponding nitrobenzoic acids but oxidation of all three isomeric nitrobenzyl alcohols did occur in isolated mitochondria. An extensive literature search revealed few studies on the metabolism of aralkyl alcohols by mitochondrial preparations. 4-(3-Morpholinopropyl)benzyl alcohol is metabolized to the corresponding benzoic acid by preparations of rat liver mitochondria [24], and there have been two reports describing the histological localization of alcohol dehydrogenase activity in mitochondrial membranes [25, 26]. The presence of this type of activity in mitochondria deserves further characterization.

Attempts to use the kinetic parameters determined here to model the metabolism of either the nitrotoluenes or the nitrobenzyl alcohols in isolated hepatocytes failed. The models yielded rates of formation of metabolites which were much more similar than were actually found in hepatocytes. Nevertheless, inspection of the parameters shows that they qualitatively predict that glucuronidation is the preferred pathway for metabolism of 2-nitrobenzyl alcohol, that sulfation is the preferred pathway for metabolism of 4-nitrobenzyl alcohol, and that oxidation to nitrobenzoic acid is the preferred pathway for 3-nitrobenzyl alcohol. Since glucuronidation with subsequent excretion in the bile is essential to the eventual formation of metabolites of 2-nitrotoluene which covalently bind to rat hepatic macromolecules [7], a study comparing the metabolism of the mononitrobenzyl alcohols by human hepatic microsomes and cytosol may be useful to the extrapolation of toxicity data in rats to humans.

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