Interactions between drugs and chemicals in industrial societies.
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ENZYME INDUCTION BY ENVIRONMENTAL AGENTS: EFFECT ON DRUG KINETICS

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INTRODUCTION

In humans, the rate of biotransformation of xenobiotics is closely related to both an individual's daily habits and environmental factors. When a particular factor increases the rate of biotransformation, that is, acts as an enzyme inducer, the turnover of endogenous substances will be altered and the clearance of xenobiotics may increase. This would enhance production of toxic metabolites or, in the case of drugs, reduce the desired clinical effect if the dose is not adjusted.

There is evidence suggesting that regular physical activity enhances hepatic metabolism of such low clearance drugs as aminopyrine and antipyrine (1). Nutrition (or malnutrition) can also influence hepatic metabolism: moderate malnutrition induces the biotransformation of xenobiotics while severe malnutrition depresses it (2). On the opposite end of the scale, a high protein, low carbohydrate diet appears to increase the clearance of antipyrine and theophylline (3,4). Cruciferous vegetables, such as brussel sprouts, cabbage, turnips and cauliflower, have enzyme-inducing properties, which are attributable to their indole content (5).

The manner in which food is cooked clearly influences drug metabolism. For example, charcoal-broiled beef may contain as much as 8 μg of benzopyrene per kilogram of beef as well as other polycyclic aromatic hydrocarbons (6). These substances significantly increase the clearance of antipyrine and theophylline (7,8).

Chronic administration of alcohol to animals and man produces a proliferation of the smooth endoplasmic reticulum and increases the activity of the cytochrome P-450 isozymes, as evidenced by an increase in the clearance of several commonly used drugs such as phenytoin, warfarin, tolbutamide, meprobamate and antipyrine (9).

The effects of smoking on the rate of metabolism of xenobiotics have been clearly established in humans. The polycyclic aromatic hydrocarbons, nicotine, cadmium and certain pesticides contained in the tobacco act as potent enzyme inducers (10,11). The influence of smoking on drug kinetics is considerable. For example, due to an increase in theophylline clearance

the area under the theophylline plasma concentration - time curve (AUC) decreased by 40% in individuals smoking over 20 cigarettes per day (12). Such individuals would therefore require higher daily doses of theophylline. Also in smokers, propranolol clearance was reported increased, while plasma concentrations and bioavailability decreased (13). Cigarette smoking has also been related to a decrease in the antianginal effects of nifedipine, propranolol and atenolol (14).

In the modern industrialized world, hardly anyone can escape the exposure to potent enzyme inducers. For example, the polyhalogenated cyclic hydrocarbons are widely used. Chlorinated organic chemicals, such as the highly toxic 2,3,7,8-tetrachlorodibenzeno-dioxin, are well known inducers of the mixed function oxidase system (MFOS) in animals and man (15,16). Fungicides, such as hexachlorobenzene (17) and pentachlorophenol (18), are also effective enzyme inducers. Various herbicides, including 2,4-dichloro- or 2,4,5-trichlorophenoxyacetic acid, induce the activity of some very specific enzymes located in the peroximes (19). Halobenzenes (20), polychlorinated and polybrominated biphenyls (21,22), naphthalenes and dibenzofurans (23) are other potent enzyme inducers.

All of these compounds, as well as smoking, affect the activity of aryl hydrocarbon hydroxylase, which is controlled by the Ah locus. There is growing evidence that this particular cytochrome P-450 isozyme is associated with the production of reactive metabolites known to be mutagenic and carcinogenic (24). Consequently, polyhalogenated cyclic hydrocarbons increase the metabolic clearance of many drugs, which decreases their effect or increases the production of toxic metabolites (23).

Certain occupational factors, such as organic solvents, induce the MFOS. In fact, it was demonstrated more than a decade ago that acetone and isopropanol increased the activity of hepatic microsomes (25). More recent studies have confirmed this report (26) and showed that other ketones could also induce the hepatic MFOS activity (27-29). In our laboratory, we have demonstrated that acetone, methyl n-butyl ketone or methyl iso-butyl ketone have definite but different inducing capacities (30). The known consequences of MFOS induction by ketones include potentiation of haloalkane-induced hepato- and nephrotoxicity (31). These developments acquire an urgent relevance because of the large number of people exposed to ketones and haloalkanes every day. The danger has been dramatically illustrated with the outbreaks of hepatitis among industrial workers of an isopropyl alcohol packaging plant who were exposed to carbon tetrachloride (32).

Although very little is known about the effects of solvents on drug disposition, Dossing (33) has shown in a recent report that spray painters who

are exposed to multiple solvents, cleared antipyrine differently than did controls.

The first aim of the present series of studies was to characterize the effect of ketones on the hepatic MFOS in the rabbit. Secondly, we investigated how ketones influence the kinetics of several drugs in the same animal model.

MATERIAL AND METHODS

In all the studies we used New Zealand male rabbits weighing 2 to 3 kg, purchased from Laka Biological Supplies (St-Basile-le-Grand, Québec). They were maintained on Purina pellets and water ad libitum in individual, well ventilated cages for at least seven days before any experimental work was undertaken.

In vitro studies: Effect of three ketones on hepatic MFOS

In preliminary studies we determined the minimal effective dose as well as tolerance to different dosages of acetone (A & C, American Chemicals Ltd., Montréal, Québec), methyl n-butyl ketone (MnBK) (Aldrich Chemical Co., Milwaukee, Wisconsin) and methyl iso-butyl ketone (MiBK) (Fisher Scientific Co, Fair Lawn, New Jersey).

Single doses of 15, 10 or 7.5 mmol/kg of the ketones, administered by gavage, increased the activity of the MFOS, however tolerance was very poor. A single dose of 5 mmol/kg was well tolerated but did not affect MFOS acitivity. It was finally decided that 5 mmol/kg would be administered by gavage daily for 3 consecutive days since tolerance was acceptable and the effect of each solvent on the MFOS was clearly measurable. The ketones were administered in corn oil in a final volume of 10 mL/kg; control animals received the same amount of the vehicle.

The rabbits were sacrificed on the fourth day. Their livers were immediately extirpated, excised, washed in a solution of 1.5% KCl (w/v) and blotted dry. Liver homogenates (20%) were prepared in ice-cold sucrose (0.25 M) and centrifuged at 600xg for 10 min. The supernatant was centrifuged at 12000xg for 10 min., and to each 10 mL of the resulting supernatant 0.1 mL of $CaCl_2$ (1.0 M) was added (34). Following centrifugation at 27000xg for 15 min., the pellet was resuspended in 1.15% KCl (w/v) and centrifuged at 27000xg for 15 min. The resulting pellet was covered with sucrose 0.25 M and kept frozen at -40°C. All the above operations were performed at 4°C.

Protein content in the microsomal preparation was determined by using the technique described by Lowry et al. (35). The concentration of total cytochrome P-450 was determined by optical difference spectrophotometry, according to the technique described by Omura and Sato (36).

The effect of the ketones on the activity of the hepatic MFOS was assessed by measuring the rates of aminopyrine N-demethylation, of 7-ethoxycoumarin O-dealkylation and of aniline hydroxylation in the microsomal preparation. These measures were carried out by determining the rate of formation of formaldehyde, using the colorimetric method described by Holtzman et al. (37), by determining the rate of formation of 7-hydroxycoumarin using the fluorimetric method described by Jacobson et al. (38) and by measuring the rate of formation of para-aminophenol using the colorimetric method described by Brodie and Axelrod (39).

In vivo studies: Effect of three ketones on drug kinetics

The effect of acetone, MnBK and MiBK on the kinetics of warfarin was assessed in 3 groups of 6 rabbits. The animals received 5 mmol/kg of one of the ketones by gavage daily for 3 consecutive days. On the fourth day, they received warfarin intravenously. Control animals received the same volume of vehicle (2 mL/kg). Warfarin was given in a dose of 0.375 mg/kg. Blood samples were withdrawn at 0, 0.17, 0.33, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 24 and 27 hours.

Warfarin in plasma was assayed as follows: to 0.25 mL of plasma were added 0.1 mL of a solution containing the internal standard (indomethacin, 0.515 mg/mL) and 0.65 mL of acetonitrile. After mixing and centrifuging, 0.6 mL of the supernatant was evaporated to dryness and the residue recovered with 0.2 mL of the mobile phase. An aliquot of 0.1 mL was injected into the HPLC (Waters Associates). The mobile phase consisted of 0.01 M phosphate buffer: acetronitrile:methanol (49:47:4). The absorbance was measured with a 441 Detector (Waters Associates) at 214 nm with a zinc lamp. The column used was a Radial Bondapack C_{18} 10 μ (Waters Associates).

The effect of MnBK on the kinetics of antipyrine and diphenylhydantoin was studied in 2 other groups of rabbits. These animals were pretreated with MnBK at a daily dose of 7.5 mmol/kg administered subcutaneously for 3 days.

Antipyrine was administered at a dose of 20 mg/kg. Blood samples were withdrawn at 0, 15, 30, 45, 60, 90 and 120 minutes. Plasma concentrations of antipyrine were determined by spectrophotometry with a Beckman Model 25 Spectrophotometer, according to the technique described elsewhere (40).

Diphenylhydantoin was administered at a dose of 10 mg/kg. Blood samples were withdrawn at 0, 0.5, 1, 1.5, 2, 3, 4 and 5 hours. Diphenylhydantoin plasma concentrations were assayed by GLC as described elsewhere (41).

Preliminary studies were carried out to ascertain that, at the dose used, the kinetics of the test drugs were linear. The kinetic parameters were calculated using model independent equations as described by Gibaldi and Perrier (42).

The effects of each ketone on the activity of hepatic cytochrome P-450 and on the pharmacokinetic parameters of the test drugs were compared to the control groups using a one-way analysis of variance for parallel groups. The statistical difference was determined using the Dunnett's distribution table (43). The level of significance was established at $p < 0.05$.

RESULTS

In vitro studies

The daily administration of 5 mmol/kg of the ketones by gavage for 3 days affected the MFOS rather uniformly. The administration of acetone increased the rate of dealkylation of 7-ethoxycoumarin and the rate of hydroxylation of aniline. However, it did not affect either the rate of N-demethylation of aminopyrine or the concentration of total cytochrome P-450 (table I). MnBK and MiBK enhanced the rates of oxidation of the three substrates, but only MiBK increased the concentration of total cytochrome P-450. Dosages of 5 mmol/kg of the ketones were always well tolerated.

TABLE I. Influence of 5 mmol/kg of acetone, MnBK or MiBK, administered daily for 3 days, on the activity of the mixed-function oxidase (mean ± SEM)

	Aminopyrine N-Demethylation (mmol/mg/min)	7-Ethoxycoumarine dealkylation (mmol/mg/min)	Aniline Hydroxylation (mmol/mg/min)	Total cytochrome P-450 concentration (mmol/mg)
Controls	3.23 ± 0.41	0.38 ± 0 09	0.33 ± 0.02	0.86 ± 0 12
Acetone	3.66 ± 0 56	0.77 ± 0.10*	0.55 ± 0.05**	0.87 ± 0 10
MnBK	5.67 ± 0.50*	0.82 ± 0.13*	0.54 ± 0.06*	1 17 ± 0.22
MiBK	9 80 + 0 75**	0.89 ± 0 08**	0 50 ± 0.02**	2.15 ± 0 13**

* $p < 0.05$
** $p < 0.01$

In vivo studies

Exposure of the rabbits to the ketones did not affect the plasma concentrations of warfarin or the slope of decay of these concentrations (figure 1). As a consequence, the estimated values of the warfarin apparent total body clearance and apparent volume of distribution remained rather constant (figure 2).

The subcutaneous administration of 7.5 mmol/kg of MnBK daily for 3 days was well tolerated by the rabbits, despite the fact that some animals exhibited an inflammatory reaction at the injection site. MnBK decreased antipyrine plasma concentrations (figure 3), secondary to an increase in

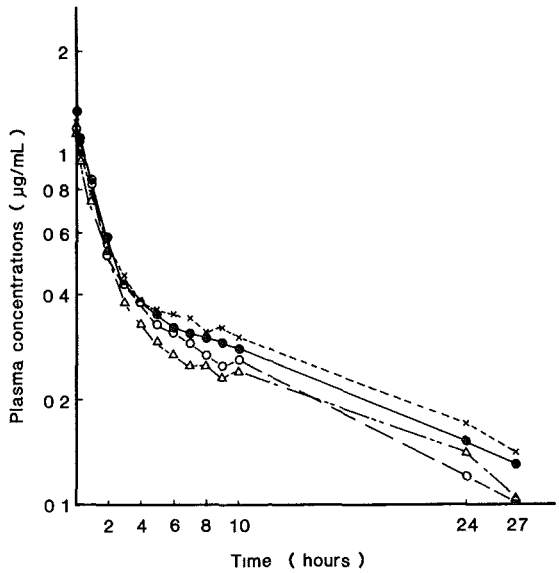

Fig. 1. Mean warfarin plasma concentrations as a function of time in groups of rabbits (n = 6) treated by gavage with corn oil (●) or 5 mmol/kg of acetone (×), methyl n-butyl ketone (○) or methyl iso-butyl ketone (△) daily for 3 days.

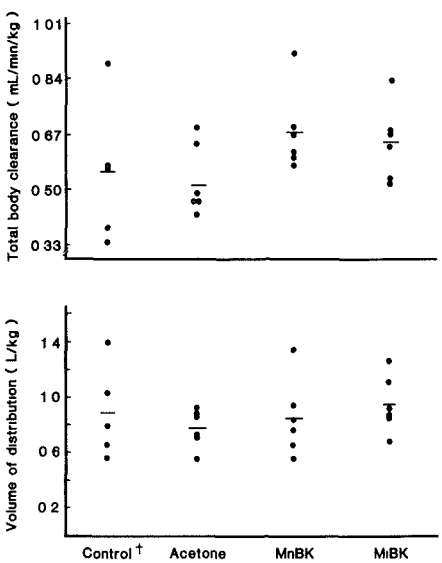

Fig. 2. Warfarin apparent total body clearance and volume of distribution in rabbits (n = 6) treated by gavage with corn oil (†) or with 5 mmol/kg of acetone, methyl n-butyl ketone (MnBK) or methyl iso-butyl ketone (MiBK), daily for 3 days. Horizontal bars represent the mean value.

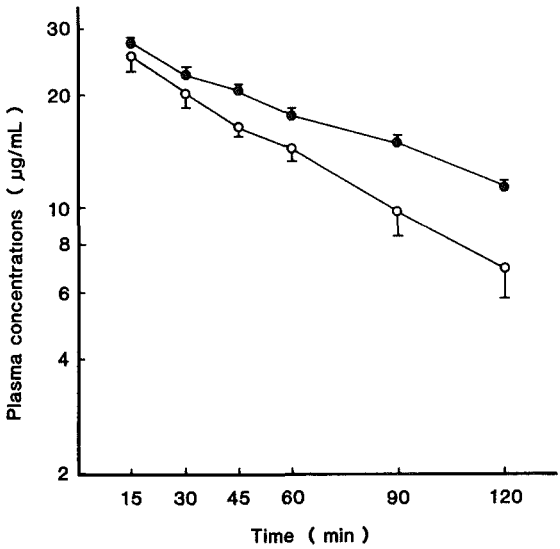

Fig. 3. Mean (± SEM) antipyrine plasma concentrations as a function of time in groups of rabbits (n = 6) treated with corn oil (●) or 7.5 mmol/kg methyl n-butyl ketone (○) administered subcutaneously daily for 3 days.

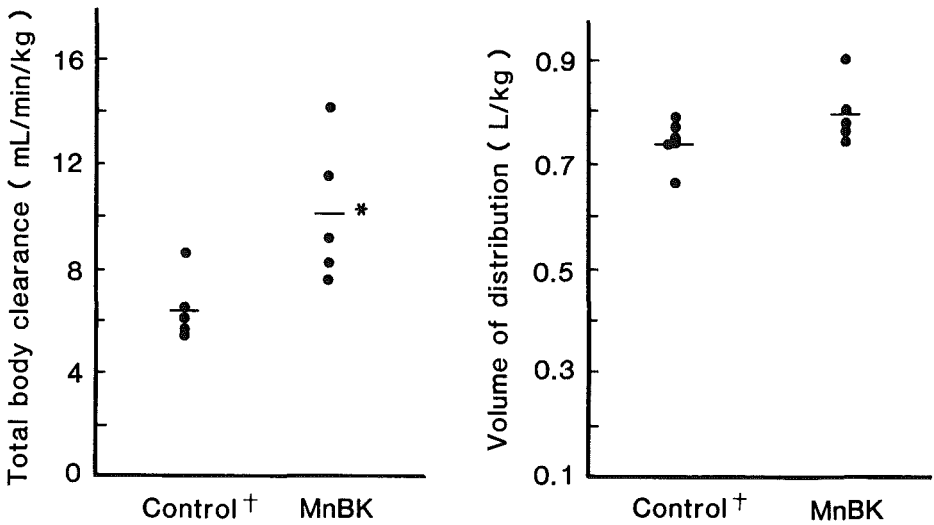

Fig. 4. Antipyrine apparent total body clearance and volume of distribution in rabbits treated subcutaneously with corn oil (†) or with 7.5 mmol/kg of methyl n-butyl ketone (MnBK), daily for 3 days. Horizontal bars represent the mean value. * $p < 0.05$.

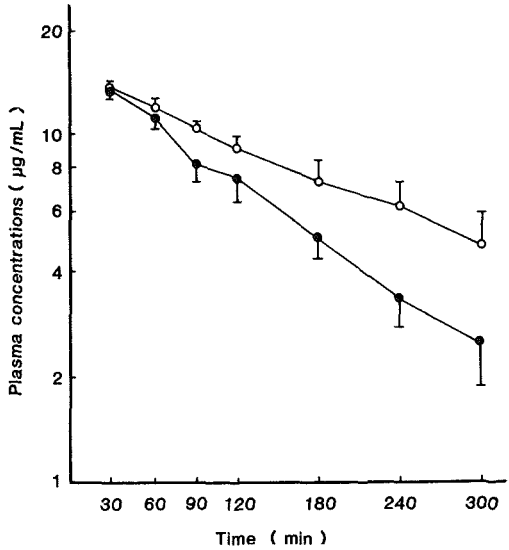

Fig. 5. Mean (± SEM) diphenylhydantoin plasma concentrations as a function of time in rabbits (n = 6) treated subcutaneously with corn oil (●) or 7.5 mmol/kg methyl n-butyl ketone (○), daily for 3 days.

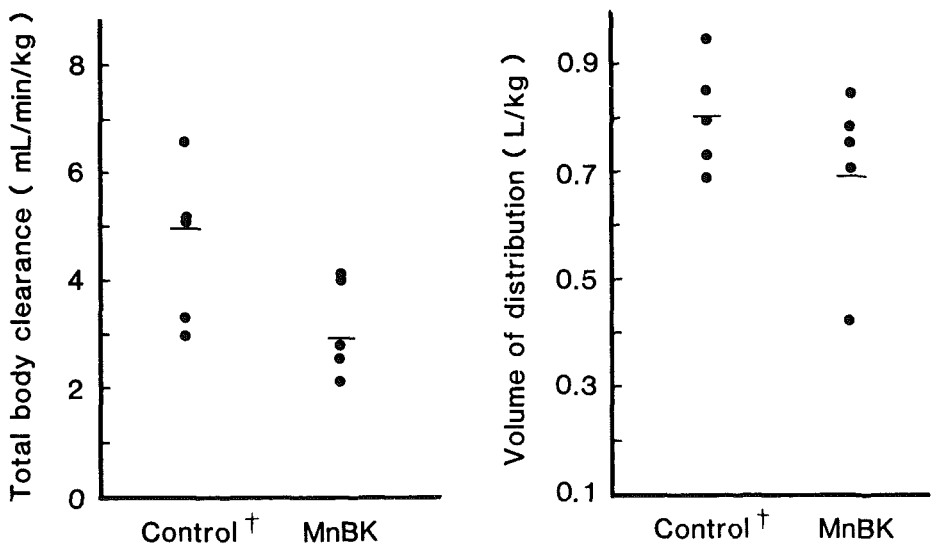

Fig. 6. Diphenylhydantoin apparent total body clearance and volume of distribution in rabbits treated subcutaneously with corn oil (†) or with 7.5 mmol/kg of methyl n-butyl ketone (MnBK), daily for 3 days. Horizontal bars represent the mean value.

antipyrine apparent total body clearance (figure 4). MnBK did not modify the antipyrine apparent volume of distribution. The antipyrine half-life was significantly decreased from 1.4 ± 0.1 hours in the controls to 0.9 ± 0.1 hours in animals exposed to MnBK ($p < 0.01$).

The pharmacokinetic parameters of phenytoin were not significantly altered by the subcutaneous administration of MnBK, although the plasma concentrations were slightly increased (figure 5). These changes appear to be related to a decreasing trend in the apparent total body clearance of phenytoin (figure 6). The phenytoin half life was not significantly influenced (1.9 ± 0.3 hours in control rabbits versus 2.4 ± 0.3 hours in rabbits exposed to MnBK).

DISCUSSION

Our results confirm the enzyme inducing capacity of ketones, but suggest that this capacity is non-specific, as ketones probably affect several P-450 isozymes. Furthermore, the effect on cytochrome P-450 activity seemed to depend on the type of ketone, the method of exposure and on the animal species. In fact, a single oral administration of any one of the ketones used exerted only minimal effects on the rabbit as compared to the rat (30). However, when lower doses (5 mmol/kg) were administered daily for 3 days, the changes in the rabbit MFOS were similar to thoses observed in the rat following a single 15 mmol/kg oral dose. Under this experimental condition, MiBK was a more potent inducer than MnBK, which, in turn was more potent than acetone.

The potentiation of chloroform and carbon tetrachloride hepatotoxicity and nephrotoxicity is a well documented consequence of hepatic enzyme induction by ketones (26-28). Interestingly, this potentiating effect varies with different ketones, an increasing response being observed with the longer chain solvents (31, 44, 45). Our results corroborate these reports, as MiBK was shown to be a more potent enzyme inducer than MnBK and acetone.

Another consequence of enzyme induction due to acetone may be an increase in the hepatotoxicity of trihalomethanes other than chloroform such as bromodichloromethane and dibromochloromethane, two compounds that are ubiquitous contaminants of municipal water supplies (46).

Exposure to acetone can also increase the hepatotoxicity of N-nitrosodimethylamine, probably by enhancing the biotransformation of this nitrosamine to reactive metabolites (47). Nitrosamines are found in the general environment as well as in food, cigarette smoke and alcoholic beverages. They can also be synthesized endogenously in the body. It has been widely documented that the toxic and carcinogenic effects of nitrosamines appear only after their metabolic activation.

Aliphatic hexacarbons, such as n-hexane, MnBK and MiBK, produce central and peripheral axonopathies, MnBK being the most potent (48). Interestingly, n-hexane metabolism generates MnBK (49). Exposure to MiBK also increases the neurotoxicity of n-hexane, probably by enhancing cytochrome P-450 activity and increasing the generation of the more toxic MnBK (29).

Other industrial organic solvents such as xylene and xylene isomers, are potent inducers of several P-450 isozymes in the liver, kidney and lung (50, 51). Metabolic induction by xylene may be a cause of embryotoxicity, since p-xylene, a component of xylene, appears to decrease blood levels of progesterone and of 17-beta-estradiol (52).

Toluene also increases the metabolic activity of liver microsomal cytochrome P-450 (51). Occupational coexposure to xylene and toluene is common. Therefore, Wallen and coll. (53) investigated the possible implications of such coexposure in healthy volunteers. They reported that simultaneous exposure to xylene and toluene decreased the clearance of both solvents by almost 40% and 21% respectively. Interestingly, CNS toxicity did not appear to increase after exposure to both these solvents for 4 hours (54).

Benzene and its methyl derivatives are also potent inducers of the MFOS (55). The consequences of exposure to benzene may be relevant, as it has been shown to increase the rates of N-demethylation of aminopyrine and of hydroxylation of aniline (55). Both pathways have been associated with the hepato- and nephrotoxicity of haloalkanes (30).

Other recent studies have shown that toluene and benzene increase the activity of the aromatic-L-aminoacid decarboxylase in the brain (56). This observation may have interesting clinical ramifications, as this enzyme mediates the decarboxylation of p-tyrosine to p-tyramine, the latter being a neurotransmitter or neuromodulator that may be implicated in a variety of pathophysiological states ranging from migraine to hepatic encephalopathy (57).

A variety of highly volatile solvents, such as those used for anesthesia (diethyl ether, isopropyl ether, ethyl vinyl ether, halothane, enflurane and fluroxene), increase the hepatic microsomal enzyme activity in animal models (58), in normal volunteers (59), in patients undergoing surgical procedures (60) and in anesthetists (61). In addition, like ketones and benzenes, these anesthetics appear to increase the rate of hydroxylation of aniline (62). This effect may be related to their well known toxic effects on the liver and kidney.

In the foregoing discussion, we have shown that exposure to solvents can adversely affect health. However, little is known about the possible influence of these solvents on drug disposition. In the first phase of our study, we characterized the effect of acetone, MnBK and MiBK on the activity

of hepatic cytochrome P-450. In the second phase, we explored how these enzyme inducers influenced the disposition of three substrates in vivo.

The elimination of antipyrine was greatly increased in animals which received MnBK. This observation is important since antipyrine is very frequently used as a reference substrate to estimate the activity of the cytochrome P-450.

The disposition of warfarin was not affected by any of the solvents, despite the fact that both MnBK and MiBK tended to increase warfarin total body clearance.

MnBK decreased the total body clearance of diphenylhydantoin by 44%, however, this difference was not significant. It is not clear why this solvent exerted such an unexpected effect on the disposition of this drug. One reason could be the subcutaneous route of administration of the solvent since we know that an abcess induced by a subcutaneous administration of turpentine decreases the activity of cytochrome P-450 (63). On the other hand, since subcutaneously administered MnBK induced the metabolism of antipyrine, it is not likely that the effect of MnBK on phenytoin metabolism was due to the route of administration.

In summary, we have confirmed that ketonic solvents alter the activity of the hepatic MFOS and that the ability of these solvents to induce such changes differs widely. We have also shown in rabbits that this enzyme induction can modify the disposition of selected drugs. At the present time we are unable to predict which drugs will be affected by exposure to organic solvents. Moreover, it remains to be established whether the effects observed in the rabbit will extend to humans who are exposed to solvent concentrations lower than the TLV for prolonged periods.

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DISCUSSION - Enzyme induction by environmental agents.

G. Zbinden

You have described an enzyme induction by ketones given by gavage. Would you expect a different effect of these compounds when given by inhalation?

P. du Souich

No. Dr's Plaa group has shown in the rat that these compounds have exactly the same effect when given by inhalation or by gavage.

A dose of 15 mmol/kg in the rat produces an effect on cytochrome P 450 similar to that of 5 mmol/kg for 3 days in the rabbit. I do not think that the route will change the response.

P.G. Watanabe

I presume that one may identify, in studies with experimental animals, many types of interactions of environmental chemicals and drugs. However, what we should be most concerned with is how to identify relevant interactions relative to what humans may be potentially exposed to. Would you like to comment on a strategy for this identification?

P. du Souich

I am afraid that none is available at present. One of the main problems is that the response is quite different when dealing with individual drugs : variable in the case of phenytoin, either no response or inhibition in the case of tolbutamide or warfarin and clear induction in the case of theophylline. Until we are able to identify the isozymes involved in all the drug biotransformation pathways we cannot envisage any start in this direction.

G.L. Plaa

A point that deserves consideration is the direct toxicity of the ketone. Thus, in the case of MnBK its great potential neurotoxicity eliminates it from further studies, since the threshold limit values are such that the concentrations found in occupational setting would be low. Probably the most interesting

agent in the context of these studies is acetone itself, because the doses needed to induce microsome enzymes are not very far from the threshold limit value now accepted. I would say that the interest of ketones as inducers present in the environment is inversely correlated to their inherent toxicity.

R. Lauwerys

It is somehow surprising that there are so few examples of clearly demonstrated interactions between environmental agents, outside of tobacco smoke, and drugs. Of course, there have been some clear-cut results, but in general the studies are not very convincing. I wonder whether this is so because the exposure level is too low or because the design of these studies is not correct enough.

P. du Souich

Since the effect on the isozymes differs among the agents, as we have shown with solvents, it is rather difficult to select the right combination. Perhaps antipyrine metabolism is not the best marker.

L.F. Prescott

We should be concerned here with the possible effects of these solvents on drug metabolism in man, and I think that the question of occupational exposure is minor compared to some circumstances in clinical medicine where there may be very high levels of ketones in the blood. I refer to uncontrolled diabetes and to patients who are not eating normally and suffering relative starvation. A few studies of drug metabolism in diabetes have been published, but the results have not been very impressive. It would be of interest to know whether drug metabolism is abnormal in patients with ketosis.

M.M. Reidenberg

Some time ago we measured the rate of metabolism of antipyrine and tolbutamide in a group of individuals before and during substantial fasting, so that they had ketonuria. We did not find any change in the half life or volume of distribution of either of these drugs. (Reidenberg MM, Vesell ES. Clin Pharmacol Ther 17 : 650, 1975).

G.L. Plaa

Let me just mention something. Dr. du Souich's results and those of our work refer mostly to effects of ketones on cytochrome P 450. I think that effects of these agents on other pathways of drug metabolism should also be evaluated.

H. Vainio

To what extent are changes due to increased synthesis of protein and to in vivo activation?

P. du Souich

I cannot give an exact figure, but we have gathered data indicating that there is increased synthesis of proteins, particularly of those with a molecular weight between 52 and 60000 daltons.

J.V. Castell

Could you give us some details about the time course of enzyme induction after exposure to ketones?

P. du Souich

I cannot, since we have not done the appropriate studies to answer this question. What we know is that, for instance, the induction of aniline hydroxylation is very short lasting. Induction is evident 24 hours after a single dose and has disappeared 24 hours later. In the case of other pathways, such as antipyrine demethylation, the turnover rate of the isozyme involved seems to be much slower.

O. Pelkonen

I just want to mention that some specific isozymes, such as LM3a from rabbit liver and P 450j from rat liver have now been purified, and that antibodies against these isozymes are available. Therefore, it is quite easy to detect their presence, and this could be an approach of interest in the studies of enzyme induction by ketones.

M.M. Reidenberg

I think it would be interesting if you would look at the kidney and not just the liver. The oxidative pathways are important, at least for nephrotoxicity, and in studies that we did evaluating the urinary excretion of lysosomal enzymes as an index of renal effect we found that people with occupational exposure to volatile organic solvents had high levels of urinary enzyme excretion. I believe that these people would have had normal levels of the various liver enzymes in their serum at the levels of exposure they had. (Meyer BR et al, Amer J Med 76 : 989, 1984).

P. du Souich

We are simultaneously working on hepatic and renal isozymes. The problem with the kidney is the location of the isozymes and their amount, much smaller that in the liver.