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SELECTIVE ALTERATION OF CONSTITUTIVE HEPATIC CYTOCHROME P-450 ENZYMES IN THE RAT DURING PARENTERAL HYPERALIMENTATION

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Abstract—Decreased drug metabolism and hepatic cytochrome P-450 levels have been shown previously to occur in rats receiving total parenteral nutrition (TPN) compared to animals receiving the same hyperalimentation solution enterally (TEN). In the present studies, animals received a 7-day infusion of a 25% glucose–2.75% crystalline amino acid solution via a catheter in the jugular vein or stomach; hepatic microsomal levels of four major constitutive cytochromes P-450 were determined subsequently by immunoquantitation and correlated with metabolism of selected substrates biotransformed by these enzymes. TPN resulted in a marked decrease in apoprotein of two constitutive cytochromes P-450, P-450_{UT-A} and P-450_{PCN-E}, compared to TEN experiments (for P-450_{UT-A}, 11.0 ± 1.8 vs 44.7 ± 6.5% of total cytochrome P-450 measured by CO-difference spectra, $P > 0.001$; for P-450_{PCN-E}, 15.4 ± 4.4 vs 30.2 ± 7.6%, $P < 0.01$), but apoprotein levels of two other constitutive cytochromes P-450, P-450_{PB-C} and P-450_{UT-F}, showed relatively little change. Concordant reductions in metabolism of benzphetamine, ethylmorphine and erythromycin were seen in TPN animals. While the mechanisms responsible for these selective changes in the synthesis and function of individual cytochromes P-450 remain to be elucidated, altered gene transcription due to differences in portal blood composition elicited by intravenous versus enteral feeding is a possible hypothesis. These studies also provide information which should be valuable in designing studies to probe further the clinical question of whether TPN induces significant alterations in human drug metabolism.

Several laboratories have demonstrated that continuous intravenous administration of amino acid–glucose mixtures to rats results in impaired *in vivo* clearance of a number of drugs primarily metabolized by the liver [1, 2]. Subsequent studies revealed that levels of cytochrome P-450 and capacity for demethylation of meperidine and hydroxylation of pentobarbital by hepatic microsomes obtained from livers of rats receiving total parenteral nutrition (TPN) were reduced significantly compared to animals receiving the same amino acid–glucose mixture via a gastric catheter (TEN) or chow-fed animals [3]. In these later studies, qualitative differences between hepatic microsomal protein obtained from livers of TPN animals versus those of enterally-alimented or chow-fed animals were seen in the cytochrome P-450 region (M_r = 45,000–60,000) by sodium dodecyl sulfate–polyacrylamide electrophoresis [3]. It was also observed that the reduction in meperidine demethylase activity produced by TPN (approximately 5-fold) exceeded the 2-fold decrease in total hepatic cytochrome P-450 levels seen during parenteral hyperalimentation [3]. Since "hepatic cytochrome P-450" is a family of enzymes rather than a single protein [4–6], these data suggested that TPN may influence the regulation of specific forms of cytochrome P-450 rather than exerting a generalized effect on cytochrome P-450-dependent drug-metabolizing systems.

Certain cytochrome P-450 enzymes are found in rat liver in significant quantities only after treatment of animals with various inducing agents such as phenobarbital or β-naphthoflavone [4–6], while other "constitutive" forms can be identified in liver of untreated animals [4, 7]. Previous immunochemical determination of individual forms of cytochrome P-450 in liver microsomes of untreated rats revealed that four enzymes‖, P-450_{UT-A}, P-450_{PB-C}, P-450_{PCN-E} and P-450_{UT-F}, can account for as much as 90% of constitutive male rat liver cytochrome P-450 [4]. The purpose of the present investigation was to determine the effect(s) of parenteral hyperalimentation on constitutive cytochrome P-450 enzymes in rat liver and to correlate changes in individual cytochrome P-450 enzymes with alterations in hepatic drug-metabolizing activity induced by TPN.

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‖ A nomenclature developed in one of our laboratories, which includes an abbreviation referring to the inducing agent utilized in initial studies, is used to designate enzymes. For comparison of purified preparations to others described in the literature see Refs. 8–10. Briefly, some other preparations similar to cytochrome P-450_{UT-A} are designated P-450 2c, P-450 h, P-450 male and RLM5; to P-450_{PB-C}—P-450 PB-1; to P-450_{PCN-E}—P-450p and P-450 PB-2a; and to P-450_{UT-F}—P-450a and P-450 PB-3.

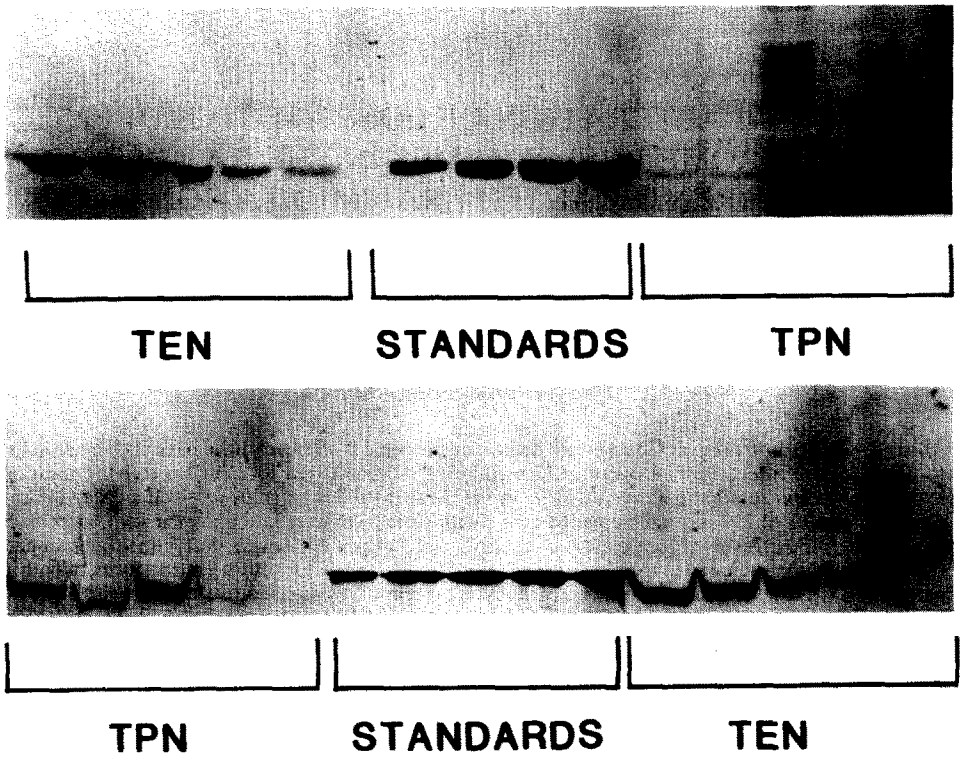

Fig. 1. Immunoblots of hepatic microsomal protein after staining with polyclonal antibodies to specific rat cytochrome P-450 enzymes. TPN = total parenteral nutrition; TEN = total enteral nutrition. Top panel: Staining with antibody to cytochrome $P\text{-}450_{UT\text{-}A}$. Twenty pmol of total microsomal cytochrome P-450 from individual animals was run in each experimental lane. Standards (left to right) contained 5–20 pmol of purified cytochrome $P\text{-}450_{UT\text{-}A}$. Bottom panel: Staining with antibody to cytochrome $P\text{-}450_{PCN\text{-}E}$. Thirty pmol of microsomal cytochrome P-450 was run in each lane. Purified cytochrome $P\text{-}450_{PCN\text{-}E}$ standards (1–5 pmol) were electrophoresed concomitantly.

MATERIALS AND METHODS

Male Sprague–Dawley rats (Charles River Laboratory, Wilmington, MA), weighing 250–300 g, were lightly anesthetized with ether, and a silastic catheter was placed in either the jugular vein or stomach; a 2.75% amino acid* −25% glucose solution containing multivitamins and electrolytes was subsequently administered for 7 days (see Ref. 3 for additional details of the infusion technique and exact composition of the hyperalimentation solution). A third group of animals had surgical placement of either a jugular vein or gastric catheter and was allowed free access to standard laboratory chow (Ralston Purina Co., No. 5001).

After 1 week of hyperalimentation, animals were again anesthestized with ether and killed by exsanguination. Hepatic microsomes were prepared by standard methods and stored at −20° in 10 mM Tris acetate buffer, pH 7.4, containing 1 mM EDTA and 20% (v/v) glycerol. Various enzyme activities and cytochrome P-450 levels are stable under these storage conditions for a minimum of 6 months; all analyses were performed within 3 months of microsome preparation. Glucose-6-phosphate dehydrogenase (Type VI), $NADP^+$, D-glucose-6-phosphate, benzphetamine, erythromycin, Tris acetate and bovine serum albumin were obtained from the Sigma Chemical Co. (St Louis, MO). Protein concentrations were determined by the method of Sutherland *et al.* [11], and total cytochrome P-450 levels were assayed in microsomes according to the method of Omura and Sato [12]. Rates for N-demethylation of benzphetamine, erythromycin, and ethylmorphine were measured by the rate of formaldehyde formation according to the method of Nash [13].

Individual rat cytochrome P-450 enzymes were isolated from phenobarbital, β-naphthoflavone- or pregnenolone-16α-carbonitrile-treated rats as described elsewhere [4]; antisera were raised in female New Zealand white rabbits per the method of Kiminsky *et al.* [14]. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of microsomal protein samples containing 20–30 pmol of total cytochrome P-450 was performed, as described by Laemmli [15], using 7.5% (w/v) acrylamide gels. Resolved proteins were electrophoretically transferred from gels to sheets of nitrocellulose (Schleicher & Schuell, Inc., Keene, NH), and immunoblotting was performed as previously described [16] with the exception that 4-chloro-1-naphthol was used instead of 3,3'-diaminobenzidine

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Table 1. Effects of parenteral (TPN) and enteral (TEN) hyperalimentation on rat hepatic microsomal cytochrome P-450 levels and mixed-function oxidase activity*

Alimentation regimen	Cytochrome P-450 (nmol/mg)	Dealkylase activity (nmol/min/mg)		
		Benzphetamine	Ethylmorphine	Erythromycin
TPN	0.474 ± 0.140	1.1 ± 0.4	5.6 ± 1.0	0.35 ± 0.15
TEN	0.908 ± 0.220†	4.0 ± 1.2†	9.5 ± 1.2†	0.60 ± 0.06†

* Values are means ± SD, N = 5 for each group.
† Significantly higher than the TPN group at $P < 0.01$ or greater.

Table 2. Immunochemical quantitation of four major constitutive cytochrome P-450 enzymes in hepatic microsomes of rats receiving parenteral (TPN) or enteral (TEN) hyperalimentation*

Alimentation regimen	Cytochrome P-450 enzymes† (% of total cytochrome P-450 electrophoresed)			
	$P\text{-}450_{UT\text{-}A}$	$P\text{-}450_{PCN\text{-}E}$	$P\text{-}450_{PB\text{-}C}$	$P\text{-}450_{UT\text{-}F}$
TPN	11.0 ± 1.8	15.4 ± 4.4	28.8 ± 2.8	14.9 ± 1.2
TEN	44.7 ± 6.5‡	30.2 ± 7.6‡	19.2 ± 0.4‡	12.0 ± 1.3

* Values are means ± SE (N = 5 for each group) of total electrophoresed cytochrome P-450 (as determined by CO-difference spectra); 20 pmol of microsomal cytochrome P-450 was used for UT-A and PB-C experiments and 30 pmol of cytochrome P-450 for PCN-E and UT-F immunoblots. Purified samples of each enzyme were run concomitantly as standards.
† Respective values obtained for hepatic microsomes from chow-fed animals (N = 4) were: UT-A, 44.7 ± 0.8; PCN-E, 37.3 ± 15.2; PBC, 39.9 ± 6.6; and UT-F, 19.3 ± 4.9.
‡ Significantly different from TPN group at $P < 0.01$ or greater.

for staining. Quantitation of individual cytochrome P-450 enzymes in electrophoresed microsomes was performed by densitometry, utilizing an LKB Ultra Scan XL Laser Densitometer with Gel Scan XL software, and comparison of values with those obtained for concomitantly electrophoresed standards of the purified individual cytochrome P-450 enzymes.

RESULTS

Hepatic levels of total cytochrome P-450 and activities for benzphetamine, ethylmorphine and erythromycin N-dealkylation are shown in Table 1. $P\text{-}450_{UT\text{-}A}$ is the constitutive cytochrome P-450 with the highest activity for benzphetamine demethylation and $P\text{-}450_{UT\text{-}A}$ and $P\text{-}450_{PB\text{-}C}$ are the two constitutive cytochromes P-450 with high activity for demethylation of ethylmorphine [4]; $P\text{-}450_{PCN\text{-}E}$ is the primary enzyme responsible for erythromycin metabolism [17]. Significant decreases in total liver cytochrome P-450 and drug-metabolizing activity for all three substrates were seen for hepatic microsomes obtained from TPN animals compared to those from TEN experiments.

Immunoblots of liver microsomal protein from TPN and TEN animals after staining with antisera raised to $P\text{-}450_{UT\text{-}A}$ and $P\text{-}450_{PCN\text{-}E}$ are shown in Fig. 1. Hepatic levels of apoprotein for both constitutive cytochrome P-450 enzymes from TPN experiments were reduced markedly compared to those in microsomes from TEN animals. Similar immunoblots developed after staining with antisera to $P\text{-}450_{PB\text{-}C}$ and $P\text{-}450_{UT\text{-}F}$ did not display marked differences between the TPN and TEN microsomes (blots not shown). Immunoquantitation data obtained for the individual constitutive cytochrome P-450 enzymes expressed as a percentage of total cytochrome P-450 in liver microsomes are shown in Table 2. Highly significant reductions in apoprotein for cytochromes $P\text{-}450_{UT\text{-}A}$ and $P\text{-}450_{PCN\text{-}E}$ were seen in TPN experiments compared to TEN studies. No significant change was seen between the percentages of cytochrome $P\text{-}450_{UT\text{-}F}$ present in TPN and TEN microsomes, and the percentage of cytochrome $P\text{-}450_{PB\text{-}C}$ in TPN microsomes was actually increased compared to the TEN group. Since total cytochrome P-450 levels in liver microsomes from TPN animals were only approximately 50% of those seen in TEN studies (Table 1), data from immunoquantitations were also used to calculate amounts of individual cytochrome P-450 enzymes/mg microsomal protein. Mean values ± SE for cytochromes $P\text{-}450_{UT\text{-}A}$ and $P\text{-}450_{PCN\text{-}E}$ in TPN experiments were 0.050 ± 0.014 and 0.062 ± 0.033 nmol/mg protein, respectively, versus 0.399 ± 0.182 and 0.261 ± 0.175 nmol/mg protein, respectively, for TEN microsomes ($P < 0.01$ for both comparisons). Levels of cytochrome $P\text{-}450_{PB\text{-}C}$/mg liver microsomal protein were not reduced significantly by TPN compared to TEN (0.138 ±

0.048 versus 0.159 ± 0.034 nmol/mg protein, respectively). Although TPN did result in a statistically significant decrease in nmol cytochrome P-$450_{UT\text{-}F}$/mg of microsomal protein compared to TEN experiments (0.069 ± 0.018 versus 0.096 ± 0.010 nmol/mg protein respectively), this decrease (28%) was of much lesser magnitude than those seen for cytochromes P-$450_{UT\text{-}A}$ and PCN-E (87% and 76% respectively).

DISCUSSION

TPN with glucose–amino acid mixtures has been shown to decrease hepatic drug metabolism of a variety of substrates including meperidine, pentobarbital and antipyrine in rats [1–3]. However, the magnitude of decrease for these different substrates has varied considerably, ranging from 5-fold for meperidine [3] to 4- and 2-fold for antipyrine and pentobarbital respectively [2, 3]. These observations have led to speculation that TPN may produce differential effects on enzymes with the cytochrome P-450 family, and this speculation has been confirmed in the present study. While apoprotein levels for two major constitutive rat hepatic cytochromes P-450 (P-$450_{UT\text{-}A}$ and P-$450_{PCN\text{-}E}$) were depressed markedly in microsomes from rats receiving TPN, changes in the levels of the other two most prominent constitutive enzymes (P-$450_{PB\text{-}C}$ and P-$450_{UT\text{-}F}$) were relatively minor. Metabolism of several substrates selected to probe the function of specific cytochromes P-450 was also concordant with immunoblot data. P-$450_{UT\text{-}A}$ is the most active constitutive cytochrome P-450 with regard to benzphetamine metabolism in the rat [4], and microsomal benzphetamine demethylation was reduced severely concomitant with the reduction in P-$450_{UT\text{-}A}$ apoprotein (Table 1). Ethylmorphine is demethylated by both P-$450_{UT\text{-}A}$ and P-$450_{PB\text{-}C}$ [4], so the less severe reduction in ethylmorphine demethylation observed during TPN is explained by the relative lack of effect TPN had on P-$450_{PB\text{-}C}$. Finally, erythromycin demethylation appears to be selectively performed in the rat by proteins in the PCN-E-inducible P-450 gene family [17, 18], and decreased erythromycin demethylation parallelled the reduction seen in immunoreactive P-$450_{PCN\text{-}E}$.

Although TPN depresses hepatic drug metabolism in rats, studies examining the effects of TPN on drug metabolism in humans have produced less consistent results. Pantuck *et al.* found that antipyrine metabolism in severely malnourished patients was improved from baseline by parenteral administration of a glucose–amino acid solution [19], but Burgess and co-workers found that clearance of the same drug was decreased 30% in surgical patients who received similar infusions postoperatively compared to patients not receiving TPN [20]. While the results of the current investigations in rats cannot be transposed to humans, they do provide a possible explanation for different outcomes produced by TPN in the forementioned human studies and suggest several additional considerations which must be taken into account when examining the effects of parenteral nutrition on drug metabolism in humans. If nutritional permeations differentially affect individual cytochrome P-450 enzymes in humans, then the cytochrome P-450 profile of severely malnourished patients may be significantly different at the start of TPN than that of relatively healthy surgical patients unable to tolerate oral nutrients in the immediate postoperative period. Also, if TPN has variable effects on the different human cytochromes P-450 as was demonstrated in these animal studies, the substrate chosen to probe the response of hepatic drug metabolism to TPN may profoundly influence the outcome of the study.

Intravenous alimentation produces alterations in release of several gastrointestinal hormones such as insulin and gastrin when compared with enteral nutrition [21, 22], and these observations represent one possible mechanism to explain the findings of the current studies. There is a mounting body of evidence that expression of constitutive cytochromes P-450 in the rat is differentially influenced by permeations in physiology such as hormone secretion. The importance of androgens on levels of expression of P-$450_{UT\text{-}A}$ in adult male rats has been demonstrated [23, 24]. Induction of diabetes in rats has been reported to produce a marked decrease in immunodetectable P-450 RLM5 (synonymous with P-$450_{UT\text{-}A}$) in rat hepatic microsomes and a concomitant reduction in testosterone 16α-hydroxylase activity [25]; insulin administration partially reversed this alteration in expression and function. Although not directly examined by immunoquantitation, assays of testosterone 6β- and 7α-hydroxylase activity in the same diabetic rats suggest that diabetes does not alter the levels of two other constitutive rat hepatic cytochromes P-450, P-$450_{UT\text{-}F}$ and P-$450_{PCN\text{-}E}$ [25]. Chronic administration of pentagastrin, a gastrin analogue, has also been reported to increase levels of total hepatic cytochrome P-450, but these studies did not pursue the effects of gastrin on individual forms of hepatic cytochrome P-450 [26]. Preliminary data obtained for a small group of TPN and TEN animals in this laboratory have shown modestly higher levels of both insulin (+10%) and gastrin (+50%) in TEN animals [3], but much additional work is needed to further examine effects of gastrointestinal hormones on hepatic cytochromes P-450.

Reduced transcription of genes responsible for production of the P-$450_{UT\text{-}A}$ and P-$450_{PCN\text{-}E}$ apoproteins is one attractive hypothesis to explain the decrease seen in P-$450_{UT\text{-}A}$ and P-$450_{PCN\text{-}E}$ observed in TPN experiments at the molecular level. In a preliminary report, Traber and Gumucio [27] demonstrated that diversion of portal blood from the liver decreases the inductive effect of phenobarbital on hepatic transcription of the cytochromes P-450 b and e genes. This observation suggests that some factor(s) in portal blood regulates transcription of cytochrome P-450 genes in the liver; gastrointestinal hormones present in portal blood of animals receiving enteral nutrition, but absent or in reduced concentrations in portal blood of TPN animals, are one candidate factor. Further support for a regulatory interrelationship between portal blood and cytochrome P-450 is provided by recent studies of Farrell and Koltai [28] demonstrating that portal bypass decreases testosterone 16α- and 2α-hydroxylation, activities catalyzed almost exclusively by P-$450_{UT\text{-}A}$.

Long-term parenteral and enteral nutrition are being used with increasing frequency in clinical medicine. These animal studies demonstrate that TPN produces selective alterations in both production of individual cytochrome P-450 enzymes and mixed-function oxidase activity. If similar alterations in drug metabolism occur in humans, they would have profound clinical implications. Future work in this area should be directed toward defining the mechanism(s) responsible for TPN-induced alterations of drug metabolism in animals and using data gained in animal studies to design appropriate experiments to examine this important clinical question in humans.

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