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A Preliminary Evaluation of Drug Biotransformation in Hepatocytes of Genetically Defined Rat Strains

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Summary

This study was directed to use the genetically developed isoprenaline-sensitive (S), isoprenaline-resistant (R) and spontaneous hypertensive rats (SHR) as standard diseased animal models for *in vitro* liver function evaluation of drug biotransformation. Hepatic hexobarbital hydroxylase and glutathione transferase (GST) were evaluated by using hexobarbital and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates, at concentrations of 0.21 mmol/l and 1 mmol/l, respectively. The assay was conducted by using isolated hepatocytes in suspension and hepatocytes in a bioreactor configuration. The data demonstrate that there are certain cellular pharmacokinetic differences in hexobarbital hydroxylase and GST activities in hepatocytes obtained from Wistar, SHR, R and S strains which can be better demonstrated, when using the model of perfused and immobilized hepatocytes.

Key words

Hepatocytes – Spontaneously hypertensive rat – Isoprenaline-sensitive rat – Isoprenaline-resistant rat

Introduction

Drug metabolizing enzymes are of great importance in drug detoxification as well as chemical mutagenesis, carcinogenesis and toxicity *via* metabolic activation. The genetically determined differences in the activity of these enzymes can influence the individual response to adverse drug reactions, drug-induced diseases and certain types of chemically induced cancers (Eichelbaum *et al.* 1992). Progress during the last thirty years has enabled thorough studies in genetic polymorphism of human drug metabolizing enzymes as N-acetyltransferase and some cytochrome P-450 isozymes based on the metabolic handling of certain probe drugs. Moreover, animal studies demonstrated that Gunn rats exhibited kernicterus and lack of glucuronidation similar to patients with the Crigler-Najjar syndrome (Kalow 1968). This emphasizes the importance of the selective breeding of various genetically defined strains of rats that enables the elaboration of animal models which are of great value in the study of the pathophysiology of diseases. The spontaneously hypertensive rats (SHR) have been obtained (Kurtz *et al.* 1991) and the rat TNFα gene was mapped (Pravenec *et al.* 1991). It was suggested that TNFa may be functionally related to abnormalities associated with the k haplotype in SHR. In addition, strains of rats which are sensitive and resistant to the cardiotoxic effects of isoprenaline were selectively bred at our institute and some differences between these two strains in the body composition were found (Mráz *et al.* 1986). The liver as the main biotransformation organ and its possible abnormality has not been addressed in various rat strains that are selectively bred at our institute. The present study, therefore, is carried out with the specific aims of (i) using the genetically developed isoprenaline-sensitive, isoprenaline-resistant and SHR rats as standard diseased animal models for *in vitro* liver function evaluation of drug biotransformation, and (ii) maximizing the use of the cellular model by applying the developed technique of perfused immobilized hepatocytes for comparison of xenobiotic biotransformation kinetics in liver cells obtained from different rat strains.

Methods

Male Wistar rats (controls) and genetically defined rat strains with various cardiovascular abnormalities including spontaneously hypertensive rats (SHR), isoprenaline-sensitive (S) and isoprenaline-resistant rats (R) were used in the present study. The rats weighed between 220–300 g. Throughout the period of investigation, the animals were allowed free access to water and a commercial diet. Each experimental group contained 5–6 animals. Rats were anaesthesized with ether and the hepatocytes were isolated, immobilized and perfused as described in an earlier article of this issue.

In vitro evaluation of hepatic hexobarbital hydroxylase and glutathione transferase was carried out as representatives of phase I and phase II of drug metabolism, respectively. The assay was conducted by using the two hepatocyte experimental models – cells in suspension and cells in the bioreactor configuration. In previous experiments we found that the initial concentrations of 0.21 mmol/l (50 mg/l) of hexobarbital and 1 mmol/l (204 mg/l) of 1-chloro-2,4-dinitrobenzene (CDNB) are appropriate for the present biotransformation studies. The disappearance of hexobarbital as a representative substrate of cytochrome P-450 linked drug metabolism was followed up in the perfused hepatocyte system for 2 h using liver cells of the Wistar and SHR strains. The hepatocyte bioreactor obtained from the same two strains was used for following up the conjugation reaction of the substrate CDNB with reduced glutathione as a measure of glutathione transferase activity up to 1 h. Moreover, both substrates were tested in the hepatocyte suspensions that were prepared from Wistar, SHR, S and R strains. The time course of hexobarbital disappearance or CDNB glutathione conjugate appearance was measured as previously described.

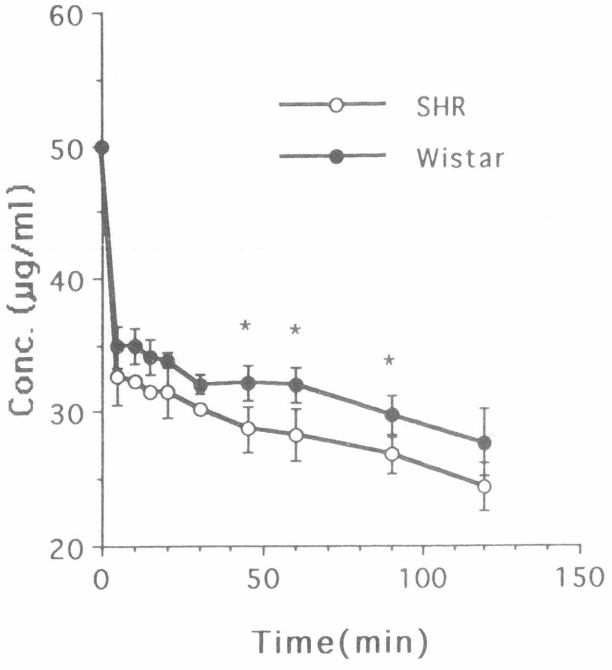

Fig. 1
The time course of hexobarbital disappearance in the perfusion media of immobilized hepatocytes of Wistar and SHR rats (mean ±S.E.M., n=4). * indicates significant difference ($P<0.05$).

Results

The time course of hexobarbital disappearance in immobilized perfused hepatocytes of Wistar and SHR rats is illustrated in Fig. 1. During the first 10 min of measurements there was a rapid fall of hexobarbital concentration followed by a slower phase in hepatocytes of both Wistar and SHR strains. The concentrations of hexobarbital at most time intervals was, however, significantly lower in the perfusate obtained from SHR as compared to Wistar rats. Thus perfused immobilized hepatocytes of SHR apparently exhibited a higher rate of hexobarbital disappearance. The time course of hexobarbital disappearance in the hepatocyte suspensions prepared from Wistar, SHR, S and R strains is shown in Fig. 2. Hexobarbital disappearance in these hepatocytes did not reveal any significant differences between the four strains.

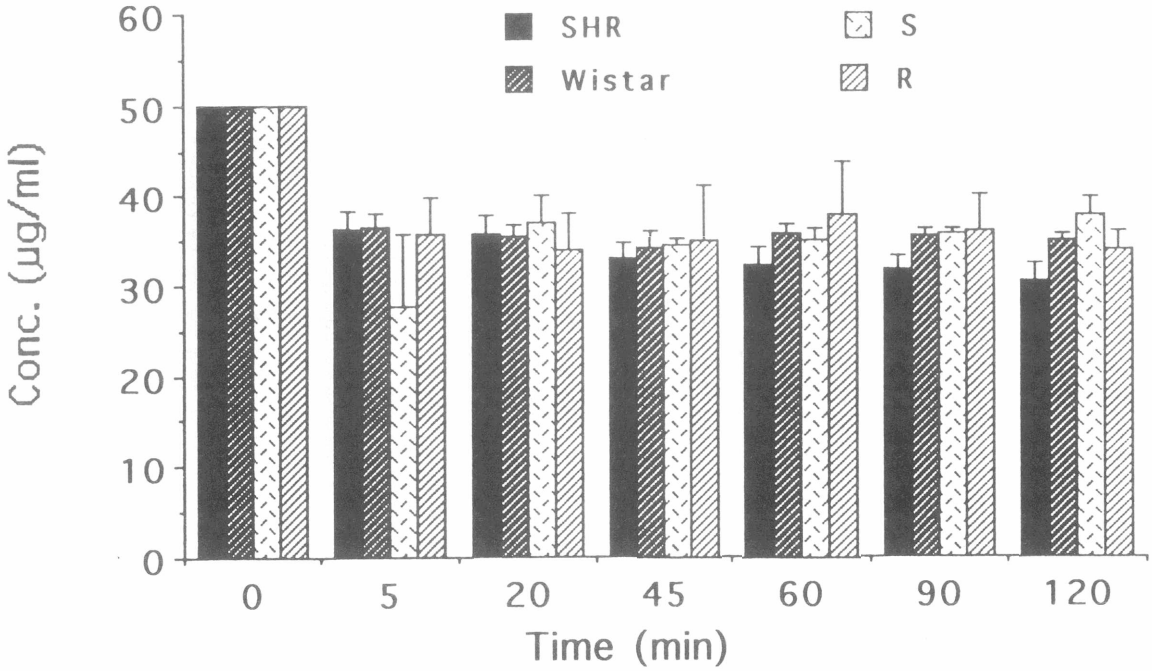

Fig. 2
The time course of hexobarbital disappearance in hepatocyte suspension media of Wistar, SHR, S, and R rats. (mean ±S.E.M., n=6).

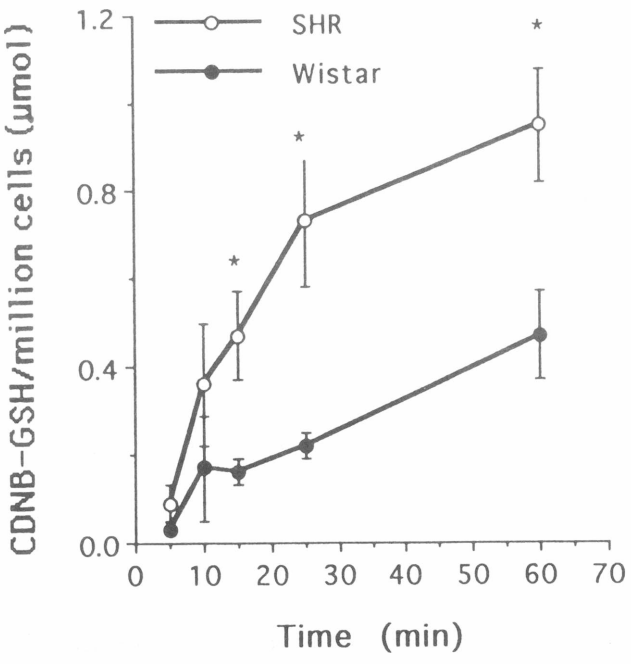

Fig. 3
The time course of the cummulative CDNB-GS conjugate in the perfusion media of immobilized hepatocytes of Wistar and SHR rats (mean ±S.E.M. n=5). * indicates significant difference ($P<0.05$).

The cummulative CDNB conjugation in hepatocytes of Wistar and SHR strains is demonstrated in Fig. 3. Again, SHR liver cells exhibited a higher activity of CDNB conjugation at all time intervals tested. SHR hepatocytes metabolized CDNB more than twice as much as Wistar hepatocytes. Even when hepatocyte suspension was used to compare GST activity, significant differences were observed. Fig. 4 illustrates the time course of the cummulative conjugation of CDNB in suspensions of rat hepatocytes of different strains. At 25 and 60 min incubation, both R and SHR hepatocytes exhibited a higher rate of CDNB conjugation as compared to cells obtained from the Wistar and S strains. However, there was no significant difference between GST activity in SHR and R strains, especially after 60 min incubation.

Discussion

From the very beginning pharmacogenetics have been associated with drug metabolism. Most pharmacogenetic conditions represent genetically determined differences in the rate of drug metabolism. There have been relatively few examples of genetic differences modifying pharmacodynamic properties of the drugs, a notable exception being hereditary resistance to coumarin anticoagulants (Kalow 1962, La Du 1983).

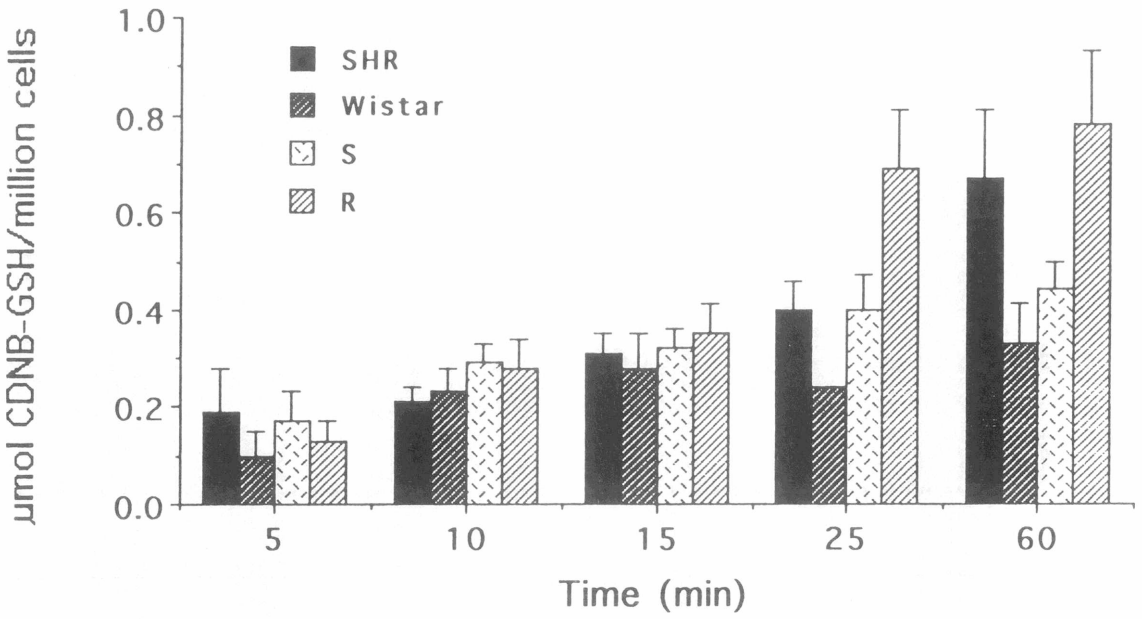

Fig. 4
The time course of the cummulative CDNB-GS conjugate in the hepatocyte suspension media of various rat strains (mean ±S.E.M., n=6).

Most pharmacogenetic conditions shift the concentration of a drug delivered or removed from the site of action; thus these differences are pharmacokinetic rather than pharmacodynamic in nature. Indeed, the present data demonstrate that there are certain cellular pharmacokinetic differences in hexobarbital hydroxylase and GST activities in hepatocytes obtained from Wistar, SHR, R and S strains. These differences can be better demonstrated, especially when using the more physiological model of perfused and immobilized hepatocytes. When hepatocyte suspensions were used for hexobarbital hydroxylation evaluation, no differences in its activity could be encountered between the SHR and Wistar strains. In addition, GST activity was not different in hepatocytes of all the studied strains during the first 30 min of incubation, while this was evident in the bioreactor model. Thus important genetic differences in drug biotransformation can be masked unless an efficient cellular model is used which is reflected in the present study by using the bioreactor as contrasted to cellular suspension in different strains. This study is an attempt to demonstrate difference(s) in xenobiotic biotransformation in some genetically defined rat strains. The significant higher hexobarbital hydroxylase and glutathione transferase activities in hepatocytes obtained from the SHR or R strains as compared to the Wistar or S strains are noteworthy. At present, information about the gene expression of these metabolizing enzymes in the relevant strains is lacking. Therefore, the metabolic capacity of liver cells as obtained from the R and SHR strains versus those obtained from the S or Wistar strains deserves further studies.

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