IMPAIRED ELIMINATION OF PROPRANOLOL DUE TO RIGHT HEART FAILURE: DRUG CLEARANCE IN THE ISOLATED LIVER AND ITS RELATIONSHIP TO INTRINSIC METABOLIC CAPACITY

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ABSTRACT:

It is unclear if reduced hepatic drug elimination in congestive heart failure is primarily due to impairment of enzyme function as a result of tissue hypoxia, to the direct effects of hepatic congestion, or to changes intrinsic to the liver, such as reductions in enzyme content and activity. We therefore compared propranolol clearance in perfused rat livers from animals with right ventricular failure (RVF) with that from control animals. Despite the fact that both groups were perfused at comparable flow rates, perfusion pressures, and levels of oxygen delivery, hepatic extraction of propranolol was significantly reduced in RVF livers (0.688 ± 0.122 versus 0.991 ± 0.006 ml/min/g of liver in controls, P < .001). This effect was reflected in a 97% reduction in propranolol intrinsic clearance in RVF livers (5 ± 4 versus 172 ± 82 ml/min/g of liver in controls, P < .01). In RVF livers, total hepatic CYP expression was reduced by 19% compared with controls, whereas cytochrome P450 isoenzymes 1A1/2 and 2D1 were reduced by 41 and 26%, respectively. Despite the 97% reduction in propranolol intrinsic clearance in perfused RVF liver, intrinsic clearance in microsomal preparations from the same livers was reduced by only 48% compared with controls (P < .05). These findings suggest that impaired propranolol clearance in RVF is not primarily accounted for by reduced hepatic oxygen delivery or by changes in hepatic content and activity of drug-metabolizing enzymes.

A number of studies have provided evidence that hepatic elimination of drugs via oxidative metabolism is impaired in patients with congestive heart failure (Hepner et al., 1978; Ueda and Drindzio, 1978; Baughman et al., 1980; Rissam et al., 1983; Jenne, 1986; Patel et al., 1990; Huber et al., 1992). However, the mechanisms responsible have not been clearly defined. There is considerable evidence from in vitro studies that oxidative drug metabolism is likely to be sensitive to relatively mild reductions in hepatic oxygen supply (Jones, 1981; Angus et al., 1995). Using an experimental model of right ventricular failure (RVF), we have previously examined whether in congestive heart failure there may be a sufficient reduction in hepatic oxygen delivery to result in impairment of hepatic oxidative drug elimination (Ng et al., 1995). We found that in the presence of hepatic congestion due to right ventricular failure, there were significant changes in the splanchnic circulation resulting in decreased perfusion and oxygenation of the liver, and these changes appeared to correlate with the degree of impairment of hepatic drug elimination (Ng et al., 1995). However, other factors, including reduced enzyme content, reduced enzyme activity, or direct physical effects of liver congestion on the hepatocyte, may also be important.

If reduced hepatic oxygen delivery is largely responsible for impaired hepatic drug clearance in RVF, it would be predicted that reduced hepatic drug clearance in vivo would be restored when livers from animals with RVF were perfused with normal amounts of oxygen. Similarly, if some direct effects of hepatic congestion were the most important factor, drug clearance should be returned to normal when the liver was perfused in the absence of obstruction of hepatic venous outflow. In contrast, if drug metabolism remained abnormal when the liver was perfused with normal amounts of oxygen and at normal outflow pressures, changes in drug metabolism in vivo are likely to reflect changes intrinsic to the liver, such as a reduction of hepatic drug-metabolizing enzyme expression and/or activity. Therefore, in the present study, we have removed and perfused livers from animals with RVF and examined the ability of these livers to metabolize drugs under controlled flow and oxygen delivery conditions. Propranolol was chosen as the marker of oxidative drug elimination because the drug is eliminated almost entirely via oxidation by CYP enzymes (Fujita et al., 1993; Masubuchi et al., 1993), is highly extracted by the liver (Fenyves et al., 1993), and has a relatively short half-life, thus allowing quick attainment of steady state.

To determine the contribution of changes in hepatic enzyme content and activity to changes in hepatic propranolol clearance, we compared intrinsic hepatic clearance calculated from data obtained in the iso-
lated perfused rat liver (IPRL) with intrinsic clearance \( (V_{\text{max}}/K_{\text{m}}) \) in microsomes prepared from the same livers. The hepatic expression of total CYP as well as those isoenzymes directly involved in propranolol metabolism (CYP 1A1/2 and 2D1) were also measured.

Materials and Methods

Chemicals. The hydrochloride salt of \( \beta \)-propranolol was purchased from Imperial Chemical Industries Ltd (Cheshire, England). Rat CYP 1A1/2 antibody was a gift kindly donated by Dr. Michael Owens (Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR). Glucose-6-phosphate dehydrogenase and NADP were purchased from Boehringer Mannheim (Mannheim, Germany). \([4^{-3}\text{H}]\)Propranolol (784 GBq/mmol) was purchased from NEN DuPont (Boston, MA), and scintillation cocktail (Ready Organic) was from Beckman Instrument, Inc. (Fullerton, CA). All other chemicals used were analytical grade.

Right Heart Failure Model. Sprague-Dawley rats weighing between 90 and 110 g and aged between 4 and 5 weeks were randomized into sham or pulmonary artery-constricted (PAC) groups. Rats in the PAC group \((n = 9)\) underwent constriction of the pulmonary artery via a left-sided thoracotomy as previously described (Ng et al., 1995). Rats in the sham group \((n = 8)\) underwent the same surgery but without the pulmonary artery constriction. There was no significant difference \((P > 0.05)\) between the initial body weight of the sham \((n = 8, \text{mean weight} 104 \pm 8)\) and the PAC groups \((n = 9, \text{mean weight} 101 \pm 9)\).

IPRL. Experimental preparation. Fifteen to 17 weeks after pulmonary artery constriction or sham operation, rats of the two experimental groups were anesthetized with pentobarbital (60 mg/kg). Before the removal of the liver, the left jugular vein was cannulated, and the mean central venous pressure was measured by an electronic pressure monitor unit (78205A; Hewlett Packard, Palo Alto, CA). Using standard surgical techniques, the common bile duct, the portal vein, and the inferior vena cava were cannulated. The liver was then dissected free from the abdominal cavity and weighed.

Experimental design. Livers were perfused at a constant flow rate, via the portal vein, with a pump that was calibrated on the day of the experiment. Similar flow rates were used in both groups with an average of 1.5 ml/min/g of liver (Table 1). The perfusion was initially in a recirculating mode, which lasted for about 20 min to allow the liver to stabilize and its viability to be assessed, and was followed by 1 h of perfusion using a single-pass design. Both the single-pass and the recirculating perfusate consisted of Krebs-Henseleit buffer (pH 7.4) containing 20% (v/v) washed human red blood cells, 1% (w/v) bovine serum albumin, 0.1% (w/v) glucose, and 30 \( \mu \text{M} \) sodium taurocholate and were maintained at 37°C in a humidified cabinet. This composition of perfusate resulted in a free fraction \( (f_d) \) of 0.66. Single-pass perfusate was spiked with \( \beta \)-propranolol HCl to a concentration of 2 \( \mu \text{g/ml} \) (6.76 \( \mu \text{M} \)). Bile was continually collected into preweighed tubes at 30-min intervals.

The viability of liver preparations was assessed by macroscopic appearance, oxygen consumption, and perfusion pressure. All livers were homogeneously perfused as indicated by the even color of the liver lobes during the perfusion, had oxygen consumption of greater than 4 \( \mu \text{mol/min/g} \) of liver, and had perfusion pressure of no greater than 12 cm of H\(_2\)O at the end of the perfusion and less than 2 cm of H\(_2\)O difference in the perfusion pressure between the hepatic expression of CYP 1A1/2 and 2D1 were also measured.

Measurement of hepatic CYP Enzyme(s) Expression and Activity. Preparation of hepatic microsomes and measurement of total CYP expression. Microsomes were prepared (Aitio and Vainio, 1976) from five livers (2 g each) randomly chosen from the sham group and livers of the PAC rats that had evidence of RVF \((n = 5)\). Microsomal protein content was measured by a standard Lowry assay (Lowry et al., 1951) using bovine serum albumin as the protein standard. Using this method, the mean microsomal protein recovery per gram of liver was 10.8 \( \pm 1.4 \) mg in the sham group and 14.6 \( \pm 2.7 \) mg in the RVF group \((P < 0.05)\). Total CYP concentration in the microsomes was measured by dithiobis-thiocarbamyl spectroscopy as previously described (Matsumura et al., 1976). Total CYP content of the RVF group was then expressed as a percentage of the mean intensity of bands for that isoenzyme from the sham group.

The linearity of the band intensity with protein concentration was checked with a pooled sample of liver microsomes from sham-operated rats. The intensity of the band was found to be linearly related to the amount of protein loaded in each lane over the range of 2.5 and 6 \( \mu \text{g} \) of microsomal protein loaded in each lane (standard amount 3 \( \mu \text{g} \)).

Measurement of Propranolol Intrinsic Clearance in the Microsomes. The decline in propranolol concentration in the microsomal mix was described by a monoexponential decline:

\[
[S] = [S]_0 \cdot e^{-kt}
\]

where \([S]_0\) and \([S]\) is the propranolol concentration at time 0 and time \( t \), respectively, and \( k \) is the exponential rate constant describing the decline in concentration.

Intrinsic clearance of propranolol in the microsomes was measured by dividing the amount of drug in the tube at the initiation of the reaction \(([S]_0\), \( V \), where \( V \) is the volume) by the area under the curve from zero to infinity. Because the decline in concentration was monoexponential, the area under the curve was calculated by dividing the initial concentration by the slope \(( [S]_0/k)\)

\[
CL_{\text{int}} (\text{microsomes}) = \frac{[S]_0 \cdot V}{[S]_0} = \frac{V \cdot k}{k}
\]

The incubation reaction mixture (1-ml reaction volume) consisted of 50 \( \mu \text{L} \) of microsomes (final protein concentration range, 6–20 \( \mu \text{g/ml} \)), 0.505 ml of 1.15% KCl, and 0.4 ml of 0.2 M potassium phosphate buffer (pH 7.4) containing the NADPH-generating system (10 \( \mu \text{M} \) of glucose 6-phosphate, 0.5 \( \mu \text{M} \) of NADP, 2 units of glucose-6-phosphate dehydrogenase, and 8 \( \mu \text{mol} \) of MgCl\(_2\)). The rate of propranolol elimination was found to be linearly related with microsomal protein concentration from 5 to 25 \( \mu \text{g/ml} \). After incubating the mixture under air at 37°C for 3 min, the reaction was commenced by the addition of 45 \( \mu \text{L} \) of the 1:1000 diluted \([4^{-3}\text{H}]\)propranolol.
**Right Heart Failure Model.** At 15 weeks, five of the nine rats that underwent the pulmonary artery constriction developed RVF as evidenced by a 10-fold increase in the mean central venous pressure (Table 1) and an engorged liver at laparotomy. In these animals, there was evidence of cardiac hypertrophy with a mean increase in right ventricular weight of 57%. Although the other four rats that underwent the pulmonary artery constriction also developed cardiac hypertrophy (mean right ventricular weight, 1.18 ± 0.13 g), there was no evidence of RVF as indicated by the near normal mean central venous pressure (3 ± 1 mm Hg) and the absence of hepatic congestion. The RVF rats did not have increased lung weight to suggest pulmonary congestion or edema due to reduction of left ventricular function (Table 1). There was no significant difference in the mean body weight between the sham and the RVF groups.

**Evidence of Hepatic Congestion.** All livers from the five animals with RVF showed macroscopic evidence of hepatic congestion. When examined under the light microscope, livers from these animals showed sinusoidal dilatation and congestion. There were features of hepatocyte degeneration around the pericentral congested regions, but hepatic fibrosis was absent. None of the livers from the sham group showed hepatic congestion under light microscopy. The mean liver weight of the RVF rats was not significantly different from those of the sham group (Table 1).

**Viability of the Isolated Liver Preparation.** Physiological parameters of the isolated perfused livers from the sham and the five RVF rats are summarized in Table 1. The data illustrate that liver preparations in both groups were perfused at similar flow rate and oxygen delivery. All preparations were viable, with no difference in oxygen extraction and consumption between the two groups.

**Extraction and Hepatic Clearance of Propranolol.** The outflow concentration had reached steady state by 20 min in all liver preparations. RVF significantly reduced hepatic extraction of propranolol, as illustrated by markedly elevated outflow perfusate concentrations and reduction of extraction ratio of the drug, from a mean of 0.991 in the sham group to 0.688 in the RVF group, P < .001 (Table 1). In parallel with the reduced extraction ratio, hepatic clearance of the drug in RVF was reduced (1.07 ± 0.21 ml/min/g of liver in RVF versus 1.52 ± 0.25 ml/min/g of liver in sham, P < .01) (Table 1). The mean total CYP expression in livers of the RVF group was 19.1 ± 3.9% less than that in the sham group (P < .05). The mean CYP 1A1/2 and 2D1 contents were reduced by 41.5, 39.2, and 21.3% of the control value, respectively (P < .01).

**Propranolol Intrinsic Clearance.** The mean CLint of propranolol in the IPRL and in the microsomes is summarized in Fig. 1. The fall in mean hepatic clearance of the drug from the control value of 1.52 ml/min/g of liver to the value of 1.07 ml/min/g of liver in the IPRLs from animals with RVF (Table 1) was reflected in a 97% fall in intrinsic clearance (from 257 ± 123 ml/min/g of liver in sham to 7.0 ± 5.9 ml/min/g of liver in RVF; P < .01) when calculated by the venous equilibrium model. However, in microsomes from animals with RVF, the mean CLint (microsomes) was only 48% less than that in shams (6.9 ± 1.2 versus 13.1 ± 2.5 ml/min/mg of microsomal protein; P < .05).

**Discussion**

The present study demonstrates that in the isolated liver, hepatic elimination of propranolol is impaired in livers from animals with right ventricular failure. Hepatic elimination of propranolol is considered to be flow-dependent because the extraction ratio of the drug is very high (>0.9) (Wilkinson and Shand, 1975). Thus, if perfusion of the liver was reduced in heart failure (Ng et al., 1995), it might be expected to result in impairment of hepatic clearance of the drug. The present finding that hepatic clearance of propranolol was significantly reduced in RVF rat livers further supports this notion.
propranolol in RVF cannot be fully accounted for by a reduction in the intrinsic clearance calculated by the venous equilibrium model was decreased in RVF to a much greater extent in the RVF than in microsomes. This suggests that reduced clearance of propranolol in RVF can be fully accounted for by a reduction in the intrinsic metabolic capacity of the enzymes involved in propranolol metabolism.

Reduced propranolol metabolism was associated with a decrease in hepatic oxygen supply that are likely to occur in vivo (Elliott et al., 1993). Indeed, previous studies have shown that hepatic elimination of propranolol is impaired in RVF independent of changes in blood flow. Propranolol is metabolized through four different pathways: ring oxidation, side chain oxidation, glucuronidation, and O-dealkylation (Bargar et al., 1983). Side chain oxidation, which forms N-desisopropylypropranolol, is mainly responsible for propranolol elimination in humans (Fujita et al., 1993; Masubuchi et al., 1993). In humans, 4- and 5-hydroxylation and N-desisopropylation are the major propranolol elimination pathways (Masubuchi et al., 1994; Yoshimoto et al., 1995). Propranolol metabolism may be sensitive to reductions in hepatic oxygenation that occur in heart failure (Ng et al., 1995) because oxygen is utilized directly as a substrate in drug oxidation (Jones, 1981; Angus et al., 1995) and CYP isoforms are predominantly localized in the acinar zone 3 hepatocytes (Baron et al., 1973; Gooding et al., 1978; Ratnasavanh et al., 1991) where hepatic oxygen concentrations are lowest (Lemasters et al., 1981; Matsumura et al., 1986). Indeed, previous studies have shown that hepatic elimination of propranolol is impaired with minor reductions in hepatic oxygen supply that are likely to occur in vivo (Elliott et al., 1993). However, the present finding of markedly reduced hepatic clearance of propranolol in isolated livers from animals with RVF in the face of normal levels of hepatic perfusion and oxygenation, supports the view that reduction in hepatic oxygen delivery that may occur in heart failure (Ng et al., 1995) is unlikely to be the most important determinant of impaired elimination of propranolol.

The level of expression and the activity of the enzymes determine the intrinsic metabolic capacity of the liver. Total expression of CYP and expression of CYP 1A1/2 and 2D1 (Fujita et al., 1993; Masubuchi et al., 1993), the isoforms responsible for propranolol metabolism in rats, were reduced in livers from the RVF animals by 19, 41, and 26%, respectively. The reduction in \( CL_{int} \) of propranolol in liver microsomes (48% reduction; 6.9 versus 13.1 ml/min/mg of microsomal protein) was commensurate with the reduced level of CYP expression. However, the magnitude of the reductions in CYP content and \( CL_{int} \) in liver microsomes from RVF animals was very much less than the reduction in hepatic \( CL_{int} \) observed in the IPRL (97% reduction; 257 ml/min/g of liver in shams to 7.0 ml/min/g of liver in RVF). It is not possible to directly predict drug clearance in the intact liver from microsomal data without using scaling methods derived from healthy rats (Houston and Carlile, 1997) which may not be applicable to the diseased liver. However, the relatively modest reductions in hepatic CYP content and enzyme activity in microsomes from animals with RVF appear insufficient to explain the very large reduction in intrinsic clearance by the intact liver.

There are major differences between the isolated perfused liver and the microsomal preparations that may explain these findings. In the latter, there is no limitation of drug access to the drug-metabolizing enzymes, and all cofactors (such as NADPH) are supplied in excess. Thus, it is possible that in the intact liver in RVF, impaired drug uptake or depletion of cofactors may be primarily responsible for impaired propranolol clearance; because these factors are not rate limiting in microsomes, intrinsic clearance is less severely affected.

Histological examination of the perfused livers from the RVF animals showed that there was sinusoidal dilatation and congestion, which was not relieved after the livers had been removed from their in vivo source of congestion. It is possible that this congestion leads to disturbance of the microcirculation and impaired access of drugs to hepatocytes, as has been suggested to occur in cirrhosis (Gariepy et al., 1993). Also, although fibrosis was not detected by light microscopy in RVF livers, a previous electron microscopic study demonstrated that chronic passive congestion leads to deposition of collagen in the space of Disse and development of a basement membrane (Safran and Schaffner, 1967). It is possible that similar changes occur in RVF and contribute to impaired hepatic drug elimination.

In conclusion, the current study demonstrates that hepatic elimination of propranolol is impaired in RVF independent of changes in hepatic blood and oxygen delivery. The reduction in propranolol elimination could not be solely attributed to reductions in hepatic expression or activity of the drug-metabolizing enzymes, suggesting that other factors such as impaired drug uptake or deficiencies of cofactor supply may be involved.

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PROPRANOLOL CLEARANCE IN RIGHT HEART FAILURE


