Influence of Amino Acids on the Kinetics of Metoprolol Metabolism Using the Isolated, Perfused Rat Liver: A Study Related to the Food Effect

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To

my parents

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Abstract

Metoprolol, 1-[4-(2-methoxyethyl)phenoxy]-3-isopropylamino-2-propanol, is a relatively selective β_1-adrenergic blocking drug. Metoprolol is among several lipophilic, basic drugs which are highly extracted on the first passage through the liver. Its AUC_{oral} is increased by an average of 40% when concomitantly administered with a protein-rich meal. Research during the past two decades has shown that a reduction in the first-pass metabolism of certain lipophilic, highly extracted drugs could be the main factor that accounts for the substantial increase in the AUC_{oral}. By ruling out changes in the splanchnic-hepatic blood flow and the fraction of unbound drug in plasma after the meal, it was speculated that a transient inhibition in hepatic intrinsic clearance may contribute to the food effect. In this research, it was hypothesized that amino acids as released into the portal vein, after ingestion of food, could cause a transient inhibition of metoprolol hepatic metabolism and changes to the pooled V_{max} and K_m values of Michaelis-Menten kinetics of metoprolol metabolism as well as each metabolic pathway.

This research project consisted of two parts. The initial task was to develop an assay method. By using a C_{18} column, a mobile phase consisting of water-acetonitrile-triethylamine (91: 9: 0.3, vol/vol/vol, adjusted to pH 3 with H_3PO_4) and a fluorescence detector with an excitation wavelength of 224 nm and no emission filter, chromatographic resolution was achieved for α-hydroxymetoprolol, *O*-demethylmetoprolol, metoprolol acid, nadolol (used as internal standard) and metoprolol. The quantitization of metoprolol and its three metabolites in aqueous samples was accomplished based on the ratio of peak area or height of analyte to that of nadolol. The calibration ranges for the quantitization of

metoprolol, α-hydroxymetoprolol, *O*-demethylmetoprolol, and metoprolol acid were 0.04 to 20 μg/mL, 0.06 to 4.05 μg/mL, 0.06 to 2.6 μg/mL, and 0.1 to 5 μg/mL, respectively. The analytical method was validated in that the intraday and interday coefficients of variation (CV) for the assay of each analyte at each of three concentration levels were less than 5%. The accuracy for all analytes concerned were in the range of 95 to 105%. The HPLC method developed provided a simple, quick and accurate method for the quantitization of the four analytes.

The isolated, perfused rat liver (IPRL) was used in this research. The perfusion in the IPRL was conducted in three consecutive phases. The perfusion in the IPRL was carried out first with metoprolol-oxygenated Krebs bicarbonate buffer for 50 min, then with metoprolol-Krebs buffer containing metoprolol as well as a mixture of amino acids (diluted from Abbott AMINOSYN* Amino Acids Injection 10%) for 40 min, and finally with Krebs buffer containing only metoprolol for 40 min. Twenty-four male Sprague-Dawley rats were randomly divided into six groups and each was used as a liver donor. Each group was studied at one of six metoprolol inlet concentrations (1.0, 1.2, 1.5, 2.0, 5, and 12.0 μg/mL). From the beginning of the metoprolol infusion, liver outlet samples were collected for 20 seconds at 1, 2.5, 5, 7.5, 10 min and then every 5 min until 130 min. The samples were analyzed for all four analytes.

It was found that within 20 min, the outlet concentrations of metoprolol, α-hydroxymetoprolol, and *O*-demethylmetoprolol reached steady state for all six metoprolol inlet concentrations. The metoprolol acid outlet concentrations rose along the course of infusion and never reached steady state. The amount of metoprolol hepatic tissue binding,

based on the metoprolol outlet concentrations from time zero to time to steady state, was between 17 and 280 nMol/g liver over the range of the inlet concentrations. In contrast with propranolol, the relatively small amount of metoprolol hepatic binding is a favorable characteristic for a drug used to study the food effect.

The analysis of steady-state outlet concentrations of metoprolol and its metabolites in the three phases showed that co-infused amino acids caused immediate, significant, and reversible inhibition of the metoprolol hepatic metabolism. Co-infusion with amino acids caused the metoprolol extraction ratios to significantly decrease by 36% at 1 μg/mL to 54% at 12 μg/mL. Correspondingly, the formation clearances for α-hydroxymetoprolol were decreased by 27% at 1.2 μg/mL to 55% at 5 μg/mL and the formation clearances for *O*-demethylmetoprolol were decreased by 17% at 1.2 μg/mL to 50% at 12 μg/mL. The co-infusion caused the availabilities of metoprolol to significantly increase by 21% at 12 μg/mL to 93% at 1 μg/mL. At the lowest inlet concentration of 1 μg/mL, the 93% increase in the availability of metoprolol is relevant to the human situation in which an average 40% increase in AUC_{oral} of metoprolol was observed when the drug was ingested with food.

Based on the assumptions of the well stirred and parallel-tube models, the reaction velocities versus the metoprolol concentrations were fitted to the Michaelis-Menten equation using PCNONLIN. It was found, regardless of the model used, that the co-infused amino acids decreased the pooled V_{max} values of metoprolol metabolism and the α-hydroxylation and *O*-demethylation pathways by about 50%. No consistent alteration was made to the K_m values in the presence of amino acids. The striking changes to the pooled V_{max} values ruled

out competitive inhibition as a mechanism of the observed effects and suggested that the availability of NADPH and O_2 required in metoprolol metabolism might be limited. Hence, the results in this *in vitro* study implicate nutrients, specificially amino acids in the regulation of metoprolol hepatic metabolism during the food effect.

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Symbols and Abbreviations

AUC	area under the concentration *vs.* time curve
AUC_{oral}	area under the concentration *vs.* time curve after an oral single dose
$C_{portal\ vein}$	concentration of drug in the portal vein
CL	clearance
CL_{int}	hepatic intrinsic clearance
C_{liver}	concentration in the liver water
C_{in}	inflow concentration
C_{out}	outflow concentration
$C_{ss,\ oral}$	the steady-state drug concentration
E	the extraction ratio during drug passage through the liver
EM	extensive metabolizer
F	availability, the fraction of drug dose that escaped (first-pass) metabolism
f_u	fraction of unbound drug in the blood
GC	gas chromatograph(y)
GI	gastrointestinal
HPLC	high performance liquid chromatograph(y)
IPRL	isolated, perfused rat liver
I.S.	internal standard
NADPH	reduced nicotinamide adenine dinucleotide
PM	poor metabolizer

Q_h	hepatic blood flow
Q	liver perfusion rate
SD	standard deviation
SE	standard error of the mean
TEA	triethylamine
$t_{½}$	half-life
t_{max}	time at which C_{max} is reached

Contents

1.0 Introduction

1.1 Interaction between Food and First-Pass Metabolized Drugs

The effect of food on drug absorption and disposition, especially the interaction of orally administered drugs and food, has been recognized for a long time as a major factor of pharmacokinetic and biopharmaceutic variability (Welling, 1977, 1984). Previous observations of a decrease in bioavailability if certain drugs were administered with food were rationalized on the basis of physical binding of the drug to meal components, e.g., tetracycline and/or the degradation of acid labile drugs due to retention in a gastrointestinal region of low pH for a long period of time, e.g., penicillin (Welling, 1977).

After the 1977 finding of a food-related effect on propranolol and metoprolol bioavailabilities, much attention was focused on this food-drug interaction. It was reported that concomitant food intake caused a striking increase in the AUC_{oral} of propranolol and metoprolol by 50% and 40%, respectively, compared with that in the fasted state (Melander et al., 1977). Since then, it has been found that food intake causes a marked increase in the AUC_{oral} of propranolol (Melander et al., 1977), metoprolol (Melander et al., 1977), labetalol (Daneshmend and Roberts,1982), propafenone (Axelson et al., 1987), dixyrazine (Liedholm et al., 1985) and zuclopenthixol (Aaes et al., 1987). For propranolol, the increase in the AUC_{oral} caused by co-intake of food has been confirmed by six teams of researchers (McBride et al., 1980; McLean et al.,1981; Melander et al., 1977; Walle et al., 1981; Liedholm and Melander, 1986; Olanoff et al., 1986), and co-administration of food resulted in a mean increase of 63% in the AUC_{oral} after an oral dose in 47 subjects. Only five subjects

for whom individual data were available exhibited a decrease in the AUC_{oral}. The most pronounced effect of food occurred with propafenone. On average, the AUC_{oral} after administration increased 120% in the fed compared with the fasted state (Axelson et al., 1987). In one subject, the increase was more than 600%. The increase in propafenone's AUC_{oral} was greatest in efficient metabolizers and insignificant in poor metabolizers of debrisoquine.

The increase in AUC_{oral} caused by co-intake of food (the "food effect") appears to primarily occur with drugs which are subject to high first-pass metabolism and which are weakly basic, lipophilic (Melander and McLean., 1983). However, not all high first-pass metabolized drugs are affected by food in this way. The AUC_{oral} of nortriptyline and zimelidine were not affected by food co-ingestion (Melander et al., 1986; Wahlen et al., 1984).

1.1.1 Mechanisms of the Food Effect

The AUC_{oral} of orally administered drugs, which reflects systemic bioavailability of drugs, is determined by a series of complex processes that are affected by many factors, including the physicochemical properties of the drug, gastrointestinal transit time, and intestinal and hepatic metabolism. Several mechanisms for the food effect have been suggested. The increased AUC_{oral} could result from:

1. Increased absorption from the gut;
2. Decreased removal of drug from liver during the initial transfer from gut to the systemic circulation (first-pass metabolism);

3. Decreased rate of drug removal after entry into the general circulation (systemic clearance).

After oral administration of a drug, the AUC_{oral} reflects the amount of drug that reaches the systemic sampling site. Incomplete gastrointestinal absorption and first-pass elimination are primarily responsible for a bioavailability of less than unity. For drugs subject to high first-pass metabolism, the bioavailability (the relative amount of administered dose that reaches the systemic circulation) is different from the extent of drug absorption from the gastrointestinal tract. Following ingestion, drugs must be dissolved in gastrointestinal fluids to be absorbed. As weak bases or acids, drugs usually exist in various states of ionization. The extent of gastrointestinal absorption depends upon the relative degree of ionization and degree of lipid solubility. Because of these fundamental characteristics, it is generally found that in the fasting state these lipophilic drugs which have the food effect are virtually completely absorbed from the gastrointestinal tract lumen into the splanchnic circulation. For instance, the extent of absorption has been reported to be almost complete for propranolol and metoprolol (90% and 95% of dose, respectively, Shand and Rango, 1972; Shand, 1974; Regardh et al., 1974; Ervik, 1975; McBride et al.,1980). The observations of virtually complete gastrointestinal absorption of propranolol and metoprolol invalidate the first possible mechanism that the increased bioavailability is due to increased extent of absorption. Even if there had been enhancements to the extent of propranolol or metoprolol absorption due to food co-ingestion, the potential quantitative influence of such an effect by food on the extent of absorption would be much less than the average change of 60% in AUC_{oral} documented for these drugs. In the case of ^{14}C-labeled propranolol co-

administered with food (McLean et al., 1981), radioactive recovery from urine indicated that the absorption of propranolol was 85% and showed little change after food. Hence, changes in absorption could not account for the substantial increase in AUC_{oral} or bioavailability. The complete absorption of propafenone in man (Connolly et al., 1984) suggests that the conclusion about the extent of absorption for other compounds would most likely be similar (Melander et al., 1988).

In the food effect phenomenon, it was found that the plasma/serum concentrations of these drugs showing the food effect were higher than in the fasting state after oral administration with a protein-rich meal, in both the absorption and post-absorption phases. The enhanced plasma/serum drug concentrations could result from the decreased apparent systemic clearance due to the presence of food. However, in the cases of propranolol (McBride et al., 1980; McLean et al., 1981; Melander et al., 1977; Walle et al., 1981; Liedholm and Melander, 1986; Olanoff et al., 1986), metoprolol (Melander et al., 1977) and propafenone (Axelson et al., 1987), the $t_{1/2}$ in the post-absorption phase was almost identical in both conditions. Furthermore, no change in plasma protein binding of propranolol after a meal was documented (Feely et al., 1983); similar results were observed with lidocaine (Elvin et al., 1981).

The intrinsic capacity of the liver to remove these drugs showing the food effect from the portal circulation is very high. Substantial first-pass metabolism leads to the small fraction of the dose which reaches the systemic circulation. For example, as little as 25% of propranolol and 30% of metoprolol entering the liver via the portal circulation would reach the hepatic vein and then the general systemic circulation.

Based on the data obtained so far, it can be reasoned that the most likely mechanism of the food effect is a reduction in first-pass hepatic metabolism of these highly extracted drugs during the initial transfer from the gut to the systemic circulation. This reasoning now seems to be shared by most of the researchers on this topic.

1.1.2 Possible Mechanisms of the Reduction in First-Pass Metabolism

Physiological models describing drug removal by the liver made it possible to elucidate the mechanisms for the reduction in the first-pass hepatic metabolism of highly extracted drugs. The characteristics of the hepatic models for theoretical assessment of hepatic drug removal have been critically reviewed by Wilkinson (Wilkinson, 1987) and Morgan (Morgan and Smallwood, 1990). The two most studied models are the well-stirred model (the venous equilibrium model) and the parallel-tube model (the undistributed sinusoidal perfusion model). In these models, the hepatic sinusoids are assumed to be anatomically and functionally homogeneous.

The well-stirred model is an empirical model in which the liver is conceived as a single, well-stirred compartment. The basic assumptions of the well-stirred model are instantaneous, uniform drug distribution and therefore all cells are exposed to a concentration of free substrate which is in equilibrium with the venous outflow concentration (C_{out}). In other words, the concentration of substrate throughout the hepatic compartment is assumed to be uniform and equal to the hepatic outflow concentration ($C_{liver} = C_{out}$). Although this assumption does not have a physiological basis, the model has been justified in terms of its

ability to simply and accurately relate hepatic clearance with its 3 determinants (Wilkinson, 1987; Morgan and Smallwood, 1990):

$$CL_h = \frac{Q_h f_u CL_i}{Q_h + f_u CL_i} \quad (1)$$

where Q_h is liver blood flow rate, CL_i is the intrinsic clearance of drug from liver water, f_u is the fraction of drug unbound in the blood, and CL_h is the hepatic clearance.

In the parallel-tube model, the liver is viewed as a set of identical tubes representing the sinusoids, which are arranged in parallel to each other with drug metabolizing enzymes distributed evenly along them. Blood flow is assumed to be undistributed in the sense that it is the same in all the tubes. Uptake by the hepatocytes is a function of concentration, and the greatest uptake takes place at the portal venous end of the tubes. Concentration, and therefore uptake, is held to decline monoexponentially along the tubes in the direction of flow. At any point along the tubes, unbound drug species in the sinusoids and hepatocytes are in equilibrium. In this model, the average concentration of substrate within the liver is taken as the logarithmic average of inflow and outflow concentrations (C_{liver}= $\ln(C_{in}/C_{out})/(C_{in}-C_{out})$) (Wilkinson, 1987; Morgan and Smallwood, 1990).

$$CL_h = Q_h(1 - e^{-f_u CL_i/Q_h}) \quad (2)$$

The well-stirred and parallel-tube models represent the extremes in the average drug concentration assumed to apply in the liver (i.e., logarithmic average of inflow and outflow

at one extreme, and outflow at the other). Therefore, these contrasting assumptions lead not only to many quantitative differences in behaviour between the well-stirred and parallel-tube models but also to diametrically opposed predictions as to the influence of alterations in hepatic blood flow both on the AUC_{oral} and on the steady-state drug concentration ($C_{ss, oral}$) during constant oral dosage delivery by this route (Pang and Rowland, 1977).

In the well-stirred model, E, F, AUC_{oral}, and $C_{ss, oral}$ are given by the following equations:

$$E=\frac{f_u CL_i}{Q_h+f_u CL_i} \tag{3}$$

$$F=\frac{Q_h}{Q_h+f_u CL_i} \tag{4}$$

$$AUC_{oral}=\frac{Dose}{f_u CL_i} \tag{5}$$

$$C_{ss,oral}=\frac{Dose}{\tau f_u CL_i} \tag{6}$$

where CL_i represents intrinsic hepatic clearance, f_u represents unbound fraction, and τ represents the dosing interval. This model concedes that hepatic extraction is sensitive to changes in hepatic blood flow, decreasing as the flow increases. The prediction of flow independence of AUC_{oral} and $C_{ss, oral}$ with this model is explained by the availability (F) and the hepatic clearance (CL) varying in direct proportion to flow, thus making the ratio F/CL

flow independent. Any change in oral bioavailability of high clearance drugs must then be a reflection of altered enzyme activity within the liver (i.e. a change in CL_i or f_u).

On the other hand, the corresponding relationships based on the parallel-tube model are:

$$E = 1 - e^{-f_u CL_i / Q_h} \tag{7}$$

$$F = e^{-f_u CL_i / Q_h} \tag{8}$$

$$AUC_{oral} = \frac{Dose(e^{-f_u CL_i / Q_h})}{Q_h(1 - e^{-f_u CL_i / Q_h})} \tag{9}$$

$$C_{ss,oral} = \frac{Dose(e^{-f_u CL_i / Q_h})}{\tau Q_h(1 - e^{-f_u CL_i / Q_h})} \tag{10}$$

The parallel-tube model dictates an exponential dependence of hepatic extraction on blood flow, and thus the AUC_{oral} and $C_{ss,\ oral}$ predicted by the approach would be vastly different to those predicted by the well-stirred model, especially for highly first-pass metabolized drugs (Pang and Rowland, 1977). Indeed, the parallel-tube model predicts sensitivity of oral bioavailability to hepatic blood flow. According to this model, changes in oral bioavailability of high clearance drugs evoked by possible external or internal factors could be caused by alterations in hepatic blood flow, hepatic enzyme activity, or in plasma protein binding.

Identification of the mechanism of the increase in AUC_{oral} in the food effect, however, relies on the use of a model of hepatic elimination. According to the well-stirred model, the

increase in AUC_{oral} could arise from changes in CL_i or f_u (equation 5), but the parallel-tube model indicates that it could be due to all of the 3 determinants: Q_h, CL_i, and f_u (equation 9). Because of limitations on experimentation, there are not enough data to judge which hepatic model is better suited for the removal of drugs involved in the food effect even though there is supportive evidence that the well-stirred model precisely describes the hepatic elimination of propranolol (Jones et al., 1984). The study of these proposed physiological models, however, is of great assistance in exploring possible factors contributing to the reduction in first-pass metabolism in the presence of food. By exclusion of the change in the drug plasma protein binding (Feely et al., 1983), the reduction in first-pass metabolism is attributed to the alterations in Q_h and/or CL_i. Extraction ratio is more sensitive to flow changes in the parallel-tube model than in the well-stirred model.

1.1.3 Role of Hepatic Blood Flow (Q_h) in First-Pass Metabolism

Previous studies on the mechanisms of increased oral bioavailability have largely focused on a possible role of increased splanchnic blood flow after a meal. It has been shown that in man estimated splanchnic blood flow is enhanced from 1160 to 1570 $mL/min/m^2$ during the first hour after a meal of high-protein liquid and starts to return 30 min later (Brandt et al., 1955). The first hypothesis to explain the increased AUC_{oral} of propranolol caused by the co-ingestion of food was a transient increase in splanchnic blood flow rate (of which Q_h is a part) in response to a protein-rich meal during drug absorption (McLean et al., 1978). Because the hepatic extraction ratio is a monotonic decreasing function of Q_h according to the well-stirred model (McLean et al., 1978) or a nonlinear

decreasing function of Q_h according to the parallel-tube model, a transient increase in hepatic blood flow could cause more drug to survive hepatic first-pass metabolism during the initial passage from gut epithelium to the systemic circulation. Once the drug had entered the general circulation, much of the increased "dose" of drug would be eliminated from the body after Q_h had returned to near basal levels (i.e., an increased real "dose" coupled with time-averaged near normal clearance). This phenomenon evokes a mechanism involving hemodynamic changes including mesenteric vasodilation. This flow hypothesis is only a speculation. Although the mechanism by which food increases Q_h is unknown, there is considerable evidence that such transient changes in Q_h do occur after eating in human (Brandt et al., 1955; Orrego et al., 1965; Marigold et al., 1981). Computer simulations indicate that enhanced bioavailability is achieved without the systemic clearance being changed by setting a higher level of hepatic blood flow in the absorption phase, and is speculated as the mechanism for the food effect (McLean et al., 1978). Qualitatively, the role of hepatic blood flow in hepatic plasma clearance is well supported by the evidence that the kinetics of intravenously administered lidocaine and propranolol have been shown to be highly dependent on hepatic blood flow (Elvin et al., 1981; Feely et al., 1983; Olanoff et al., 1986). In addition, the fact that the vasodilator, hydralazine, which is known to enhance splanchnic blood flow, also enhances propranolol oral bioavailability is consistent with this hypothesis (McLean et al., 1980; Jackman et al., 1981; Heinzow et al., 1984; Schneck and Vary, 1984). The results of computer simulations (McLean et al., 1978), using unrealistically high hepatic blood flows (such as change of Q_h from 1.5 to 2.5 and 4.5 L/min), do not truly represent the actual magnitude of the food effect observed. This hypothesis was undermined

by later investigations. Taking into consideration the role of changes to hepatic blood flow in the food effect, calculations for propranolol show that the food-induced increase in hepatic blood flow would cause only a 14% increase in AUC_{oral} according to the well-stirred model, and that the flow-induced increase in AUC_{oral} would be 30% according to the parallel-tube model (Svensson et al., 1983). It was shown that the magnitude of postprandial change in hepatic blood flow was not high enough to trigger the observed 50% increase in AUC_{oral} of propranolol.

The breakfast used in previous studies of the food effect consisted of 150 ml low fat milk, 100 ml orange juice, 1 egg, 2 pieces of toasted bread, 5 g margarine, 20% orange marmalade, 20 g cheese and 100 ml non-sweetened black coffee or tea. This equalled 20 g (20%) protein, 17 g (35%) fat and 50 g (45%) carbohydrates and a total energy of 440 calories and is treated as a protein-rich meal (Melander et al., 1977; Liedholm and Melander, 1986; Axelson et al., 1987). Similar protein-rich meals or steak were served in experiments by others (Walle et al., 1981; Olanoff et al., 1986; Feely et al., 1983). However, when propranolol was taken with carbohydrate-rich meal such as cooked potatoes, which is presumed not to have the effect on splanchnic blood flow that protein-rich food does, an average 40% increase of propranolol AUC_{oral} over the fasting state was still achieved (McLean et al., 1981; Jackman et al., 1981) (Note: the baked potatos served in the study are not pure carbohydrate, but contain some protein). It was previously found that 100 gm glucose had no effect on splanchnic blood flow while a protein-rich meal increased splanchnic blood flow up to an average of 35% within the first hour (Brandt et al., 1955). Similarly, a protein-rich meal was observed to increase estimated Q_h an average of 91% 2