Systemic and Splanchnic Hemodynamic Effects of Molsidomine in Rats with Carbon Tetrachloride–induced Cirrhosis

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Molsidomine, a long-acting vasodilator mainly used as an antianginal agent, was reported to decrease the portohepatic venous pressure gradient in patients with alcoholic cirrhosis. This study investigated the effects of linsidomine, the active metabolite of molsidomine, on systemic and splanchnic hemodynamics in rats with CCl_4-induced cirrhosis using the microsphere technique. Compared with placebo-treated rats, linsidomine-treated animals were found to have a significant decrease in portal venous pressure (−18%, $p < 0.01$) and in mean arterial pressure (−16%, $p < 0.01$), smaller peripheral resistances ($p < 0.01$), greater portal venous inflow ($p < 0.05$), smaller splanchnic arteriolar resistances ($p < 0.01$) and smaller portocollateral resistances ($p < 0.05$). Cardiac output, hepatic arterial blood flow, portal blood flow and estimated hepatic blood flow were not significantly different between the two groups of animals. Linsidomine-treated rats exhibited a trend toward greater collateral blood flow compared with controls, but this difference was not significant. We conclude that linsidomine decreases portal venous pressure by reducing portocollateral resistances without affecting liver blood flow. These effects should be beneficial for patients with cirrhosis and portal hypertension. (HEPATOLOGY 1991;13: 1181-1184.)

Molsidomine was reported to decrease portohepatic venous pressure gradient (PHVPG) in patients with alcoholic cirrhosis (1, 2). This vasodilator has a predominant effect on the venous system and is used mainly as an antianginal agent.

The mechanisms of its PHVPG-reducing effect are not known. PHVPG decrease could be a result of the reduction of mean arterial pressure (MAP). As a vasodilator molsidomine could also decrease splanchnic resistances, portocollateral resistances or both. Finally, as reported when using organic nitrates (see "Discussion"), this drug could elicit splanchnic arteriolar vasoconstriction as a resultof sympathetic reflexes in response to venous pooling of blood.

To clarify this issue, we studied the effects of linsidomine (Corvasal Intracoronaire, Hoechst AG, Frankfurt, Germany), the active metabolite of molsidomine, on systemic and splanchnic hemodynamics in rats with CCl_4-induced cirrhosis.

MATERIALS AND METHODS

The study was performed using male Sprague-Dawley rats (IFFA- CREDO, L'Arbresle, France). Cirrhosis was induced by phenobarbital and CCl_4 as described by Proctor and Chatamra (3). Hemodynamic studies were performed 1 wk after discontinuation of both agents. Only rats with cirrhosis (a total of 25) were used for this study.

Surgical Procedure. After an overnight fast with free access to water, the rats were anesthetized with intramuscular ketamine HCl (100 $mg \cdot kg^{-1}$ body wt). Rectal temperature was maintained at 37° ± 0.5° C by means of a heating lamp. A polyethylene catheter (PE-50, Becton Dickinson, Parsippany, NJ) was advanced through the right carotid artery into the left ventricle under continuous pressure recording; it was also used for microspheres injection. After the laparotomy, an ileocolic vein was catheterized with a PE-50 tube to measure portal venous pressure (PVP) and to inject microspheres into the portal vein. This catheter was fixed to the mesentery using cyanoacrylate glue, and the abdomen was closed with surgical sutures.

A third PE-50 catheter was pushed in the left jugular vein and then advanced into the right atria for pressure measurements and medication infusion. Finally, the left femoral artery was cannulated for MAP measurement. The catheters were connected to highly sensitive pressure transducers (P23XL, Gould Electronique, France).

Blood pressures were registered with a multichannel recorder (13-4615-50, Gould Electronique, Ballainvilliers, France). The external zero reference for all pressure measurements was placed at the level of the heart.

Three rats died during this procedure.

Microsphere Technique. Cardiac output (CO) was determined using the radioactive microsphere technique (4-6). A reference sample was withdrawn from the femoral artery catheter for 75 sec at an approximate rate of 1 $ml \cdot min^{-1}$ using a continuous withdrawal pump. Fifteen seconds after the withdrawal started, 40,000 to 60,000 ^{141}Ce-radiolabeled microspheres (15 ± 3 μm diameter, 10 $mCi \cdot gm^{-1}$ specific

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TABLE 1. Pressure measurement in rats with CCl_4-induced cirrhosis[a]

Hemodynamic parameter	Placebo-treated animals		Linsidomine-treated animals	
	Before	After	Before	After
PVP	13.1 ± 2.33	13 ± 2.6	15.5 ± 3.2	12.7 ± 2.6[b]
MAP	113.1 ± 14.0	116.7 ± 14.3	110.0 ± 6.9	92.1 ± 9.2[b]
RAP	1.6 ± 1.3	1.2 ± 1.4	1.2 ± 0.9	0.7 ± 1.3

[a]Measured in mm Hg.
[b]$p < 0.01$ vs. baseline value.

activity, Dupont New England Nuclear Research Products, Boston, MA) were injected into the left ventricle.

Portosystemic shunting (PSS) was assessed using ^{51}Cr-labeled microspheres (15 ± 3 μm diameter, 31 $mCi \cdot gm^{-1}$ specific activity, Dupont New England Nuclear Research Products) injected into the portal vein (7).

After the rats died, the heart, lungs, kidneys, testes and splanchnic organs were dissected. The radioactivity of each organ and of the reference sample was calculated using a gamma counter (Minaxi Gamma-5000 series, Packard Instrument Co., Rungis, France). The ^{51}Cr radioactivity (energy window = 240 to 400 KeV) interference into the ^{141}Ce channel (energy window = 100 to 165 KeV) was corrected using ^{51}Cr and ^{141}Ce standards (6, 8).

Experiments were discarded whenever less than 400 microspheres were found in the withdrawn blood (three rats) and/or more than a 10% difference in radioactivity was found between the two kidneys and/or testes (four rats).

Calculations. CO and regional blood flows were calculated from the ^{141}Ce counting as: injected radioactivity (cpm) × reference blood flow ($ml \cdot min^{-1}$)/reference blood radioactivity (cpm). To calculate regional blood flows, injected radioactivity was replaced in the previous equation by the radioactivity of each organ. Portal venous inflow (PVI) ($ml \cdot min^{-1} \cdot 100$ gm body wt^{-1}) was defined as the sum of stomach, spleen, small and large intestines, pancreas and mesentery arterial blood flows. PVI therefore represents the total blood flow entering the portal venous system. Collateral blood flow (CBF) ($ml \cdot min^{-1} \cdot 100$ gm body wt^{-1}) was estimated as PVI × PSS (%)/100. Portal blood flow (PBF) to the liver ($ml \cdot min^{-1} \cdot 100$ gm body wt^{-1}) = PVI − CBF. Estimated hepatic blood flow (EHBF) ($ml \cdot min^{-1} \cdot 100$ gm body wt^{-1}) was calculated as: hepatic arterial flow (HAF) ($ml \cdot min^{-1} \cdot 100$ gm body wt^{-1}) + PBF.

Resistances in various vascular beds were calculated according to the general formula:

$$R\ (mm\ Hg \cdot ml^{-1} \cdot min^{-1} \cdot 100\ gm\ body\ wt^{-1}) = \Delta P\ (mm\ Hg)/Q\ (ml \cdot min^{-1} \cdot 100\ gm\ body\ wt^{-1})$$

where R = resistances, ΔP = pressure gradient across the vascular territory and Q = blood flow in this territory.

For peripheral resistances, ΔP = MAP − right atrial pressure (RAP); Q = CO. Splanchnic arteriolar resistances (SARs) were calculated as: (MAP − PVP)/PVI. For portocollateral resistances (i.e., the sum of resistances within the collateral vessels and within the portohepatic vascular bed), ΔP = PVP − RAP; Q = PVI. Portal venous resistances were calculated as: PVP − RAP = ΔP, and PBF = Q.

Experimental Design. Cirrhotic rats were randomly assigned to two groups: group 1 was injected with linsidomine ($0.6\ mg \cdot kg$ body wt^{-1}); group 2 was injected with the same quantity of normal saline.

After the animals recovered from surgery, we obtained baseline values of MAP, PVP and RAP over 10 min. Ten minutes after the injection of linsidomine or normal saline, microspheres were injected into the left ventricle and the portal vein. The animals were then killed using saturated intravenous KCl. Animal protocols conformed to the guidelines of the Institut national de la Santé et de la Recherche Médicale.

Statistical Analysis. Results were expressed as mean ± S.D. Comparisons were performed using the Mann-Whitney U test or paired t test as required. Correlations were assessed using Spearman's rank correlation coefficient; $p < 0.05$ was considered the significance level.

RESULTS

The two groups of rats had comparable basal values for hemodynamic parameters (Table 1). Linsidomine significantly lowered MAP (−16%, $p < 0.01$) and PVP (−18%) (Table 1). Compared with placebo-treated animals, linsidomine-treated rats were found to have significantly smaller peripheral vascular resistances ($p < 0.01$), greater PVI ($p < 0.05$), smaller SARs ($p < 0.01$) and smaller portocollateral resistances ($p < 0.05$) (Table 2).

In the group of linsidomine-treated animals, no correlation was found between MAP decrease and PVP decrease. However, SARs and PVI were inversely correlated ($r = 0.786$, $p < 0.05$).

CO, HAF, PBF, EHBF and portohepatic resistances were not significantly different between the two groups of animals (Table 3). Linsidomine-treated rats were found to have a trend toward greater CBF as compared with controls (Table 2). However, owing to a large dispersion of data, the difference did not reach the level of statistical significance.

DISCUSSION

To assess the mechanism of the molsidomine-induced decrease in PHVPG, we investigated its effects on splanchnic and systemic hemodynamics in rats with CCl_4-induced cirrhosis using microspheres to measure regional blood flows.

All rats were found to have cirrhosis and portal hypertension. However, portocollateral shunting was low (mean shunting in placebo-treated rats = 2.7% ± 2.3%). Most experimental studies on portal hypertension in rats were performed using the portal-vein–ligated model. However, this latter model is unsuitable for assessing the effects of vasodilators on portohepatic resistances. Because such an action is supposed to be the most relevant in the PVP-reducing effect of vasodilators,

TABLE 2. Effects of linsidomine on systemic and splanchnic hemodynamics in rats with CCl_4-induced cirrhosis

Hemodynamic parameter	Placebo-treated animals	Linsidomine-treated animals	p
Peripheral-vascular resistances (mm Hg · ml^{-1} · min^{-1} · 100 gm body wt^{-1})	5.730 ± 2.462	3.379 ± 1.461	< 0.01
SARs (mm Hg · ml^{-1} · min^{-1} · 100 gm body wt^{-1})	1.813 ± 0.705	0.847 ± 0.269	< 0.01
PVI (ml · min^{-1} · 100 gm body wt^{-1})	4.276 ± 1.812	7.096 ± 1.910	< 0.05
Portocollateral resistances (mm Hg · ml^{-1} · min^{-1} · 100 gm body wt^{-1})	0.202 ± 0.779	0.127 ± 0.497	< 0.05
CBF (ml · min^{-1} · 100 gm body wt^{-1})	0.145 ± 0.175	1.273 ± 1.389	NS

TABLE 3. Effects of molsidomine on CO, HAF, PBF, EHBF and portohepatic resistances in rats with CCl_4-induced cirrhosis

Hemodynamic parameter	Placebo-treated rats	Linsidomine-treated rats	p
CO (ml · min^{-1} · 100 gm body wt^{-1})	22.862 ± 7.712	30.941 ± 11.778	NS
HAF (ml · min^{-1} · 100 gm wt^{-1})	1.829 ± 0.639	2.215 ± 0.842	NS
PBF (ml · min^{-1} · 100 gm body wt^{-1})	4.131 ± 1.656	5.820 ± 1.749	NS
EHBF	5.960 ± 2.085	8.035 ± 1.991	NS
Portohepatic resistances (mm Hg · ml^{-1} · min^{-1} · 100 gm body wt^{-1})	3.161 ± 1.066	2.378 ± 1.355	NS

NS = not significant.

we believe these drugs should be tested in cirrhotic animals. It can be argued that different types of experimental cirrhoses have been shown to exhibit different kinds of hemodynamic reactions after vasoactive drug administration (9). Our results may therefore be model dependent. However, CCl_4-induced cirrhosis is a micronodular cirrhosis as is alcohol-induced cirrhosis.

Molsidomine is a pro-drug metabolized by the liver in linsidomine, an active metabolite (10). For this experimental study, animals were given intravenous linsidomine because the pro-drug is not available for parenteral administration. The dose we used was determined so as to decrease MAP because molsidomine was reported to lower PHVPG in humans with alcoholic cirrhosis at doses that reduced MAP (1, 2) but had little or no effect at lower doses (2).

In this study, linsidomine was shown to reduce both PVP (or PHVPG) and MAP; however, these two parameters were not correlated. This is in accordance with findings for molsidomine-treated patients with alcoholic cirrhosis (1, 2). This indicates that the PVP-reducing effect of linsidomine cannot be totally ascribed to the hypotensive action of the drug. Actually, SARs were decreased. As a consequence, PVI increased. Such hemodynamic changes should have increased PVP. However, PVP was found to be reduced because the rise in PVI was overcompensated by a drop in portocollateral resistances. EHBF (i.e., the sum of portal and arterial hepatic blood flows) remained unchanged. This is in accordance with previously published studies that showed that organic nitrates (11-14) and molsidomine (1, 2) decreased PVP without any reduction in total liver blood flow. Accordingly, intrinsic hepatic clearance of indocyanine green was not lowered after molsidomine ingestion in patients with alcoholic cirrhosis (1).

A trend toward an increase in CBF was observed, but it was not statistically significant. Increased PVI and increased CBF could be detrimental in patients with cirrhosis because pressure in collateral veins, in particular in esophageal varices, might increase. Such a phenomenon could be overcome by the decrease in portocollateral resistances. This, however, clearly needs to be assessed by direct measurement of variceal pressure in patients with cirrhosis.

Linsidomine was injected at a dose that elicited a 16% decrease in MAP. Using such a dose, we observed a reduction of SARs. This result is in accordance with the mechanism of action of organic nitrates. Actually, organic nitrates were shown to reduce portal pressure in portal vein–ligated rats and in humans (15). At low doses, their action was ascribed to a rise in SARs secondary to sympathetic baroreflex vasoconstriction elicited by arterial pressure reduction (16). At higher doses, nitrates were found to dilate splanchnic arteries. (16).

Finally, our results raise the question of associating molsidomine with β-blockers. If the portocollateral resistances' reducing effect of molsidomine is associated with the splanchnic vasoconstricting action of β-blockers, these two kinds of drugs should potentiate each other to reduce PVP. However, the splanchnic arterial resistances' decreasing action of molsidomine could counterbalance the α-1–adrenergic receptors' mediated splanchnic arteriolar vasoconstriction induced by β-blockers. This issue should also be addressed for patients with cirrhosis.

We conclude that linsidomine decreases PVP by reducing portocollateral resistances without affecting liver blood flow. These effects should be beneficial for patients with cirrhosis. However, a trend toward an increase in CBF was also noted. The consequences of this action on pressure in esophageal varices need to be checked before molsidomine can be recommended for the treatment of portal hypertension.

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