Lack of effect of verapamil and isosorbide dinitrate on the hepatic clearance of indocyanine green in cirrhosis

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1 Vasodilators are currently under investigation as possible therapeutic agents in the treatment of portal hypertension. Among them calcium-channel antagonists and organic nitrates have been of particular interest. Few and conflicting data, however, have been reported on their effect on liver function.

2 Twenty patients with biopsy-proven alcoholic cirrhosis were studied before and 25–35 min following the acute administration of 5 mg verapamil i.v. (eight patients) or 5 mg isosorbide dinitrate sublingually (12 patients). The plasma clearance of indocyanine green (ICG) and its intrinsic hepatic clearance were used as indices of liver function. Seven further patients were investigated before and after a placebo infusion according to the same methodology.

3 No significant change in ICG kinetics was found after verapamil or isosorbide dinitrate.

Keywords liver cirrhosis portal hypertension indocyanine green liver metabolism

Introduction

The ideal drug for the treatment of portal hypertension should decrease portal pressure without affecting liver function. Recently, vasodilators such as organic nitrates (Blei *et al.*, 1988; Freeman *et al.*, 1985; Groszmann *et al.*, 1982; Hallemans *et al.*, 1983) and calcium-channel antagonists (Freeman *et al.*, 1985; Kong *et al.*, 1986; Reichen *et al.*, 1986) have been investigated as possible therapeutic agents in portal hypertension. They were reported to decrease portal pressure both in experimental animals (Blei & Gottstein, 1986; Groszmann *et al.*, 1982; Reichen *et al.*, 1986) and in patients with cirrhosis (Freeman *et al.*, 1985; Garcia-Tsao *et al.*, 1987; Groszmann *et al.*, 1982; Hallemans *et al.*, 1983; Kong *et al.*, 1986; Merkel *et al.*, 1987a; Staritz *et al.*, 1985), and are currently being evaluated either in combination with vasopressin in the treatment of acute variceal bleeding (Gimson *et al.*, 1986; Tsai *et al.*, 1986), or as chronic therapy for preventing variceal bleeding (Freeman *et al.*, 1985; Garcia-Pagan *et al.*, 1988; Kong *et al.*, 1986).

However, improvement (Kong *et al.*, 1986; Miotti & Reichen, 1985; Reichen & Le, 1986), or impairment (Lay *et al.*, 1988), or no effect (Navasa *et al.*, 1988) on liver function have been alternatively reported in patients receiving verapamil. Organic nitrates have been also reported to impair liver function in normal man (Svensson *et al.*, 1985) and in animals with experimental cirrhosis (Gelman & Ernst, 1982).

The aim of this study was to investigate the effect of verapamil and isosorbide dinitrate (ISDN), on liver function in patients with cirrhosis, using the plasma and intrinsic hepatic clearance of indocyanine green (ICG) as markers of liver function.

Methods

Twenty patients with biopsy-proven alcoholic cirrhosis were studied. All admitted drinking at least 100 g per day of alcohol for 5 years or longer. At the time of the study all patients had

Table 1 Clinical and biochemical data of the patients with cirrhosis

Case	Age (years)	Ascites	Previous g.i. bleeding	Oesophageal varices (§)	Albumin ($\mu\text{mol l}^{-1}$)	Bilirubin ($\mu\text{mol l}^{-1}$)	Prothrombin index (%)
1	59	—	+	F3	421	18.8	48
2	26	—	—	F1	624	27.4	39
3	50	—	+	F3	609	15.4	56
4	38	—	+	F3	537	49.7	46
5	34	+	—	F1	421	149.0	31
6	50	—	+	F2	479	18.8	47
7	46	—	+	F3	508	24.0	61
8	58	—	—	F2	522	25.7	59
9	39	—	+	F3	609	13.7	52
10	51	—	+	F2	449	15.4	46
11	47	—	+	F2	464	12.0	55
12	53	—	—	F3	421	13.7	66
13	58	—	+	F3	459	10.3	58
14	56	—	+	F2	522	46.2	35
15	69	—	+	F3	493	30.9	59
16	34	—	+	F3	493	27.4	39
17	37	—	+	F3	490	39.3	37
18	41	—	+	F2	319	42.8	41
19	56	—	+	F3	466	53.0	42
20	61	—	+	F3	406	29.1	68

(§) according to the Japanese classification (*Jap. J. Surg.*, 10, 84, 1980):

F1 = straight varices; F2 = tortuous varices occupying less than one third of the oesophageal lumen;

F3 = larger sized tortuous varices occupying more than one third of the oesophageal lumen.

Reference range for albumin = 507–724 $\mu\text{mol l}^{-1}$; bilirubin = 2.0–17.0 $\mu\text{mol l}^{-1}$; prothrombin index = 80–100%.

Cases 1–8: patients treated with verapamil; Cases 9–20: patients treated with isosorbide dinitrate.

oesophageal varices and a history of previous gastro-intestinal bleeding was present in sixteen patients. One patient had mild ascites, none had clinical signs of hepatic encephalopathy. Pertinent clinical and biochemical data are given in Table 1. After full explanation of possible risks of the procedure, informed consent was obtained from all subjects. Patients were studied during a haemodynamic investigation of the splanchnic circulation which included hepatic vein catheterization for the measurement of occluded and free hepatic vein pressures, a procedure which is routinely offered at our Institution to all patients with cirrhosis and oesophageal varices, as part of the evaluation of the disease. A constant i.v. infusion of ICG was administered as described by Merkel *et al.* (1986). Briefly, following an i.v. bolus of 3.5 mg ICG m^2 body surface, ICG was infused at a constant rate of 0.25 $\text{mg min}^{-1} \text{m}^{-2}$ body surface. After a delay of 60 min to allow the establishment of steady state blood dye concentration, blood samples were drawn simultaneously from

an artery and a hepatic vein at 60, 65, 70, 75 min. Steady state was considered to have been achieved when the difference between arterial blood dye concentrations did not exceed 4%.

After baseline measurements, 5 mg verapamil was infused intravenously over 5 min in eight patients. In twelve patients 5 mg ISDN was given sublingually. In all patients three pairs of blood samples were drawn from an artery and the hepatic vein at 25, 30, 35 min after drug administration.

Plasma ICG concentrations were measured using a thermostated spectrophotometer (Beckman DU-30) at a wavelength of 800 nm, using the plasma of the patient drawn before the beginning of the experiment as a blank. The turbidity of plasma was corrected for according to Nielsen (1963). The limit of the assay was 0.1 mg l^{-1} and the coefficient of variation for replicate determination in 16 plasma samples in the range 0.2–2 mg l^{-1} was 3.1%.

The plasma clearance of ICG ($\text{CL}(\text{ICG})$) and its intrinsic hepatic clearance ($\text{CL}_{\text{int}}(\text{ICG})$) were

calculated according to the 'sinusoidal perfusion' model (Bass & Winkler, 1980) using the following equations:

$$\text{CL}_{\text{int}}(\text{ICG}) = \frac{\text{CL}(\text{ICG})}{\text{CL}_{\text{int}}(\text{ICG})} = \frac{R_o}{C_A} \cdot \frac{C_A - C_{Hv}}{C_A} \cdot [-\ln(1 - E)]$$

where R_o = rate of infusion; C_A = arterial ICG concentration; C_{Hv} = hepatic vein ICG concentration; E = ICG extraction ratio $(C_A - C_{Hv})/C_A$. The results of the haemodynamic investigation in these patients were reported elsewhere (Merkel *et al.*, 1987a; 1988). Since in one patient infused with verapamil and one infused with ISDN, ICG concentrations in hepatic veins were below the detection limit of the assay, E and $\text{CL}_{\text{int}}(\text{ICG})$ could not be calculated in these subjects.

In order to exclude the possibility that changes in clearances of ICG might be influenced by the one-way sequence design of the study, results were compared with those obtained in a previous group of seven patients with cirrhosis investigated before and after a placebo infusion. In these patients $\text{CL}(\text{ICG})$ increased by 1% on average, and $\text{CL}_{\text{int}}(\text{ICG})$ decreased by 2% on average. The results of this experiment were described by Merkel *et al.* (1987b).

All results are expressed as mean $\pm$ s.e. mean. Comparisons between data before and after drug administration were made using the paired rank sum test. Percentage variations in hepatic function parameters found after giving drugs and placebo were compared using the Mann-Whitney test. The null hypothesis was rejected at the 0.05 significance level, and, when the null hypothesis was not rejected, the power of the test was calculated according to conventional techniques.

Results

Concentrations of ICG in artery and hepatic venous plasma in patients receiving verapamil or ISDN are shown in Figures 1 and 2, respectively. Steady state concentrations of the dye were achieved in all patients both before and after administration of the drugs.

In patients treated with verapamil the extraction ratio of ICG (E) decreased significantly from $0.44 \pm 0.08\%$ to $0.40 \pm 0.07\%$ ($P < 0.025$). The mean percentage variation was -11% . However, values of $\text{CL}(\text{ICG})$ did not change significantly ($403 \pm 92 \text{ ml min}^{-1}$ to $396 \pm 89 \text{ ml min}^{-1}$; average percentage variation -1% ; P : NS; power = 85%). $\text{CL}_{\text{int}}(\text{ICG})$ also did not change significantly ($566 \pm 214 \text{ ml min}^{-1}$ to 522

Figure 1 Plasma ICG concentrations in an artery (upper panel) and in the hepatic vein (lower panel) of patients receiving verapamil 5 mg i.v. (shaded area); dotted line: limit of the assay.

$\pm 177 \text{ ml min}^{-1}$; average percentage variation -5% ; P : NS; power = 65%) (Figure 3).

In patients treated with ISDN no significant variation was observed in values of E (0.34 ± 0.05 to 0.34 ± 0.04 ; average percentage variation $+4\%$; P : NS; power 85%), $\text{CL}(\text{ICG})$ ($334 \pm 60 \text{ ml min}^{-1}$ to $348 \pm 74 \text{ ml min}^{-1}$; average percentage variation $+2\%$; P : NS; power = 90%), or $\text{CL}_{\text{int}}(\text{ICG})$ ($376 \pm 68 \text{ ml min}^{-1}$ to $398 \pm 89 \text{ ml min}^{-1}$; average percentage variation $+3\%$; P : NS; power = 89%) (Figure 4).

No differences were observed in the percentage variations of $\text{CL}(\text{ICG})$ or $\text{CL}_{\text{int}}(\text{ICG})$ values between placebo-treated and drug-treated patients (Figure 5).

Discussion

The results indicate that in patients with cirrhosis single doses of verapamil and ISDN do not influence hepatic function, as measured by $\text{CL}(\text{ICG})$ and $\text{CL}_{\text{int}}(\text{ICG})$.

Some investigators have reported variations in hepatic function following the administration

Figure 2 Plasma ICG concentrations in an artery (upper panel) and in the hepatic vein (lower panel) of patients receiving isosorbide dinitrate 5 mg sublingually (shaded area); dotted line: limit of the assay.

of verapamil (Kong *et al.*, 1986; Lay *et al.*, 1988; Miotti & Reichen, 1985; Reichen & Le, 1986) or organic nitrates (Gelman & Ernst, 1982; Svensson *et al.*, 1985). Nevertheless, evidence to suggest that vasodilators have an effect on liver function is equivocal. Only in rats with experimental cirrhosis or in rat isolated perfused livers was verapamil shown consistently to improve liver function as measured by aminopyrine and caffeine breath tests (Reichen *et al.*, 1986) or by antipyrine extraction ratio (Reichen & Le, 1986). In contrast, in humans with liver cirrhosis conflicting results have been reported. Thus, Miotti & Reichen (1985) found a decrease in the elimination rate constant of ICG after verapamil. However, the use of the elimination rate constant of ICG as an estimate of liver function may lead to errors due to variations in volume of distribution or in liver blood flow (Keiding, 1987), as might occur after verapamil administration (Lay *et al.*, 1988; Merkel *et al.*, 1988). Kong *et al.* (1986) observed an increase in serum albumin levels after 3 months of verapamil administration. Values of a further set of seven liver parameters did not change however in the same patients, and no allowance for multiple comparison was made in the statistical analysis of the results (Godfrey, 1985). The same group recently reported a decrease in $CL(ICG)$ and $CL_{int}(ICG)$ after acute verapamil administration in patients with post-hepatic cirrhosis (Lay *et al.*, 1988). In contrast, we did not find any significant variation in $CL(ICG)$ or $CL_{int}(ICG)$ after acute verapamil

Figure 3 Extraction ratio, plasma clearance, and intrinsic hepatic clearance of ICG before and after verapamil administration in eight patients with alcoholic cirrhosis.

Figure 4 Extraction ratio, plasma clearance, and intrinsic hepatic clearance of ICG before and after isosorbide dinitrate administration in twelve patients with alcoholic cirrhosis.

Figure 5 Percentage variations in plasma clearance and intrinsic hepatic clearance of ICG in patients receiving placebo (●), verapamil (+), or isosorbide dinitrate (×).

administration in patients with alcoholic cirrhosis, using a similar study design. Results similar to ours were reported recently by Navasa *et al.* (1988). The reason for these discrepancies may be related to different disease aetiologies, to differences in the severity of disease, or to the higher dose of verapamil given by Lay *et al.* (1988).

Few data are available on the effect of organic nitrates on liver function. In normal man nitroglycerin was reported to decrease CL(ICG) (Svensson *et al.*, 1985), and in dogs with experimental cirrhosis sodium nitroprusside was shown to impair hepatic oxidative metabolism (Gelman & Ernst, 1982). In contrast, unchanged values of CL(ICG) after acute administration of isosorbide-5-mononitrate (Navasa *et al.*, 1987) and sodium nitroprusside (Chauvin *et al.*, 1985) were reported in humans. No data appear to be available on the effect of ISDN in patients with cirrhosis.

A further point to be considered is the possibility of a type II statistical error, i.e. the error of not recognising a real effect of the treatment. From calculated values of the power of the tests used it appears that in all but one test the probability of making a type II error was lower than 15%.

A last possibility to rule out is that the one-way sequence design we used could have prevented the recognition of a significant effect of

the drugs. A mean 6% per hour decrease in $CL_{int}(ICG)$ may be expected during prolonged infusions (Keiding & Skak, 1988). Therefore, a small increase in $CL_{int}(ICG)$ might have been masked by the study design. The data collected in patients infused with placebo can clarify this point. Thus, in substantial agreement with Keiding & Skak (1988), our patients infused with placebo exhibited a small decrease (2% after 30 min) in $CL_{int}(ICG)$ which did not reach

statistical significance. When the percentage variation after drugs was compared with that after placebo, no significant difference was noted.

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References

- Bass, L. & Winkler, K. (1980). A method of determining intrinsic hepatic clearance from the first-pass effect. *Clin. exp. Pharmac. Physiol.*, **7**, 339–343.
- Blei, A. T., Ganger, D., Fung, H. L. & Groszmann, R. J. (1988). Organic nitrates in portal hypertension. *Eur. Heart J.*, **9** suppl. A, 205–211.
- Blei, A. T. & Gottstein, J. (1986). Isosorbide dinitrate in experimental portal hypertension: a study of factors that modulate the hemodynamic response. *Hepatology*, **6**, 107–111.
- Chauvin, M., Bonnet, F., Montembaum, C., Lafay, M., Curet, P. & Viars, P. (1985). Hepatic plasma flow during sodium nitroprusside-induced hypotension in humans. *Anesthesiology*, **63**, 287–293.
- Freeman, J. G., Barton, J. R. & Record, C. O. (1985). Effect of isosorbide dinitrate, verapamil, and labetalol on portal pressure in cirrhosis. *Br. med. J.*, **291**, 561–562.
- Garcia-Pagan, J. C., Bosch, J., Navasa, H., Bru, C., Pizcueta, P. & Rodes, J. (1988). Enhancement of portal pressure reduction by the association of isosorbide-5-mononitrate (Is-5-Mn) to propranolol administration in cirrhosis (Abstract). *J. Hepatol.*, **7** suppl. 1, 34.
- Garcia-Tsao, G., Hansen, J. & Groszmann, R. J. (1987). Splanchnic hemodynamics in cirrhotic patients during nitroglycerin administration. *Hepatology*, **7**, 805–809.
- Gelman, S. & Ernst, E. A. (1982). Hepatic circulation during sodium nitroprusside infusion in the carbon tetrachloride-treated dog. *Ala. J. med. Sci.*, **19**, 371–374.
- Gimson, A. E., Westaby, D., Hegarty, J., Watson, A. & Williams, R. (1986). A randomized trial of vasopressin and vasopressin plus nitroglycerin in the control of acute variceal hemorrhage. *Hepatology*, **6**, 410–413.
- Godfrey, K. (1985). Comparing the means of several groups. *New Engl. J. Med.*, **313**, 1450–1456.
- Groszmann, R. J., Kravetz, D., Bosch, J., Glickmann, M., Bruix, J., Bredfeldt, J., Conn, H. O., Rodes, J. & Storer, E. T. (1982). Nitroglycerin improves the hemodynamic response to vasopressin in portal hypertension. *Hepatology*, **2**, 757–762.
- Halleman, R., Naeije, R., Mols, P., Melot, C. & Reding, P. (1983). Treatment of portal hypertension with isosorbide dinitrate alone or in combination with vasopressin. *Crit. Care Med.*, **11**, 536–540.
- Keiding, S. (1987). Hepatic clearance and liver blood flow. *J. Hepatol.*, **4**, 393–398.
- Keiding, S. & Skak, C. (1988). Methodological limitations of the use of intrinsic hepatic clearance of ICG as a measure of liver cell function. *Eur. J. clin. Invest.*, **18**, 507–511.
- Kong, C. W., Lay, C. S., Tsai, Y. T., Yeh, C. L., Lai, K. H., Lee, S. D., Lo, K. J. & Chiang, B. N. (1986). The hemodynamic effect of verapamil on portal hypertension in patients with post-necrotic cirrhosis. *Hepatology*, **6**, 423–426.
- Lay, C. S., Tsai, Y. T., Kong, C. W., Lee, F. Y., Chang, T. T., Lin, H. C., Yang, C. M., Lee, S. D., Chiang, B. N. & Lo, K. J. (1988). The influence of verapamil and nifedipine on hepatic indocyanine green clearance in patients with Hbs-Ag positive cirrhosis and ascites. *Clin. Pharmac. Ther.*, **44**, 453–457.
- Merkel, C., Finucci, G. F., Zuin, R., Bazzlerla, G., Bolognesi, M. & Gatta, A. (1987a). Effects of isosorbide dinitrate on portal hypertension in alcoholic cirrhosis. *J. Hepatol.*, **4**, 174–180.
- Merkel, C., Gatta, A., Bolognesi, M., Padriani, R., Finucci, G. F., Angeli, P. & Ruol, A. (1988). The calcium-channel blocker, verapamil, does not improve portal pressure in patients with alcoholic cirrhosis. *Br. J. clin. Pharmac.*, **26**, 273–277.
- Merkel, C., Gatta, A., Caregaro, L., Sacerdoti, D., Rondana, M. & Ruol, A. (1987b). Effect of somatostatin on liver blood flow and liver metabolic activity in patients with cirrhosis. *Scand. J. clin. Lab. Invest.*, **47**, 667–672.
- Merkel, C., Sacerdoti, D., Finucci, G. F., Zuin, R., Bazzlerla, G., Bolognesi, M. & Gatta, A. (1986). Effect of nadolol on liver haemodynamics and function in patients with cirrhosis. *Br. J. clin. Pharmac.*, **21**, 713–719.
- Miotti, T. & Reichen, J. (1985). Verapamil improves fractional clearance of indocyanine green in patients with liver cirrhosis (Abstract). *J. Hepatol.*, **1**, suppl. 2, 291.
- Navasa, M., Bosch, J., Chesta, J. & Rodés, J. (1987). Isosorbide 5-mononitrate reduces hepatic vascular resistance and portal pressure in patients with cirrhosis (Abstract). *J. Hepatol.*, **5** suppl., 48.

- Navasa, M., Bosch, J., Reichen, J., Bru, C., Mastai, R., Zysset, T., Silva, G., Chesta, J. & Rodés, J. (1988). Effects of verapamil on hepatic and systemic hemodynamics and liver function in patients with cirrhosis and portal hypertension. *Hepatology*, **8**, 850–854.
- Nielsen, N. C. (1963). Spectrophotometric determination of indocyanine green in plasma especially with a view to an improved correction for blank density. *Scand. J. clin. lab. Invest.*, **15**, 613–621.
- Reichen, J., Hirlinger, A., Ha, H. R. & Saegesser, S. (1986). Chronic verapamil administration lowers portal pressure and improves hepatic function in rats with liver cirrhosis. *J. Hepatol.*, **3**, 49–58.
- Reichen, J. & Le, M. (1986). Verapamil favourably influences hepatic microvascular exchange and function in rats with cirrhosis of the liver. *J. clin. Invest.*, **78**, 448–455.
- Staritz, M., Poralla, T. & Mayer zum Buschenfelde, K. H. (1985). Intravascular oesophageal variceal pressure (IOVP) assessed by endoscopic fine needle puncture under basal conditions, Valsalva's manoeuvre, and after glyceryltrinitrate application. *Gut*, **26**, 525–530.
- Svensson, C. K., Cumella, J. C., Tronolone, M., Middleton, E. & Lalka, D. (1985). Effects of hydralazine, nitroglycerin, and food on estimated hepatic blood flow. *Clin. Pharmac. Ther.*, **37**, 464–468.
- Tsai, V. T., Lay, C. S., Lai, K. H., Ng, W. W., Yeh, Y. S., Wang, J. Y., Chiang, T. T., Lee, S. D., Chiang, B. N. & Lo, K. J. (1986). Controlled trial of vasopressin plus nitroglycerin vs vasopressin alone in the treatment of bleeding esophageal varices. *Hepatology*, **6**, 406–409.

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