Journal of Hepatology 2000; **33**: 733–737
Printed in Denmark · All rights reserved
Munksgaard · Copenhagen

Journal of Hepatology
ISSN 0168-8278

Type A, but not type B, endothelin receptor antagonists significantly decrease portal pressure in portal hypertensive rats

Andrea De Gottardi, Sidney Shaw[1], Hans Sägesser and Jürg Reichen
Departments of Clinical Pharmacology and [1]Clinical Research, University of Berne, Switzerland

Background/Aim: **Endothelin-1 plays an important role in the regulation of portal hypertension; endothelin antagonists have been extensively studied in portal hypertensive animals. We aimed to evaluate the efficacy of highly selective endothelin antagonists in partial portal vein ligated (PPVL) rats.**
Methods: **Four groups of 7 male Sprague-Dawley rats were administered orally ABT-627 (ET_A-selective), A-192621 (ET_B-selective), or A-182086 (non-selective), with the fourth group serving as control. On the 3rd day after beginning treatment animals underwent PPVL. On the 11th day hemodynamics were studied and portal vein ET-1 was measured.**
Results: **In the control group portal pressure was 13.4±SD 0.2 mmHg; this increased to 14.9±1.8 ($p<0.05$) in the ET_B blocked group. In contrast, ET_A blockade improved portal hypertension (11.7±1.1, $p<0.05$), while the treatment with the non-selective antagonist had no effect (12.3±0.7 n.s.). Mean arterial pressure was not significantly affected by any treatment. Portal vein ET-1 was increased in all groups compared to controls; this increase was limited to the pre-stenotic area (79±43 *vs* 194±76 in the pre- and post-stenotic portal vein; $p<0.0025$).**
Conclusions: **Oral administration of an ET_A antagonist ameliorated portal hypertension; we suggest that long-term therapy of portal hypertension with selective ET_A antagonists may be more beneficial than mixed antagonists.**

Key words: **Endothelin receptor antagonists; Portal hypertension; Splanchnic circulation.**

Basal vascular tone is the result of the action of several factors with vasoconstrictor or vasodilator properties. Endothelin-1, a potent vasoactive peptide, is involved in this process, not only as a vasoconstrictor, but also as a vasodilator. While constriction is mediated by the endothelin type A receptor (ET_A) (1) and partially by the type B receptor (ET_B) present on smooth muscle cells (2), vasodilatation seems to be evoked specifically by ET_B through release of endothelium-derived vasodilators such as nitric oxide and prostacyclin (3,4).

Whereas an increased vascular resistance to portal flow initiates the development of portal hypertension, the maintenance phase is characterized by splanchnic vasodilation as well as increased hepatocollateral and intrahepatic vascular resistance (5). ET-1 is increased in the portal vein of rats with hepatic or prehepatic experimental portal hypertension (6) and has been shown to increase intrahepatic vascular resistance. ET_A and ET_B receptors have been identified in the rat liver (7) and in the portal vein (8), and increased expression has recently been demonstrated in superior mesenteric artery of portal hypertensive rats (9). Hence, although evidence suggests a role for endothelin-1 in the control of portal pressure, the extent to which particular receptors may influence individual contributory mechanisms remains controversial (10–12). Furthermore, since current pharmacologic treatment of portal hypertension does not always prevent or stop gastrointestinal bleeding associated with this condition, endothelin receptor antagonists may have therapeutic potential in these circumstances.

The aim of the present study was to investigate whether and by which mechanisms highly selective endothelin receptor antagonists may be effective in decreasing portal pressure in partial portal vein ligated rats.

Received 22 September 1999; revised 10 February; accepted 18 April 2000
Correspondence: Jürg Reichen, Department of Clinical Pharmacology, Murtenstrasse 35, 3010 Berne, Switzerland. Tel: 41 31 632 3570. Fax: 41 31 632 4997.
e-mail reichen@ikp.unibe.ch

Materials and Methods

Animals

Four groups of 7 male Sprague-Dawley rats (Biological Research Laboratories Ltd, Füllinsdorf, Switzerland) weighing 270–300 g were administered orally ABT-627 (ET_A-selective, 6.25 mg/kg/day), A-192621 (ET_B-selective, 15 mg/kg/day) or A-182086 (non-selective, 15 mg/kg/day) mixed separately in rat chow, the fourth group serving as control with a normal diet. Animals were kept on a 12-h light-dark cycle. The oral dose of the compounds was calculated for *in vivo* inhibition of the agonist-induced increase in mean arterial blood pressure (0.3 nmol/kg, i.v. bolus). Highly selective endothelin receptor antagonists were a kind gift of J. Wessale and T. Opgenorth (Abbott, North Chicago, USA). The animal experiments had been approved by a state-appointed board on animal ethics and were performed according to international guidelines concerning the conduct of animal experimentation.

On the 3rd day after beginning the pharmacological treatment, animals underwent partial portal vein ligation under pentobarbital anesthesia (50 mg/kg intraperitoneally) as previously described (13). On the 11th day studies were performed on anesthetized animals. A blood sample was collected for routine and liver function tests. One additional group of 7 sham-operated rats was taken into consideration for portal pressure and portal vein ET-1 measurements under basal conditions. In an additional group of 8 untreated animals, the portal vein was separated into pre- and post-stenotic area for measurement of ET-1.

Hemodynamic measurements

Mean arterial pressure was monitored by a Statham Pd23b transducer via a PE-50 tubing inserted in the right carotid artery, whereas portal pressure was measured using a saline-filled manometer introduced into the ileocolic vein. Cardiac output and organ blood flow were determined by means of the microsphere method (14,15): after placement of the carotid catheter into the left ventricle, 1.6 μCi ^{113}Tn-labeled microspheres (New England Nuclear, Boston, MA, USA) were injected and the reference sample withdrawn at a rate of 1 ml/min using a Harvard infusion pump. Then, ^{46}Sc-labeled microspheres (1 μCi) were injected into the ileocolic vein for shunt measurements. The γ-isotopes in organs were measured on a Packard COBRA-II γ-spectrophotometer with appropriate corrections for isotope spillover. Cardiac output was calculated as: radioactivity injected (cpm)/reference blood sample radioactivity (cpm)×blood sample (ml/min). The cardiac index was expressed per 100 g body weight. Regional blood flows were calculated by the following formula: organ blood flow (ml×min^{-1}×100 g^{-1})=organ activity (cpm)/radioactivity injected (cpm)×cardiac index (ml×min^{-1}×100 g^{-1}). Portal tributary blood flow was calculated as the sum of spleen, stomach, small intestine, colon and pancreas blood flows. Systemic vascular resistance (dyn×s×cm^{-5}×100 g×10^{-3}) was calculated as: mean arterial pressure (mmHg)×80/cardiac output. Portal territory vascular resistance (dyn×s×cm^{-5}×10^{-3}) was calculated as: [mean arterial pressure (mmHg)−portal pressure (mmHg)]×80/portal tributary blood flow (ml×min^{-1}). Hepatocollateral vascular resistance (dyn×s×cm^{-5}×10^{-3}) was calculated as: portal pressure (mmHg)×80/portal tributary blood flow (ml×min^{-1}). Portosystemic shunt (%) was calculated as: pulmonary radioactivity (^{46}Sc)×100/[hepatic radioactivity (^{46}Sc)+pulmonary radioactivity (^{46}Sc)]. Only experiments where there was less than 10% deviation in the cpm/g between the two kidneys were accepted.

Endothelin assay

Portal vein wall endothelin concentration was determined as previously described (16). Briefly, snap-frozen tissue was homogenized in a chloroform-ethanol 2:1 solution with 0.1% trifluoroacetic acid and 1 mM N-ethylmaleamide. Sterile distilled water (40%) was added to all tubes, which were then centrifuged at 4 °C, 5000 rpm for 15 min. The aqueous phase was collected, diluted 1:9 in 4% acetic acid and extracted on activated Sep-Pak C_{18} 500 mg cartridges (Waters Corporation, Milford, USA). The eluate (2 ml 86% ethanol/4% acetic acid) was dried overnight in a Speed-Vac centrifuge system. Endothelin-1 was analyzed by a double antibody radioimmunoassay technique. ET-1 was obtained from Sigma (St. Louis, USA), ET-1 antibodies were from Peninsula (St. Helens, England); [^{125}I]-ET-1 was obtained from Amersham International (Buckinghamshire, UK).

Data presentation and statistical analysis

Results are given as mean±standard deviation. Means of groups were analyzed by ANOVA followed by Duncan's test. A $p<0.05$ was considered statistically significant.

Results

In the control group (PPVL without any pharmacological treatment) portal pressure was significantly higher as compared to sham-operated animals (13.4±0.2 mmHg *vs.* 5.8±1.2, $p<10^{-6}$). Mean arterial pressure was significantly lower as compared to sham-operated rats (106±17 mmHg *vs.* 132±9, $p=0.01$). There was no significant difference in body weights between groups.

Liver tests such as AST, ALT, alkaline phosphatase and bilirubin were unchanged by the different treatment regimens (data not shown). Liver microsomal function, assessed by the aminopyrine breath test (17), revealed no significant differences between groups.

Hemodynamic measurements

The effects of endothelin receptor antagonists on systemic, splanchnic and renal circulation are reported in Table 1.

TABLE 1

Effect of endothelin receptor antagonists on systemic, splanchnic and renal hemodynamics in rats

	Controls	ET_A	ET_B	Non-selective
Mean arterial pressure (mmHg)	106±17	94±16	107±22	108±19
Cardiac index (ml×min^{-1}×100 g^{-1})	46±8	51±21	49±4	42±6
Systemic vascular resistance (dyn×s× cm^{-5} ×10^{-3})	67±21	60±23	63±17	70±12
Portal pressure (mmHg)	13.4±0.2	11.7±1.1*	14.9±1.8*	12.3±0.7
Portal tributary blood flow (ml×min^{-1}×100 g^{-1})	24.4±7.6	22.5±7.4	24.5±3.0	22.2±5.0
Portal territory vascular resistance (dyn×s×cm^{-5}×10^{-3})	333±132	324±132	307±93	352±80
Porto-systemic shunt (%)	69.8±13.3	65.3±24.4	73.4±9.3	68.6±16.8
Hepatocollateral vascular resistance (dyn×s×cm^{-5}×10^{-3})	47±13	45±14	49±7	46±10

Results are expressed as mean±SD.
*Significantly different from control value at $p<0.05$.

ABT-627

Administration of the ET_A selective antagonist produced a significant 14% reduction of the portal pressure compared to control rats. This treatment had no statistically significant effect on mean arterial pressure, cardiac index, systemic vascular resistance, portal tributary blood flow, portal territory vascular resistance, and hepatocollateral vascular resistance. A significant decrease of 30% in renal blood flow compared to control animals was observed.

A-192621

Selective ET_B blockade increased portal pressure by 10% without affecting systemic or splanchnic hemodynamics.

A-182086

Animals treated with the non-selective ET-antagonist showed no variation in hemodynamic parameters.

Endothelin-1 in the portal vein wall

The increase in portal pressure obtained after partial portal vein ligation was accompanied by increased levels of ET-1 peptide in the portal vein, as shown in Fig. 1A and 1B. This was limited to the pre-stenotic area of the portal vein, ET-1 levels averaging area 79±43 and 194±76 in the post- and pre-stenotic portal vein, respectively ($n=8$; $p<0.0025$). None of the pharmacological treatments significantly influenced portal vein ET-1 levels: 98±20 pgET-1/100 mg in controls, compared to 145±109 with the ET_A antagonist, 226±68 with the ET_B antagonist, and 204±106 with the non-selective endothelin antagonist.

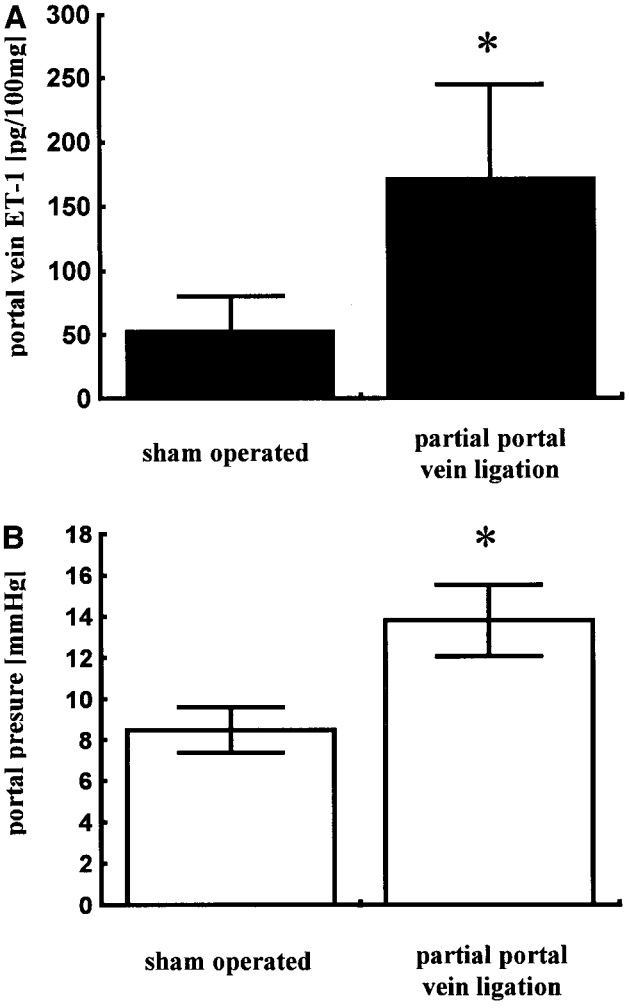

*Fig. 1. Effects of partial portal vein ligation on portal vein endothelin-1 (1A) and on portal pressure (1B). Two weeks after partial portal vein ligation in rats the amount of portal vein ET-1 was 3-fold higher and was related to the significant increase in portal pressure induced by the surgical procedure (*n=*7 for each group,* *p<*0.05).*

Discussion

The present results demonstrate that long-term oral administration of a selective ET_A receptor antagonist decreased portal pressure in an experimental model of portal hypertension induced by partial portal vein ligation. These effects occurred independently from changes in mean arterial pressure, cardiac index, systemic vascular resistance, or hepatocollateral vascular resistance. In contrast, chronic selective ET_B receptor antagonism exacerbated portal hypertension. Combined ET_A/ET_B antagonism, which presumably induced counterbalanced responses, produced no overall significant change. Finally, we demonstrate that ET-1 is increased in the pre-stenotic area in the portal vein.

Previous studies of the role of endothelin and receptor-antagonist responses in experimental models of portal hypertension have yielded conflicting and generally inconclusive results regarding the efficacy of specific antagonists or the underlying mechanisms involved. In a recent study by Cahill et al. (9), acute intravenous bolus injection of JKC-301 (a specific ET_A antagonist) in partial portal vein ligated rats was reported to slightly decrease mean arterial pressure, mesenteric artery blood flow and portal pressure. In the same study, acute ET_B blockade also decreased mesenteric artery blood flow and portal pressure but increased mean arterial pressure. Combined non-selective ET_A/ET_B -antagonism with TAK-044, was reported by Gandhi et al. to be effective in reducing portal pressure when perfused into the portal vein of cirrhotic rats (18). In an earlier acute study using the mixed antagonist Bosentan, we demonstrated a significant decrease in portal pressure in two rat models of cirrhosis (19). Sogni et al. (20) obtained similar results in cirrhotic and PPVL

rats using Bosentan. In contrast, but in keeping with the present findings, chronic oral administration of Ro 48–5695 (another mixed ET-antagonist) to cirrhotic rats failed to reduce portal pressure (21). Although the reason for the discrepancies between these observations is not clear, differences may relate to the selectivity of so-called "specific" inhibitors or to the fact that all antagonists previously shown to be effective in reducing portal pressure were administered only as an acute bolus. This may indicate intrinsic differences in physiological responses to acute and chronic administration. This could relate to long-term induction of compensatory mechanisms in peripheral vascular beds, which may alter the role of particular endothelin receptor subtypes under these conditions.

Regardless of the detailed mechanisms, the reason for the selective beneficial response to specific ET_A receptor blockade in the present study remains unclear. The absence of marked improvements in single parameters of peripheral circulatory hemodynamics known to be involved in the maintenance of portal hypertension suggests that several factors have simultaneously contributed to ameliorating portal pressure. Despite the absence of statistically significant changes at a unique site of action of ET antagonists, measurements of portal tributary blood flow and porto-systemic shunts tended to decrease in ET_A antagonist-treated animals and to increase in the ET_B-treated group (Table 1). This suggests that the action of these compounds may modulate portal blood flow in part by regulating the development of porto-systemic collaterals. The data collected in this study do not demonstrate that ET antagonists induce changes in the intrahepatic blood flow. In general, ET_A receptors tend to mediate vasoconstrictory responses while ET_B receptors also have a marked vasodilatory component to their action (1–4). Endothelin levels in portal vein tissue were found to be elevated after PPVL; this increase was restricted to the pre-stenotic segment of the portal vein, suggesting that this is a direct response to increased portal pressure. It may be speculated that selective inhibition of vasosoconstrictory ET_A at one or more sites allows a vasodilatory response to predominate. This may explain the significant decrease in portal pressure observed in the present study. If this assumption is true, blockade of potentially beneficial vasodilatory ET_B would be expected to worsen vasoconstriction and portal hypertension, as was indeed the case, while simultaneous antagonism of both receptor subtypes may neutralize the beneficial response to selective ET_A blockade alone.

In summary, the present study is, to our knowledge, the first report on chronic administration of selective and non-selective endothelin antagonists in the partial portal vein ligation model of portal hypertension in rats. We also demonstrate an increase in portal vein selective to the pre-stenotic area, suggesting that increased portal pressure is the stimulus for overproduction of ET-1. The failure to observe an effect on portal pressure with a non-selective endothelin antagonist is in accordance with recently published data (21). Selective inhibition of ET_A in the presence of intact beneficial ET_B response to endogenous ET-1 resulted in a significant decrease in portal pressure. Taken together, these data suggest that mechanisms contributing to the chronic maintenance of portal hypertension after PPVL may be either beneficially or detrimentally influenced by selective long-term blockade of ET_A or ET_B receptor subtypes, respectively. More importantly, these data suggest that long-term therapy of portal hypertension with selective endothelin type A receptor antagonists may be more beneficial than mixed ET_A /ET_B antagonists.

Acknowledgements

We thank J. Wessale and T. Opgenorth at Abbott Laboratories, Abbott Park, Illinois for their suggestions and for providing endothelin receptor antagonists and A. Koshy for helpful discussion.

Portions of this work were presented at the meeting Hepatic and Splanchnic Circulation in Health and Disease and published in abstract form (Hepatogastroenterology 1999;46:1447). This study was supported by a grant from the Swiss Science National Foundation to J. R. (no 45349.95).

References

1. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. Nature 1990; 348: 730–2.
2. Haynes WG, Strachan FE, Webb DJ. Endothelin ET_A and ET_B receptors cause vasoconstriction of human resistance and capacitance vessels *in vivo*. Circulation 1995; 92: 357–63.
3. Hirata Y, Emori T, Eguchi S, Kanno K, Imai T, Ohta K, et al. Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. J Clin Invest 1993; 91: 1367–73.
4. Tsukahara H, Ende H, Magazine HI, Bahou WF, Goligorsky MS. Molecular and functional characterization of the non-isopeptide-selective ETB receptor in endothelial cells: receptor coupling to nitric oxyde synthase. J Biol Chem 1994; 269: 21778–85.
5. Reichen J. Liver function and pharmacological considerations in the pathogenesis and treatment of portal hypertension. Hepatology 1990; 11: 1066–78.
6. De Gottardi A, Shaw S, Boden J, Hösli M, Reichen J. Vascular endothelin-1 increases selectively in the prestenotic area in partial portal vein ligation in the rat [abstract]. Hepatology 1998; 28: 461A.
7. Housset C, Rockey DC, Bissel DM. Endothelin receptors in rat

liver: lipocytes as a contractile target for endothelin 1. Proc Natl Acad Sci 1993; 90: 9266–70.
8. Filippelli A, Falciani M, Palla A, D'Amico M, Vacca C, Rossi F. Distribution of endothelin-1-receptor subtypes in rat portal vein. J Cardiovasc Pharmacol 1996; 27: 113–8.
9. Cahill PA, Ming-chih H, Hendrickson R, Wang YN, Zhang SM, Redmond EM, et al. Increased expression of endothelin receptors in the vasculature of portal hypertensive rats: role in splanchnic hemodynamics. Hepatology 1998; 28: 396–403.
10. Guimaraes CL, Calixto JB, Rae G. Potent constrictor actions of endothelin-1, -2 and -3 in rat isolated portal vein. Hypertension 1992; 19: 79–86.
11. Kaneda K, Ekataksin W, Sogawa M, Matsumura A, Cho A, Kawada N. Endothelin-1-induced vasoconstriction causes a significant increase in portal pressure of rat liver: localized constrictive effect on the distal segment of preterminal portal venules as revealed by light and electron microscopy and serial reconstruction. Hepatology 1998; 27: 735–47.
12. Wang HG, Shibamoto T, Miyahara T. Endothelin-1 selectively contracts portal vein through both ETA and ETB receptors in isolated rabbit liver. Am J Physiol 1997; 36: G1036–43.
13. Lebrec D, Blanchet L. Effect of two models of portal hypertension on splanchnic organ blood flow in the rat. Clin Sci 1985; 68: 23–8.
14. Groszmann RJ, Vorobioff J, Riley E. Splancnic hemodynamics in portal hypertensive rats: measurement with γ-labeled microspheres. Am J Physiol 1982; 242: G156–60.
15. Blanchet L, Lebrec D. Changes in splanchnic blood flow in portal hypertensive rats. Eur J Clin Invest 1982; 12: 327–30.
16. Barton M, Shaw S, Lüscher T. Angiotensin II increases vascular and renal endothelin 1 and functional endothelin converting enzyme activity *in vivo:* role of ET_A receptors for endothelin regulation. Biochem Biophys Res Comm 1997; 238: 861–5.
17. Gross JB, Reichen J, Zeltner T, Zimmermann A. The evolution of changes in quantitative liver function tests in a rat model of cirrhosis: correlation with morphometric measurement of hepatocyte mass. Hepatology 1987; 7: 457–63.
18. Gandhi CR, Nemoto EM, Watkins SC, Subbotin VM. An endothelin receptor antagonist TAK-044 ameliorates carbon tetrachloride-induced acute liver injury and portal hypertension in rats. Liver 1998; 18: 39–48.
19. Reichen J, Gerbes AL, Steiner MJ, Sägesser H, Clozel M. The effect of endothelin and its antagonist bosentan on hemodynamics and microvascular exchange in cirrhotic rat liver. J Hepatol 1998; 28: 1020–30.
20. Sogni P, Moreau R, Gomola A, Gadano A, Cailmail S, Calmas Y, et al. Beneficial hemodynamic effects of bosentan, a mixed ET_A and ET_B receptor antagonist, in portal hypertensive rats. Hepatology 1998; 28: 655–9.
21. Poo J-L, Jimenez W, Munoz RM, Bosch-Marcé M, Bordas N, Morales-Zaiz M, et al. Chronic blockade of endothelin receptors in cirrhotic rats: hepatic and hemodynamic effects. Gastroenterology 1999; 116: 161–7.
22. Okumura S, Takei Y, Kawano S, Nagano K, Masuda E, Goto M, et al. Vasoactive effect of endothelin-1 on rat liver *in vivo.* Hepatology 1994; 19: 155–61.
23. Bauer M, Zhang JX, Clemens MG. ET-1 induced alterations of hepatic microcirculation: sinusoidal and extrasinusoidal sites of action. Am J Physiol 1994; 267: G143–9.