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Effects of Tetramethylpyrazine on Portal Hypertensive Rats

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Abstract

The effects of tetramethylpyrazine, an alkaloid isolated from a Chinese herb *Ligusticum wallichii* Franch have been assessed in portal hypertensive rats.

Portal hypertension was induced by partial portal vein ligation in Sprague-Dawley rats. Two weeks after ligation, when the hyperdynamic state had stabilized, rats were anaesthetized after an overnight fast and cannulated for measurement of mean arterial pressure, portal venous pressure, cardiac index and heart rate. Tetramethylpyrazine (3·0, 9·9 and 30 mg kg^{-1}) induced dose-dependent reductions of portal venous pressure and mean arterial pressure after intravenous infusion. The maximum percentage reduction of portal venous pressure after tetramethylpyrazine was 6·0 ± 0·8, 9·3 ± 1·6 and 20 ± 2% of baseline for doses of 3·0, 9·9 and 30·0 mg kg^{-1}, respectively. Also, total peripheral resistance was significantly reduced by tetramethylpyrazine and cardiac index was slightly increased.

Our results showed that tetramethylpyrazine induced portal pressure reduction in portal hypertensive rats.

Tetramethylpyrazine (also called ligustrazine) is an alkaloid found in the Chinese medicinal plants *Ligusticum wallichii* Franch or *Ligusticum chuanxiong* Hort (Beijing Research Institute of the Pharmaceutical Industry 1977a), an African plant *Jatropha podagrica* Hook (Ojewole 1981) or in *Bacillus subtilis* culture media (Kosuqe & Kamiya 1962). Tetramethylpyrazine has been used clinically in China for treatment of ischaemic cerebrovascular disease (Beijing Research Institute of the Pharmaceutical Industry 1977b) and pulmonary hypertension (Peng & Duan 1992). Tetramethylpyrazine is a vasodilator and its mechanisms of action reportedly include calcium antagonism (Wang et al 1991; Pang et al 1996), cAMP production (Wu et al 1989) and endothelium-dependent NO-mediated relaxation (Peng et al 1996). The potential of tetramethylpyrazine as a therapeutic agent in portal hypertension has yet to be explored.

The two main pharmacological means of reducing portal hypertension are by reducing portal blood flow or by reducing portal vascular resistance, by use of vasoconstrictors or vasodilators, respectively (Reichen 1990; Lebrec 1994). Some calcium antagonists have been shown to be effective portal hypotensive agents with the benefit of reducing hepatic resistance and thereby improving hepatic microexchange function (Reichen & Le 1986; Reichen et al 1986; Reichen 1990). In this study we have assessed the effects of tetramethylpyrazine on the portal pressure of portal hypertensive rats.

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Materials and Methods

Drugs

Tetramethylpyrazine was purchased from Aldrich (Milwaukee, WI) and dissolved in 0·1 M HCl (except for doses of 30·0 mg kg^{-1}, when it was dissolved in 0·2 M HCl).

Portal hypertensive rats

Partial portal vein ligation was performed according to the method of Chojkier & Groszmann (1981) and as previously reported (Huang et al 1996). Briefly, male Sprague-Dawley rats (200–250 g) were anaesthetized with ether. A midline incision was made and the portal vein proximal to the

bifurcation was exposed. A 3-0 silk ligature was made around the portal vein and a piece of PE 50 tubing (Clay Adams, Parsippany, NJ). The PE tubing was then removed and the abdomen closed. Animal studies were approved by the Animal Experiment Committee of the National Yang-Ming University and conducted humanely.

Pressure measurement

Fourteen days after surgery, the rats were anaesthetized with ketamine 100, 50 mg kg^{-1} after an overnight fast. The ileocolic vein, femoral artery and femoral vein were cannulated with PE 50 tubing for measurement of portal venous pressure, arterial blood pressure, and for drug administration, respectively. Pressure changes and changes in heart rate were monitored by means of a Gould (Valley View, OH) RS 3400 polygraph by means of Viggo-Spectramed (Oxnard, CA) P23XL strain-gauge transducers. Cardiac output was measured by thermodilution according to the method of Albillos et al (1992) and as previously reported (Huang et al 1997a). Briefly, a thermistor was placed in the aortic arch, just distal to the aortic valve, and the thermal indicator (0·1 mL, 0·9% NaCl, maintained at 18·0 ± 0·5°C) was injected into the right atrium through an 8-cm PE 50 catheter. Temperature was maintained at 37·0 ± 0·5°C. The aortic thermistor was connected to a Columbus Instruments (Columbus, OH) Cardiotherm 500 cardiac output computer. The arithmetic mean of three thermodilution readings was taken as the cardiac output for each animal. For each rat triplicate readings from the cardiac output computer were very consistent, with intra-assay variability (coefficient of variation) generally less than 5%. After recording of a steady baseline for 30 min tetramethylpyrazine (3·0, 9·9 or 30·0 mg kg^{-1}) was infused into rats within a period of 3 min via a syringe pump (Harvard Apparatus, Millis, MA). Haemodynamic parameters including cardiac output were measured for data analysis at the following time points: baseline, 2 to 3 min after drug infusion when pressure responses to tetramethylpyrazine reached nadir. Cardiac index was calculated as cardiac output/100 g (mL min^{-1}/100 g) and total peripheral resistance (mmHg min 100 g mL^{-1}) was calculated by dividing mean arterial pressure by cardiac index. After each dose, the rat was left to recover for at least 30 min; in this way no tachyphylaxis was observed. For each dose of drug it was confirmed that infusion of vehicles (0·1 or 0·2 M HCl) did not alter any of the haemodynamic parameters (Table 1).

Data analysis

Data are expressed as means ± s.e.m. For each dose Student's paired *t*-test was used to compare parameters before and after infusion. Dose–response curves were analysed by the one-way analysis of variance. Significance was determined at $P < 0.05$.

Results

The baseline haemodynamic data are shown in Table 1. After partial portal vein ligation the haemodynamic profiles of the rats were typical of those of portal

Table 1. Haemodynamic responses to tetramethylpyrazine in anaesthetized portal hypertensive rats.

Parameter	Response after vehicle		Response after tetramethylpyrazine dose (mg kg^{-1}):					
	Before	After	3·0		9·9		30·0	
			Before	After	Before	After	Before	After
Portal venous pressure (mmHg)	15·0 ± 0·3	14·6 ± 0·3	14·6 ± 0·9	13·7 ± 1·0*	14·5 ± 0·8	13·1 ± 0·4*	14·1 ± 0·8	11·3 ± 0·4*
Change (%)		−2·6 ± 0·8		−6·0 ± 0·8		−9·3 ± 1·6†		−20 ± 2†
Mean arterial pressure (mmHg)	95 ± 3	94 ± 3	100 ± 7	93 ± 7*	97 ± 6	88 ± 6*	101 ± 3	82 ± 3*
Change (%)		−1·7 ± 0·8		−7·1 ± 0·8†		−10 ± 1†		−20 ± 2†
Heart rate (beats min^{-1})	308 ± 7	306 ± 8	348 ± 5	336 ± 9	355 ± 12	339 ± 14*	347 ± 11	323 ± 14*
Change (%)		−0·6 ± 0·3		−3·4 ± 1·4		−4·6 ± 1·0†		−7·1 ± 1·3†
Cardiac index (mL min^{-1}/100 g)	–	–	37·5 ± 0·9	37·8 ± 0·8	37·9 ± 0·8	38·5 ± 0·9*	37·8 ± 1·0	39·4 ± 1·0*
Change (%)		–		0·7 ± 0·7		1·7 ± 0·4*		4·4 ± 0·5*
Total peripheral resistance (mmHg min 100 g mL^{-1})	–	–	2·41 ± 0·10	2·35 ± 0·11*	2·44 ± 0·10	2·23 ± 0·10*	2·66 ± 0·05	2·03 ± 0·07*
Change (%)		–		−2·7 ± 0·7		−8·4 ± 0·4*		−24 ± 2*

Data were collected when the pressure responses to tetramethylpyrazine reached nadir. Cardiac index and total peripheral resistance were not measured during infusion of vehicle. Data are means ± s.e.m. (n = 7). *$P < 0.05$, significantly different from result before treatment with tetramethylpyrazine. †$P < 0.05$, significantly different from result for vehicle.

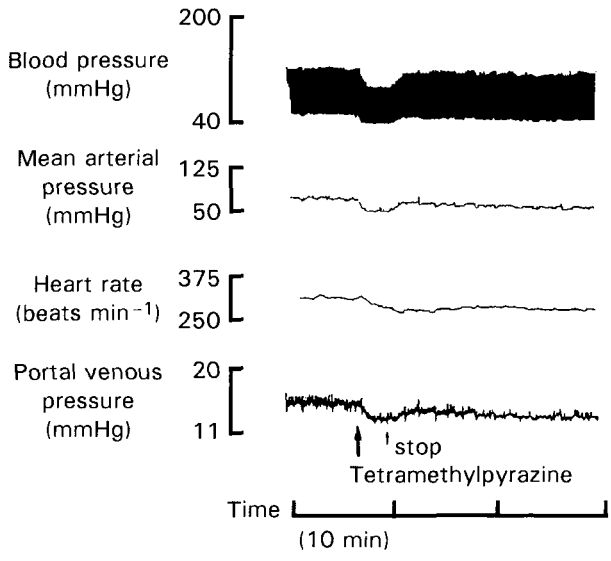

Figure 1. Traces of blood pressure, mean arterial pressure, heart rate, and portal venous pressure during the infusion of tetramethylpyrazine ($30{\cdot}0\,mg\,kg^{-1}$) to an anaesthetized portal hypertensive rat.

hypertensive animals, i.e., increased portal venous pressure ($14{\cdot}6 \pm 0{\cdot}9$ mmHg) and cardiac index ($37{\cdot}5 \pm 0{\cdot}9\,mL\,min^{-1}/100\,g$), and reduced mean arterial pressure (100 ± 7 mmHg) and total peripheral resistance ($2{\cdot}4 \pm 0{\cdot}1\,mmHg\,min\,100\,g\,mL^{-1}$) compared with sham-operated rats (Chojkier & Groszmann 1981; Huang et al 1997a). Figure 1 shows a representative example of the reduction of portal venous pressure and mean arterial pressure in a portal hypertensive rat as a result of infusion of tetramethylpyrazine ($30{\cdot}0\,mg\,kg^{-1}$). The maximum reduction of portal venous pressure and mean arterial pressure occurred 2–3 min after the start of infusion. It took several minutes for portal venous pressure and mean arterial pressure to return to nearly baseline levels after the end of infusion.

Dose–responses of portal venous pressure and mean arterial pressure to tetramethylpyrazine are shown in Table 1. Tetramethylpyrazine (3·0, 9·9 or $30{\cdot}0\,mg\,kg^{-1}$) induced dose-dependent reduction of portal venous pressure and mean arterial pressure in portal hypertensive rats. The maximum percentage reduction of portal venous pressure after tetramethylpyrazine was $6{\cdot}0 \pm 0{\cdot}8$, $9{\cdot}3 \pm 1{\cdot}6$, and $20 \pm 2\%$ of baseline for doses of 3·0, 9·9 and $30{\cdot}0\,mg\,kg^{-1}$, respectively. The maximum percentage reduction of mean arterial pressure after tetramethylpyrazine was $7{\cdot}1 \pm 0{\cdot}8$, 10 ± 1 and $20 \pm 2\%$ of baseline, again respectively, for the doses given (Table 1). Also, total peripheral resistance was significantly reduced by tetramethylpyrazine. Cardiac index was slightly increased by tetramethylpyrazine at 9·9 and $30{\cdot}0\,mg\,kg^{-1}$ (Table 1).

Discussion

Our results show that tetramethylpyrazine ($3{\cdot}0$–$30{\cdot}0\,mg\,kg^{-1}$) dose-dependently reduced portal venous pressure and mean arterial pressure in portal hypertensive rats. The percentage changes of portal venous pressure and mean arterial pressure during infusion of tetramethylpyrazine were of similar magnitude. At $30{\cdot}0\,mg\,kg^{-1}$, tetramethylpyrazine achieved 20% reduction of portal venous pressure in portal hypertensive rats. Reduction of mean arterial pressure and total peripheral resistance by tetramethylpyrazine in this study is in line with the haemodynamic effects of tetramethylpyrazine as a vasodilator (Dai & Bache 1985; Pang et al 1996). It is somewhat surprising that in this study we did not observe reflex tachycardia after tetramethylpyrazine despite reduction of systemic pressure and resistance. The slight increase in cardiac index as a result of treatment with tetramethylpyrazine suggests that the stroke volume was increased in the presence of bradycardia. The significance of this phenomenon is yet to be clarified.

We have recently reported that tetrandrine, a vasodilator and calcium-channel antagonist isolated from the Chinese herb *Stephania tetrandra* S. Moore (Wang & Lemos 1995; Liu et al 1995) induces portal hypotensive effects after acute infusion to portal hypertensive rats (Liu et al 1997) and ameliorates splanchnic hyperaemia and portal hypertension after chronic administration (Huang et al 1997b). The dose-range and magnitude of portal pressure reduction by acute infusion of tetramethylpyrazine were comparable with those previously reported for tetrandrine (Liu et al 1997). It will be interesting to investigate further the haemodynamic effects of tetramethylpyrazine after chronic oral administration in portal hypertensive rats.

There has recently been an increasing number of reports demonstrating that combination therapy of portal hypertension with drugs having different mechanisms of action has the potentially favourable effects both of reducing side effects and enhancing portal hypotensive effects (Reichen 1990; Lebrec 1994). Study is underway in our laboratory to investigate the effects of tetramethylpyrazine alone and in combination with other vasoactive drugs in portal hypertensive rats.

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