



Leukotrienes and cyclooxygenase products mediate anaphylactic venoconstriction in ovalbumin sensitized rat livers

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Abstract

Hepatic anaphylactic venoconstriction is partly involved in anaphylactic hypotension. We determined the chemical mediators responsible for anaphylaxis-induced segmental venoconstriction in perfused livers isolated from ovalbumin-sensitized rats. Livers were perfused portally and recirculatingly at constant flow with diluted blood. The portal venous pressure (Ppv), hepatic venous pressure (Phv), liver weight and hepatic oxygen consumption were continuously measured. The sinusoidal pressure was measured by the double occlusion pressure (Pdo), and was used to determine the pre-sinusoidal (Rpre) and post-sinusoidal (Rpost) resistances. After antigen injection, both Ppv and Pdo increased, resulting in 5.6- and 1.6-fold increases in Rpre and Rpost, respectively. Liver weight showed a biphasic change of an initial decrease followed by an increase. Hepatic oxygen consumption significantly decreased after antigen. Anaphylaxis-induced increase in Rpre was most extensively inhibited by 38.6% by pretreatment with ONO-1078 (100 μ M, a cysteinyl leukotriene receptor-1 antagonist), among all antagonists or inhibitors administrated individually including TCV-309 (20 μ M), AA-2414 (10 μ M), ketanserin (10 μ M) and indomethacin (10 μ M). Combined pretreatment with indomethacin and ONO-1078 exerted additive inhibitory effects and attenuated Rpre by 65.8%. However, TCV-309, a platelet activating factor (PAF) receptor antagonist, did not affect the anaphylactic response. In contrast, anaphylaxis-induced increase in Rpost was attenuated only by ONO-1078 combined pretreatment. The antigen-induced changes in liver weight and hepatic oxygen consumption were attenuated significantly when hepatic venoconstriction was attenuated. It is concluded that cysteinyl leukotrienes and cyclooxygenase products, but not PAF, are mainly involved in anaphylaxis-induced pre-sinusoidal constriction in isolated perfused rat livers.

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Keywords: Leukotriene; Cyclooxygenase; Double occlusion pressure; Hepatic vascular resistance

1. Introduction

Anaphylactic hypotension is sometimes life-threatening, and is caused by a decrease in effective circulating blood volume (Brown, 1995). It is reported that livers are partly involved in anaphylactic hypotension (Pavek et al., 1979; Shibamoto et al., 2005a). Indeed, anaphylaxis causes hepatic venoconstriction in rats (Hines and Fisher, 1992; Shibamoto et al., 2005a), guinea

pigs (Ruan et al., 2004b), rabbits (Karasawa et al., 2007) and dogs (Yamaguchi et al., 1994). This anaphylactic hepatic venoconstriction results in portal hypertension which then causes congestion of the upstream splanchnic organs, with resultant decrease in venous return and effective circulating blood volume, and finally augmentation of anaphylactic hypotension. Furthermore, if anaphylactic venoconstriction occurs substantially in the hepatic post-sinusoidal veins, hepatic congestion, i.e., pooling of blood in liver itself develops, which could contribute to anaphylactic hypotension, as observed in canine anaphylaxis (Yamaguchi et al., 1994). Measurement of the sinusoidal pressure with the vascular occlusion method in guinea pig livers revealed that hepatic congestion was also caused by significant post-sinusoidal constriction, although the pre-sinusoidal constriction was predominant (Ruan et al., 2004b). On the contrary, in rats (Shibamoto et al., 2005a) and rabbits

Abbreviations: PAF, platelet-activating factor; Tx, thromboxane; Ppv, portal venous pressure; Phv, hepatic venous pressure; Pdo, double occlusion pressure; Q, portal blood flow rate; Rt, total portal-hepatic venous resistance; Rpre, pre-sinusoidal resistance; Rpost, post-sinusoidal resistance.

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(Karasawa et al., 2007), pre-sinusoidal veins almost selectively contract with a resultant reduction of liver blood volume.

Anaphylaxis is the classic well-established immunoglobulin E dependent reaction (Montanaro and Bardana, 2002). Various inflammatory mediators, including histamine, serotonin, platelet-activating factor (PAF), and eicosanoids such as cysteinyl leukotrienes, thromboxane (Tx) A₂, and prostaglandin D₂, are involved in the initiation and propagation of the anaphylactic responses (Hagmann et al., 1992; Lieberman, 1996; Morel et al., 1991; Selig et al., 1993; Terashita et al., 1990). However, the primary mediators responsible for the local anaphylactic response may differ among species, organs and tissues involved. Concerning hepatic anaphylaxis, histamine, which is stored in mast cells and released during degranulation, caused hepatic venoconstriction in guinea pigs, but not in rats (Shibamoto et al., 2004). We have further reported that PAF and cysteinyl leukotrienes, but not histamine, serotonin or cyclooxygenase products, were mainly involved in anaphylaxis-induced hepatic venoconstriction of guinea pig livers (Shibamoto et al., 2005b). But the mediators responsible for hepatic anaphylaxis in other species including rats have not been determined. The products of the arachidonic acid cascade, such as LTD₄ (Bilzer and Lauterburg, 1993; Cui et al., 2006a; Haussinger et al., 1988; Shibamoto et al., 2005b), TxA₂ (Cui et al., 2006a; Ruan et al., 2004a) and prostaglandins (Miwa et al., 1997), and the membrane phospholipid product of PAF (Cui et al., 2006a; Ruan et al., 2004a; Shibamoto et al., 2005b), all can induce hepatic venoconstriction. Serotonin, the receptor of which was expressed on hepatic stellate cells, is involved in portal hypertension via induction of portal venoconstriction (Datte et al., 2005; Li et al., 2006). However, it is not known which mediator is mainly involved in the anaphylactic pre- or post-sinusoidal constriction and hepatic liver weight change in the rat. Thus, the purpose of the present study was to determine the roles of PAF, TxA₂, cysteinyl leukotrienes, serotonin and prostaglandins in the pre- and post-sinusoidal constriction and liver weight change during rat hepatic anaphylaxis by using the corresponding receptor antagonists or enzyme-synthesis inhibitors.

2. Materials and methods

2.1. Animals

Sixty-six male Sprague–Dawley rats (Japan SLC, Shizuoka, Japan) weighing 353 ± 2 (S.E.M.) g were used in this study. Rats were maintained at 23 °C and under pathogen-free conditions on a 12/12-hours dark/light cycle, and received food and water ad libitum. The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University.

2.2. Antigen sensitization

Rats were actively sensitized by the subcutaneous injection of an emulsion made by mixing complete Freund's adjuvant (0.5 ml) with 1 mg ovalbumin (grade V, Sigma) dissolved in

physiological saline (0.5 ml) (Cui et al., 2006b,c; Shibamoto et al., 2005a).

2.3. Isolated liver preparation

Two weeks after sensitization, the animals were anesthetized with pentobarbital sodium (60 mg/kg, ip) and mechanically ventilated with room air. The methods of isolation and perfusion of rat livers were previously described (Cui et al., 2006c). In brief, a polyethylene tube (0.5 mm ID, 0.8 mm OD) was placed in the right carotid artery. After laparotomy, the hepatic artery was ligated. At 5 min after intraarterial heparinization (500 U kg^{-1}), 8–9 ml of blood was withdrawn through the carotid arterial catheter. The intra-abdominal inferior vena cava above the renal veins was ligated, and the portal vein was cannulated with a stainless steel cannula (1.3 mm ID, 2.1 mm OD). After thoracotomy, the supradiaphragmatic inferior vena cava was cannulated through a right atrium incision with a larger stainless steel cannula (2.1 mm ID, 3.0 mm OD), then portal perfusion was begun with the heparinized autologous blood diluted with Krebs solution containing 5% bovine albumin at a Hct of 12%. The liver was rapidly excised, suspended from an isometric transducer (TB-652T, Nihon-Kohden, Japan) and weighed continuously throughout the experimental period.

The livers were perfused at a constant flow rate in a recirculating manner via the portal vein with blood that was pumped using a Masterflex roller pump from the venous reservoir through a heat exchanger (37 °C). The recirculating blood volume was 40 ml. The perfused blood was oxygenated in the venous reservoir by continuous bubbling with 95% O₂ and 5% CO₂ (perfusate PO₂ = 300 mmHg).

2.4. Measurement of hepatic vascular pressures and vascular resistances

The portal venous (Ppv) and hepatic venous (Phv) pressures were measured using pressure transducers (TP-400T, Nihon-Kohden, Japan) attached by sidearm to the appropriate cannulas with the reference points at the hepatic hilus. Portal blood flow rate (Q) was measured with an electromagnetic flow meter (MFV 1200, Nihon-Kohden, Japan), and the flow probe was positioned in the inflow line. The hepatic sinusoidal pressure was measured by the double occlusion pressure (Pdo) (Ruan et al., 2004b; Yamaguchi et al., 1994). Both the inflow and outflow lines were simultaneously and instantaneously occluded for 13 s using the solenoid valves, after which Ppv and Phv rapidly equilibrated to a nearly or identical pressure, which was Pdo. The principle of the double occlusion method to estimate the sinusoidal pressure (Yamaguchi et al., 1994) is derived from the concept of the mean circulating filling pressure (Pmcf) of the systemic circulation (Rothe, 1993). The Pmcf in the systemic circulation represents the pressure of the most compliant vascular segments, and therefore the Pdo, (i.e., the Pmcf in the isolated perfused livers) represents the pressure of the most compliant vessels in the liver, that is the sinusoids. Actually, Pdo values were obtained from the digitized data of Ppv and Phv

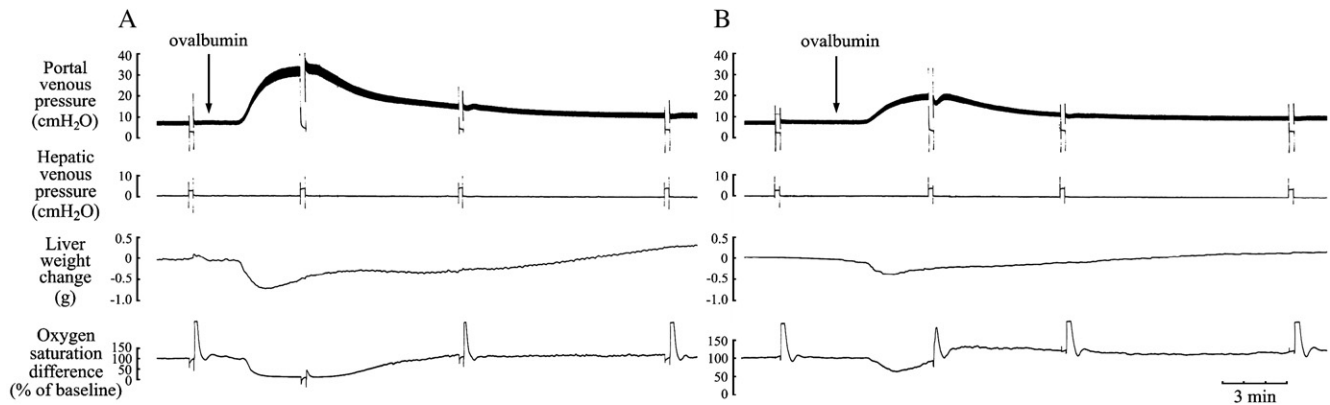


Fig. 1. Representative recordings of the changes in the portal venous pressure, hepatic venous pressure, liver weight change and oxygen saturation difference in response to the antigen of ovalbumin (0.1 mg) in the Control (A) and the ONO-1078 (B) groups. The double occlusion was performed before and at 4, 10, and 20 min after ovalbumin injection.

using an original program (LIVER software, Biomedical Science, Kanazawa, Japan). The total portal-hepatic venous (Rt), pre-sinusoidal (Rpre) and post-sinusoidal (Rpost) resistances were calculated by the following equations.

$$R_t = (P_{pv} - P_{hv})/Q \quad (1)$$

$$R_{pre} = (P_{pv} - P_{do})/Q \quad (2)$$

$$R_{post} = (P_{do} - P_{hv})/Q \quad (3)$$

where R_t represents the total portal-hepatic venous resistance, R_{pre} is the pre-sinusoidal resistance, R_{post} is the post-sinusoidal resistance, P_{pv} is the portal venous pressure, P_{hv} is the hepatic venous pressure, P_{do} is the double occlusion pressure, Q is the portal blood flow rate.

2.5. Hepatic oxygen consumption measurement

To determine the oxygen consumption, the blood oxygen saturation difference between the inflow and outflow blood (ΔSO_2) was monitored continuously with a custom-made oxygen absorption spectrophotometer (ODM-1, Biomedical Science, Kanazawa, Japan), the cuvettes and sensors of which were built in the inflow and outflow perfusion lines (Cui et al., 2006c). The measured ΔSO_2 was expressed as the ODM values (Cui et al., 2006c). ΔSO_2 was calculated using the following regression line equation as determined in our previous study (Cui et al., 2006c):

$$\Delta SO_2 = 1.5103 \times ODM - 0.0685 \quad (4)$$

Using ΔSO_2 , oxygen consumption ($\text{ml} \cdot \text{min}^{-1}$ per 10 g liver weight) was calculated according to the Fick principle by the following equation:

$$\text{Portal blood flow/liver weight (g)} \times 10 \times 1.39 (\text{ml/g}) \times \text{Hb (g/ml)} \times \Delta SO_2 \quad (5)$$

where Hb is the perfusing blood hemoglobin measured by the sodium lauryl sulfate-Hb method (Hb-B test Wako, Wako, Tokyo, Japan) with a spectrophotometer (UV-1200, Shimadzu, Japan).

2.6. Data recording

The hepatic vascular pressures, blood flow rate, liver weight and ODM values were monitored continuously and displayed through a thermal physiograph (RMP-6008, Nihon-Kohden, Japan). All outputs were also digitized by the analog–digital converter at a sampling rate of 100 Hz. These digitized values were also displayed and recorded using a personal computer for later determination of P_{do} .

2.7. Experimental protocols

Hepatic hemodynamic parameters were observed at least for 20 min after the start of perfusion until an isogravimetric state (no weight gain or loss) was obtained by adjusting the flow rate and the height of the reservoir at a P_{hv} of 0–1 cmH_2O , and at a Q of $39 \pm 1 \text{ ml} \cdot \text{min}^{-1}$ per 10 g liver wt. After the baseline measurements, the perfused livers excised from the sensitized animals were randomly assigned to one of nine groups, i.e., the Control group and eight groups for which receptor antagonists or inhibitors were pretreated.

In the Control group ($n=8$), ovalbumin (0.1 mg) was injected into the reservoir. In all other groups, at 10 min before an injection of the antigen (0.1 mg), the following receptor antagonists or inhibitors were injected into the perfusate: (1) TCV-309 ($\{3\text{-bromo-5-[N-phenyl-N-(2-\{2-(1,2,3,4\text{-tetrahydro-2-isoquinolyl-carboxyloxy)-ethyl\}carbamoyl\}ethyl)carbamyl]-l-propylpyridinium nitrate\}$; 20 μM), a PAF-receptor antagonist, for the TCV-309 group ($n=8$), (2) AA-2414 ($(\pm)\text{-7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenyl-heptanoic acid}$; 10 μM), a thromboxane-receptor antagonist, for the AA-2414 group ($n=8$) (3) ketanserin (10 μM), a serotonin-receptor antagonist, for the Ketanserin group ($n=7$), (4) indomethacin (10 μM), a cyclooxygenase inhibitor, for the Indomethacin group ($n=8$), (5) ONO-1078 (4-oxo-8-[p-(4-

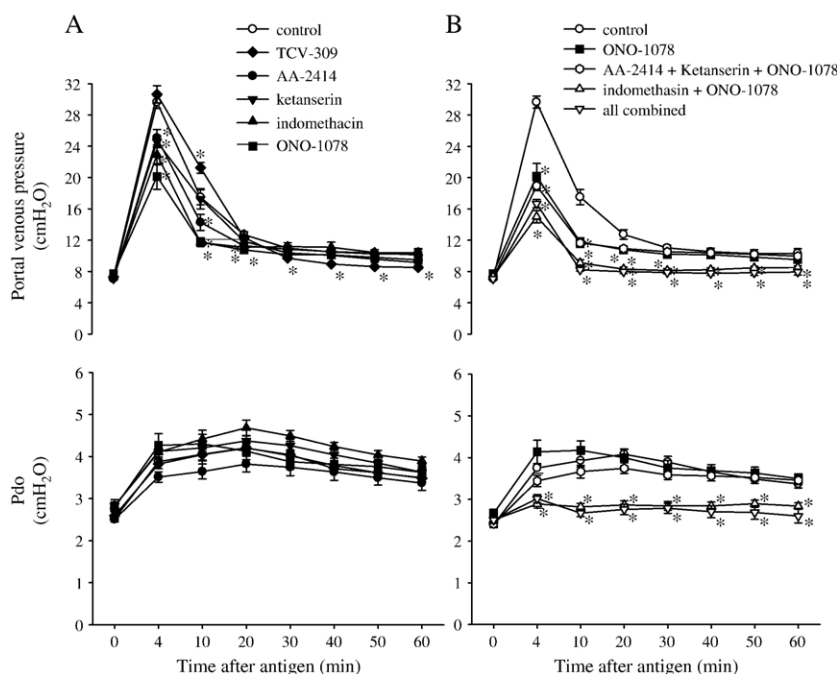


Fig. 2. Time course of the portal venous pressure (Ppv) (top) and double occlusion pressure (Pdo) (bottom) in groups of single (A) or combined (B) inhibitor or antagonist. Rpre and Rpost calculated by Eqs. (2) and (3), normalized to the baseline values, and shown as percentages. Values are means $\pm$ S.E.M.; * $P < 0.05$ vs. the Control group. Control, the Control group; TCV-309, the TCV-309 group; AA-2414, the AA-2414 group; Ketanserin, the Ketanserin group; Indomethacin, the Indomethacin group; ONO-1078, the ONO-1078 group; AA-2414 + Ketanserin + ONO-1078, the combined AA-2414, ketanserin and ONO-1078 group; Indomethacin + ONO-1078, the combined indomethacin and ONO-1078 group; All combined, the all combined group.

phenylbutyloxy) benzoylamino]-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate; 100 μ M), a specific antagonist for cysteinyl leukotriene receptor-1, for the ONO-1078 group ($n=7$), (6) combined AA-2414, ketanserin and ONO-1078 for the combined AA-2414, ketanserin and ONO-1078 group ($n=7$), (7) combined indomethacin and ONO-1078 for the combined indomethacin and ONO-1078 group ($n=7$), and (8) combined TCV-309, AA-2414, ketanserin, indomethacin and ONO-1078 for the all combined group ($n=6$).

The effects of each inhibitor or antagonist were evaluated by comparing the response of the agent-treated group with that of the Control group. The concentrations of TCV-309 (20 μ M), AA-2414 (10 μ M), ketanserin (10 μ M) and ONO-1078 (100 μ M) employed in this study were high enough to completely inhibit submaximal hepatic venoconstriction induced by 10 μ M PAF, 10 μ M U-46619 (9,11-dideoxy-9,11-epoxyme-thanoprost-5Z,13E-dien-1-oic acid; Tx_A₂ analogue), 10 μ M serotonin and 100 μ M LTD₄, respectively, in isolated rat livers in our preliminary studies. The concentrations of ketanserin and indomethacin were chosen as previously described (Martin, 1994; vom Dahl et al., 1990).

In each experimental group, Pdo was measured at baseline and 4, 6, and 10 min, and then at a 10-min interval up to 60 min after an injection of ovalbumin.

2.8. Drugs

TCV-309 and AA-2414 were kindly provided by Takeda Chemical (Osaka, Japan), and ONO-1078 by Ono Pharmaceu-

tical (Osaka, Japan). Indomethacin and ketanserin were purchased from Sigma (St. Louis, MO, USA). TCV-309 and ketanserin were dissolved in saline. ONO-1078 was dissolved in 1 N NaOH and ethanol. Indomethacin and AA-2414 were dissolved in DMSO. Concentrations of drugs were expressed as a final concentration in the perfusate.

2.9. Statistics

All results are expressed as the means $\pm$ S.E.M. Data were analyzed by one- and two-way analysis of variance, using repeated-measures for two-way comparison within groups. Comparisons of individual points between groups and within groups were made by Bonferroni's test. Differences were considered as statistically significant at P values less than 0.05.

3. Results

3.1. Antigen-induced hepatic segmental venoconstriction and liver weight change

Fig. 1A shows a representative example of the response to 0.1 mg ovalbumin in the Control group. After an ovalbumin injection, venoconstriction occurred within 1.5 min as evidenced by an increase in Ppv, which reached the peak at 4 min, and then gradually recovered. The Ppv increase was accompanied by the increase in Pdo and the decreases in oxygen saturation difference and liver weight which tended to increase

after 20 min (Fig. 1A). The summary data of changes in Ppv and Pdo after antigen in the Control and other groups are shown in Fig. 2. In the Control group, after ovalbumin injection, the Ppv increased to the peak level of 29.6 ± 0.8 cmH₂O at 4 min from the baseline of 7.1 ± 0.1 cmH₂O (Fig. 2). The Ppv-to-Phv gradient as the determinant of Rt increased to 4.3 times the baseline, indicating that Rt increased by the same degree. The double occlusion maneuver performed at 4 min after antigen revealed that the sinusoidal pressure of Pdo (3.9 ± 0.1 cmH₂O) was higher than that of the baseline (2.5 ± 0.1 cm H₂O). The Pdo-to-Phv gradient increased from the baseline of 2.4 ± 0.1 to 3.8 ± 0.1 cmH₂O, indicating an increase of 60% in Rpost. However, the increase in the Ppv-to-Pdo gradient (4.6 ± 0.1 vs. 25.8 ± 0.8 cmH₂O, baseline vs. peak) was bigger than that in the Pdo-to-Phv gradient, indicating a greater increase of 460% in Rpre than Rpost (60%), as shown in Fig. 3 (Control). The liver weight showed a biphasic change of an initial rapid decrease, reaching the bottom of -0.9 ± 0.1 g per 10 g liver wt at 4 min after antigen, followed by a gradual increase, reaching 1.0 ± 0.1 g per 10 g liver wt at the end of experiments. Hepatic oxygen consumption decreased to the nadir of $11 \pm 2\%$ of the baseline of 0.0766 ± 0.0072 ml·min⁻¹ per 10 g liver at 4 min

after antigen, then quickly recovered to the baseline level at 10 min, as shown in Fig. 4.

3.2. Effects of blockers on antigen-induced hepatic segmental venoconstriction and liver weight change

Fig. 2A shows the time courses of Ppv (top) and Pdo (bottom) in the Control group and the single blocker groups. Phv was not shown because it was kept constant due to constant flow perfusion. All the receptor antagonists or inhibitors except TCV-309 significantly attenuated the antigen-induced increase in Ppv in sensitized rat livers. The order of the efficacy of the inhibition was $\text{ONO-1078} > \text{indomethacin} > \text{ketaserin} > \text{AA-2414}$. Actually, the peak of Ppv was 68.2% in the ONO-1078 group (20.2 ± 1.6 cmH₂O), 77.4% in the indomethacin group (22.9 ± 1.3 cmH₂O), 83.1% in the ketanserin group (24.6 ± 0.8 cm H₂O) and 84.8% in the AA-2414 group (25.1 ± 1.1 cm H₂O), of that of the Control group (29.6 ± 0.8 cm H₂O).

Then, we further studied the effects of various blockers combined with ONO-1078, as also shown in Fig. 2B. The combination of indomethacin and ONO-1078, rather than that of AA-2414, ketanserin and ONO-1078, more extensively attenuated the peak value of Ppv to 15.0 ± 0.8 cmH₂O, which was only 50.7% of that in the Control group. In the all combined group, further inhibition of the antigen-induced increase in Ppv was not observed.

In contrast to Ppv, the antigen-induced increase in Pdo was attenuated slightly but significantly only in the combined indomethacin and ONO-1078 group and the all combined group, with the peaks of Pdo at 4 min after antigen in these two groups being 3.0 ± 0.1 and 3.1 ± 0.1 cmH₂O, respectively. A representative example of the response in the ONO-1078 group is shown in Fig. 1B.

Fig. 3 shows the changes in Rpre and Rpost in the absence and presence of the antagonists or inhibitors. The antigen-induced increase in Rpre was significantly attenuated in all groups except the TCV-309 group, with the same order of efficacy of the inhibition as that in Ppv. Actually, the Rpre increase was attenuated to 55.6% by pretreatment with ONO-1078, which elicited the strongest inhibitory effect among all antagonists or inhibitors administrated separately. Combined pretreatment with AA-2414, ketanserin and ONO-1078 did not further inhibit the Rpre increase after antigen, whereas combined pretreatment with indomethacin and ONO-1078 attenuated Rpre to 34.2%, i.e., exerted additive inhibitory effects on anaphylactic segmental venoconstriction. Similar to the effect on Pdo increase, Rpost increase was attenuated slightly but significantly only in the combined indomethacin and ONO-1078 group and the all combined group.

Fig. 4 shows the changes of liver weight and hepatic oxygen consumption in the single blocker groups (Fig. 4A) and the combined blockers groups (Fig. 4B). Liver weight decrease after antigen was significantly attenuated in all groups except the Ketanserin group. Actually it was completely inhibited in the Indomethacin group with the nadir of -0.06 ± 0.03 g per 10 g liver wt. The liver weight gain observed after 30 min following antigen administration was also inhibited in all groups except the

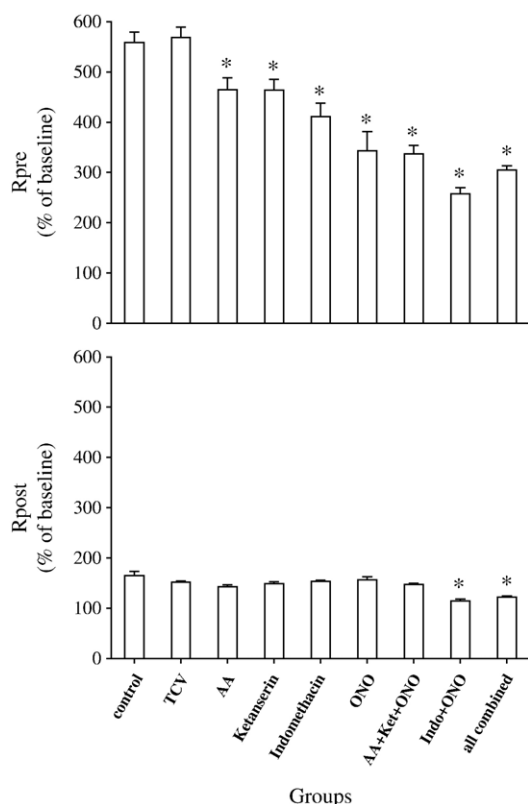


Fig. 3. The peak values of the pre-sinusoidal resistance (Rpre) and post-sinusoidal resistance (Rpost) after antigen in all groups studied. Values are means $\pm$ S.E.M.; * $P < 0.05$ vs. the Control group. Control, the Control group; TCV-309, the TCV-309 group; AA-2414, the AA-2414 group; Ketanserin, the Ketanserin group; Indomethacin, the Indomethacin group; ONO-1078, the ONO-1078 group; AA-2414+Ket+ONO-1078, the combined AA-2414, ketanserin and ONO-1078 group; Indo+ONO-1078, the combined indomethacin and ONO-1078 group; All combined, the all combined group.

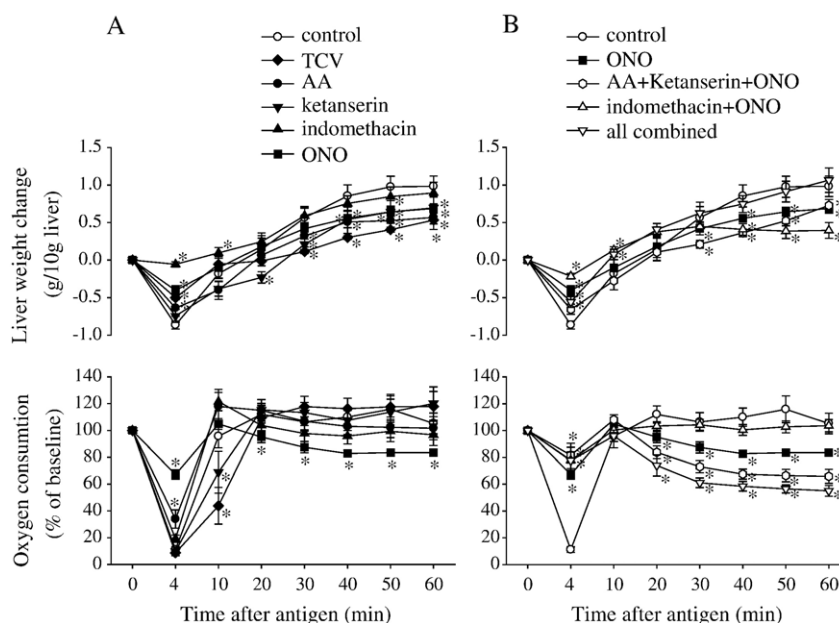


Fig. 4. The changes of liver weight and hepatic oxygen consumption after antigen in groups of single (A) or combined (B) inhibitor or antagonist. Values are means $\pm$ S.E.M.; * $P < 0.05$ vs. the Control group. Control, the Control group; TCV-309, the TCV-309 group; AA-2414, the AA-2414 group; Ketanserin, the Ketanserin group; Indomethacin, the Indomethacin group; ONO-1078, the ONO-1078 group; AA-2414+Ketanserin+ONO-1078, the combined AA-2414, ketanserin and ONO-1078 group; Indomethacin+ONO-1078, the combined indomethacin and ONO-1078 group; All combined, the all combined group.

indomethacin or the all combined group. The decrease of hepatic oxygen consumption induced by antigen was inhibited by pre-treatments with the combined AA-2414, ketanserin and ONO-1078 (by 79.3%), combined indomethacin and ONO-1078 (by 75.1%), all combined (by 74.8%), ONO-1078 (by 62.8%) and AA-2414 (by 25.6%), but not with TCV-309, ketanserin or indomethacin.

4. Discussion

In the present study, we determined the chemical mediators responsible for anaphylactic hepatic segmental venoconstriction in isolated sensitized rat livers perfused at a constant flow. The hepatic vascular resistances were assigned to the Rpre and Rpost by measuring the sinusoidal pressure with the vascular occlusion methods (Yamaguchi et al., 1994). We found that hepatic anaphylactic venoconstriction and related responses were characterized by 1) predominant pre-sinusoidal constriction, 2) biphasic change in liver weight of an initial rapid decrease followed by a gradual increase, and 3) a decrease in hepatic oxygen consumption. These results were consistent with our previous findings (Cui et al., 2006b,c). The main finding of the present study was that anaphylaxis-induced pre-sinusoidal constriction was significantly attenuated by ONO-1078 (cysteinyl leukotriene receptor-1 antagonist), indomethacin (cyclooxygenase inhibitor), ketanserin (serotonin receptor antagonist) and AA-2414 (Tx receptor antagonist), but not by TCV-309 (PAF receptor antagonist). ONO-1078 elicited the strongest inhibitory effect on anaphylactic hepatic venoconstriction among antagonists or inhibitors pretreated separately. ONO-1078 combined with indomethacin exerted the most extensive inhibitory effects on anaphylactic hepatic venoconstriction. Another major finding

was that the anaphylaxis-induced changes in liver weight and hepatic oxygen consumption became smaller when hepatic venoconstriction was attenuated by the blockers.

In the present study, ONO-1078, a specific cysteinyl leukotriene receptor-1 antagonist, most extensively inhibited the increase in Ppv and Rpre after antigen (by 44.4% and 38.6%, respectively) among blockers administered individually. This finding suggests that cysteinyl leukotrienes are main mediators of the hepatic anaphylactic venoconstriction in rats. Indeed, cysteinyl leukotrienes constrict hepatic vessels (Bilzer and Lauterburg, 1993; Haussinger et al., 1988). The present study showed that ONO-1078 significantly attenuated anaphylactic pre-sinusoidal constriction, but not the post-sinusoidal constriction (Fig. 3), suggesting that cysteinyl leukotrienes constrict significantly the pre-sinusoidal vessels of rat livers via the cysteinyl leukotriene receptor-1. This result contrasts with our previous finding on guinea pig livers perfused in the same way as in the present study, in which ONO-1078 significantly attenuated the post-sinusoidal constriction. This difference in the effects of cysteinyl leukotrienes on hepatic segmental venoconstriction between rats and guinea pigs might be attributed to the species difference in distribution of cysteinyl leukotrienes receptors on hepatic vascular segments.

Prostaglandins and TxA_2 , belonging to eicosanoids together with cysteinyl leukotrienes, are demonstrated to be other main mediators in the rat hepatic vascular anaphylaxis since pre-treatment with indomethacin or AA-2414 significantly attenuated the antigen-induced increase in Ppv (by 30.7% and 20.0%, respectively). Further the combination of indomethacin with ONO-1078 was the strongest in inhibition of anaphylactic venoconstriction. Indeed, prostaglandins could potentiate spontaneous contractions of the rat portal vein (Miwa et al., 1997);

however it is not known which vascular segment of hepatic vessels is preferentially constricted by prostaglandins. The present study showed that indomethacin selectively inhibited Rpre increase (by 26.4%), suggesting that prostaglandins significantly contract the pre-sinusoidal vessels of rat livers. In this study, TxA₂ also selectively constricted the pre-sinusoidal vessels of rat livers as suggested by the selective inhibitory effect of AA-2414 on Rpre increase (by 16.2%), which is consistent with our previous report (Cui et al., 2006a).

Although ketanserin did not attenuate anaphylactic venoconstriction in guinea pig livers (Shibamoto et al., 2005b), it inhibited increases of Ppv and Rpre after antigen by 22.3% and 17.0%, respectively, in rat livers of the present study. This result suggests that serotonin is also involved in anaphylactic hepatic vascular response and selectively constricts the pre-sinusoidal vessels of rat livers. Indeed, serotonin directly elicited constriction of portal venules and sinusoids (Reilly et al., 1984), which was mediated by activation of 5-hydroxytryptamine (5-HT) 1B/D and 5-HT_{2B} receptors subtypes on the portal vein wall (Datte and Offoumou, 2004). We did not assess the effect of histamine, another mediator released on mast cell degranulation, because histamine did not cause hepatic vasoconstriction of rats (Shibamoto et al., 2004).

Combined pretreatment with ONO-1078 and indomethacin, but not with ONO-1078, AA-2414 and ketanserin, further inhibited the anaphylaxis-induced increases in Ppv and Rpre. Moreover, even when other antagonists or inhibitors were added, they did not exert further inhibitory effects. This suggests that cysteinyl leukotrienes and cyclooxygenase products are main mediators for rat hepatic anaphylaxis, and exert the hepatic venoconstrictive actions in not synergetic but additive manner.

In the present study, TCV-309 did not attenuate the hepatic vascular response to antigen, suggesting that PAF is not involved in rat hepatic anaphylaxis. This result contrasted with the previous reports that PAF is involved in the anaphylactic hypotension of anesthetized rats, as evidenced by the inhibition of the systemic anaphylactic response by a PAF receptor antagonist (Mathison et al., 1992; Terashita et al., 1992). On the other hand, our previous study showed that PAF constricted hepatic vessels, especially the pre-sinusoidal vessels in rat livers (Cui et al., 2006a), suggesting that there are PAF receptors on the smooth muscle cell of rat portal veins. The concentration of TCV-309 administrated in the present study was high enough because it completely eliminated the venoconstriction induced by PAF 10 μ M in the same perfused rat liver preparation in our preliminary studies. Therefore, the absence of inhibitory effect of TCV-309 on anaphylactic hepatic vascular response observed in the present study indicates that production of PAF might be small in the isolated rat liver. Although there was a limited study reporting that a PAF receptor antagonist, WEB-2086, inhibited antigen-induced portal pressure increase in rat livers, the inhibitory effect was very weak: the maximal increases in portal pressure induced by antigen with and without pretreatment with WEB-2086 was 8.0 ± 0.3 vs. 9.5 ± 0.4 mmHg (Hines and Fisher, 1992).

As mentioned above, the inhibitory effects of ONO-1078, indomethacin, ketanserin and TCV-309 on hepatic venocon-

striction are different between rat livers and guinea pig livers (Shibamoto et al., 2005b), which suggested that there might be species difference in primary mediators responsible for the hepatic anaphylactic response, which might be ascribed to the different distribution of receptors of mediators on the hepatic vascular wall and different amount of mediators released from anaphylactic liver. Further studies are required in this respect.

In this study, liver weight decrease was observed within 10 min after antigen in the Control group, which might be caused by predominant pre-sinusoidal venoconstriction as evidenced by a substantial increase in Rpre. This decrease was inhibited via attenuation of increase of Rpre in all antagonists or inhibitors groups. After 30 min from antigen administration, liver weight gradually increased over the baseline in the Control group. This phenomenon might be due to the persistent high levels of Pdo, which lasted until the end of experiment; in other words, Rpost recovered slowly, while Rpre recovery was quick. This liver weight increase at the late period of experiments was attenuated as the Pdo increase was inhibited by antagonists or inhibitors.

In the present study, anaphylaxis markedly reduced hepatic oxygen consumption to 11% of baseline. This finding is consistent with previous investigations by us (Cui et al., 2006c) and by Hines and Fisher (Hines and Fisher, 1992). Hepatic oxygen consumption is regulated by various factors that affect metabolic and hemodynamic components of liver. However, the metabolic factors affect oxygen consumption during liver anaphylaxis more intensively than hemodynamic factors; even when anaphylactic hepatic venoconstriction was substantially suppressed by pretreatment with sodium nitroprusside, a potent vasodilator, the anaphylaxis-induced decrease in oxygen consumption was still preserved, as observed in our previous study (Cui et al., 2006c). Actually, ONO-1078 and AA-2414, but not TCV-309, ketanserin or indomethacin, attenuated the decrease of hepatic oxygen consumption (by 62.8% and 25.6%, respectively) in the present study, indicating that cysteinyl leukotrienes and TxA₂ decrease the anaphylactic oxygen consumption of isolated rat livers. Indeed, leukotriene C₄ and D₄ reduced hepatic oxygen consumption and these inhibitory effects were independent of changes in hemodynamic factors (Haussinger et al., 1988). The U-46619 and ONO-11113, both of which are TxA₂ analogues, also decreased oxygen consumption in perfused rat livers (Altin and Bygrave, 1988; Haussinger et al., 1988).

In summary, the chemical mediators responsible for the hepatic anaphylactic segmental venoconstriction induced by ovalbumin were determined in isolated rat livers. The cysteinyl leukotriene receptor-1 antagonist ONO-1078 was most effective, when pretreated individually, in attenuating the anaphylaxis-induced presinusoidal constriction, although cyclooxygenase inhibitor indomethacin, serotonin receptor antagonist ketanserin and thromboxane-receptor antagonist AA-2414 also significantly attenuated it. This inhibitory effect of ONO-1078 combined with indomethacin was the strongest. However, PAF receptor antagonist TCV-309 did not affect this response. We conclude that cysteinyl leukotrienes and cyclooxygenase products are mainly involved in anaphylaxis-induced hepatic venoconstriction in isolated perfused rat livers.

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