

Involvement of platelet-activating factor and leukotrienes in anaphylactic segmental venoconstriction in ovalbumin sensitized guinea pig livers

Toshishige Shibamoto*, Zonghai Ruan,
Sen Cui, Wei Liu, Yasutaka Kurata

Department of Physiology, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan

Received 5 July 2005; received in revised form 18 August 2005; accepted 19 August 2005

Available online 19 September 2005

Abstract

The hepatic anaphylactic venoconstriction is partly involved in anaphylactic hypotension, and is characterized by significant post-sinusoidal constriction and liver congestion in guinea pigs. We determined what chemical mediators are involved in anaphylaxis-induced segmental venoconstriction and liver congestion in perfused livers isolated from ovalbumin sensitized guinea pigs. Livers were perfused portally and recirculatingly at constant flow with diluted blood. 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. An antigen injection increased both the portal vein pressure and Pdo, resulting in 4.1- and 2.3-fold increases in Rpre and Rpost, respectively. Hepatic congestion was observed as reflected by liver weight gain. Pretreatment with TCV-309 (10 μ M, platelet-activating factor (PAF) receptor antagonist) or ONO-1078 (100 μ M, human cysteinyl-leukotriene (Cys-LT) receptor 1 antagonist), but not indomethacin (10 μ M, cyclooxygenase inhibitor), ketanserin (10 μ M, serotonin receptor antagonist), or diphenhydramine (100 μ M, histamine H₁ antagonist), significantly attenuated this anaphylactic hepatic venoconstriction. Anaphylaxis-induced increases in Rpre and

Abbreviations: PAF, platelet-activating factor; Cys-LTs, cysteinyl-leukotrienes; CysLT₁, cysteinyl-leukotriene receptor 1; CysLT₂, cysteinyl-leukotriene receptor 2; Tx, thromboxane; IVC, inferior vena cava; 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

* Corresponding author. Tel.: +81 76 218 8104; fax: +81 76 286 8010.

E-mail address: shibamo@kanazawa-med.ac.jp (T. Shibamoto).

Rpost were significantly inhibited by TCV-309 (by 48%) and ONO-1078 (by 36%), respectively. Combined TCV-309 and ONO-1078 pretreatment exerted additive inhibitory effects on anaphylactic hepatic venoconstriction. Anaphylactic hepatic weight gain was converted to weight loss when post-sinusoidal constriction was attenuated. It is concluded that anaphylaxis-induced pre-sinusoidal constriction is mainly caused by PAF and the post-sinusoidal constriction by Cys-LTs in guinea pig livers.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Leukotriene; Platelet-activating factor; Histamine; Hepatic circulation; Antigen; 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 [1]. It is reported that livers are partly involved in anaphylactic hypotension [2,3]. Anaphylaxis causes hepatic venoconstriction, as observed in rats [3,4], guinea pigs [5] and dogs [6,7], resulting 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, pooling of blood in liver itself develops, which could contribute to anaphylactic hypotension, as observed in canine anaphylaxis [7,8]. We have recently reported that hepatic anaphylactic venoconstriction in guinea pigs is also accompanied by liver congestion [5]. Measurement of the sinusoidal pressure with the vascular occlusion method revealed that this hepatic congestion was caused by significant post-sinusoidal constriction, although the pre-sinusoidal constriction was predominant [5].

Anaphylaxis is the classic well-established immunoglobulin E dependent reaction [9]. Various inflammatory mediators, including histamine, serotonin, platelet-activating factor (PAF), and eicosanoids such as cysteinyl-leukotrienes (CysLTs), thromboxane (Tx) A₂, and prostaglandin D₂, are involved in the initiation and propagation of the anaphylactic responses [10–14]. However, the primary mediators responsible for the local anaphylactic response may differ among organs and tissues involved. The mediators responsible for hepatic anaphylaxis in any species including guinea pigs have not been determined. Indeed, histamine, which is stored in mast cells and released during degranulation, causes hepatic venoconstriction in guinea pigs [15]. PAF [16], TxA₂ [16] and LTD₄ [17,18], all of which are the products of the arachidonic acid cascade and are synthesized upon mast cell activation, induce hepatic venoconstriction. However, it is not known which mediator is mainly involved in the anaphylactic pre- or post-sinusoidal constriction, and hepatic congestion. Thus, the purpose of the present study was to determine the role of histamine, serotonin, eicosanoids, and PAF in the pre- and post-sinusoidal constriction and hepatic congestion during guinea pig hepatic anaphylaxis by using the corresponding receptor antagonists or enzyme-synthesis inhibitors.

2. Materials and methods

2.1. Animals

The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University.

Forty-two male Hartley guinea pigs weighing 356 ± 5 (S.E.) g were used in this study. The animals were housed in a temperature-controlled animal facility with alternating 12-h light/12-h dark cycles and fed standard lab chow ad libitum with free access to water.

2.2. Antigen sensitization

Guinea pigs 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).

2.3. Isolated liver preparation

Two weeks after sensitization, the animals were anesthetized with pentobarbital sodium (35 mg/kg, i.p.) and mechanically ventilated with room air. The methods of isolation and perfusion of guinea pig livers were previously described [5]. A polyethylene tube was placed in the right carotid artery. After laparotomy, the cystic duct and the hepatic artery were ligated and the bile duct was cannulated with the polyethylene tube (1.0 mm i.d., 1.3 mm o.d.). At 5 min after intraarterial heparinization (500 U/kg), 8–9 ml of blood was withdrawn with a plastic syringe through the carotid arterial catheter. The intra-abdominal inferior vena cava (IVC) above the renal veins was ligated, and the portal vein was cannulated with a stainless cannula (2.1 mm i.d., 3.0 mm o.d.) for portal perfusion. After thoracotomy, the supradiaphragmatic IVC was cannulated through a right atrium incision with the same size stainless cannula, then portal perfusion was begun with the heparinized autologous blood that was diluted with 5% bovine albumin (Sigma) in Krebs solution (118 mM NaCl, 5.9 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1.2 mM NaH₂PO₄, 25.5 mM NaHCO₃, and 5.6 mM glucose) at Hct of 8%. The liver was rapidly excised, suspended from an isometric transducer (TB-652T, Nihon-Kohden, Japan) and weighed continuously throughout the experimental period.

The sensitized 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 height of the reservoir and the portal blood flow rate could be adjusted independently to maintain the portal and hepatic venous pressures at any desired level. The perfused blood was oxygenated in the venous reservoir by continuous bubbling with 95% O₂ and 5% CO₂. The portal venous (Ppv) and the hepatic venous (Phv) pressures were measured with pressure transducers (TP-400T, Nihon-Kohden, Japan) attached by sidearm to the appropriate cannulas with the reference points at the hepatic hilus. To occlude inflow and outflow perfusion lines simultaneously for measurement of the double occlusion pressure

(Pdo), two solenoid valves were placed in such a position that each sidearm cannula was between the corresponding solenoid valve and the liver. 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. Bile was collected drop by drop in a small tube suspended from the force transducer (SB-1T, Nihon-Kohden, Japan). One bile drop yielded 0.027 g and the time between drops was measured for determination of the bile flow rate [4]. The hepatic vascular pressures, blood flow rate, liver weight and bile weight were monitored continuously and displayed through a thermal physiograph (RMP-6008, Nihon-Kohden, Japan). Outputs were also digitized by the analog-digital converter at a sampling rate of 100 Hz. These digitized values were displayed and recorded using a personal computer for later determination of Pdo.

2.4. Experimental protocols

Hepatic hemodynamic parameters were observed for at least 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 Phv of 0–1 cmH₂O, and at a Q of 44 ± 1 ml/min/10 g liver wt. After the baseline measurements, the perfused livers excised from the sensitized animals were randomly assigned to one of the following six groups.

In the Control group ($n = 6$), ovalbumin 0.1 mg was injected into the reservoir. In all other groups, 10 min before an injection of antigen (0.1 mg), the following receptor antagonists or inhibitors were injected into the perfusate: (1) diphenhydramine HC1 (100 μ M), a histamine H₁-receptor antagonist, for the Diphenhydramine group ($n = 6$); (2) ketanserin (10 μ M), a serotonin-receptor antagonist, for the Ketanserin group ($n = 6$); (3) indomethacin (10 μ M), a cyclooxygenase inhibitor, for the Indomethacin group ($n = 6$); (4) TCV-309 (10 μ M; {3-bromo-5-[*N*-phenyl-*N*-(2-{[2-(1,2,3,4-tetrahydro-2-isoquinolyl-carbonyloxy)-ethyl]carbamoyl}ethyl)carbamyl]-1-propylpyridinium nitrate}), a PAF-receptor antagonist [19], for the TCV-309 group ($n = 6$); (5) ONO-1078 (100 μ M; 4-oxo-8-[p-(4-phenylbutyloxy) benzoylamino]-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate), a specific antagonist for human cysteinyl leukotriene receptor 1 (CysLT₁) [20], for the ONO-1078 group ($n = 6$); and (6) both TCV-309 (10 μ M) and ONO-1078 (100 μ M) for the combined TCV-309 and ONO-1078 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 (10 μ M), diphenhydramine (100 μ M), and ketanserin (10 μ M) employed in this study were high enough to completely inhibit submaximal hepatic venoconstriction induced by 10 μ M PAF, 100 μ M histamine, and 10 μ M serotonin, respectively, in the isolated guinea pig livers in our preliminary studies. The concentrations of indomethacin and ketanserin were chosen as previously described [21,22].

The hepatic sinusoidal pressure was measured by the double occlusion method [7]. 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 similar or identical pressure, which was Pdo. Actually, Pdo values were obtained from the digitized data of Ppv and Phv using an original program (LIVER software, Biomedical

Science, Kanazawa, Japan). In each experimental group, Pdo was measured at baseline and 4, 6, and 10 min, and then at a 10-min intervals up to 120 min after an injection of ovalbumin.

The total portal-hepatic venous (Rt), pre-sinusoidal (Rpre) and post-sinusoidal (Rpost) resistances were calculated by the following equations.

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

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

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

2.5. Drugs

TCV-309 and ONO-1078 were kindly provided by Takeda Chemical, (Osaka, Japan) and Ono Pharmaceutical (Osaka, Japan), respectively. Diphenhydramine-HCl, indomethacin and ketanserin were purchased from Sigma (St. Louis, MO, USA). TCV-309, ketanserin, and diphenhydramine-HCl were dissolved in saline. ONO-1078 was dissolved in 1N NaOH and ethanol. Indomethacin was dissolved in DMSO. Concentrations of drugs were expressed as a final concentration in the perfusate.

2.6. Statistics

All results are expressed as the means $\pm$ S.E. 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 hepatic weight gain

Fig. 1(left) shows a representative example of the response to 0.1 mg ovalbumin in the Control group. Within 1 min after an ovalbumin injection, venoconstriction occurred as evidenced by an increase in Ppv, which reached peak levels of 23.5 ± 1.2 cmH₂O from the baseline of 7.2 ± 0.2 cmH₂O. The Ppv-to-Phv gradient, the determinant of Rt, increased to 3.3 times the baseline, indicating that Rt increased by the same degree, as shown in Fig. 2. The double occlusion maneuver performed at 4 min after antigen revealed the sinusoidal pressure of Pdo (7.2 ± 0.4 cmH₂O) higher than that of the baseline (3.3 ± 0.1 cmH₂O). The Pdo-to-Phv gradient increased from the baseline of 2.9 ± 0.1 to 6.7 ± 0.4 cmH₂O, indicating an increase in Rpost (2.3-fold baseline). However, the increase in the Ppv-to-Pdo gradient (3.9 ± 0.2 cmH₂O versus 16.3 ± 1.1 cmH₂O, baseline versus peak) was greater than that

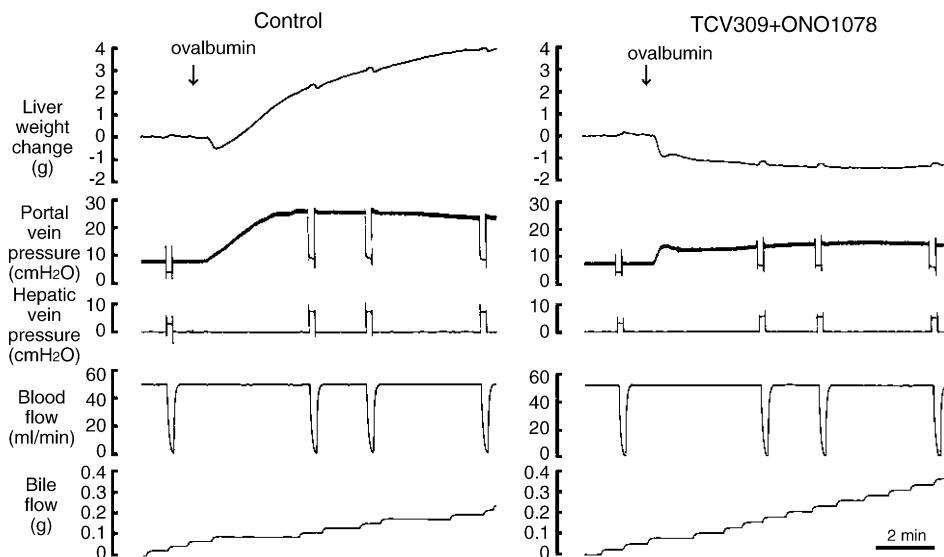


Fig. 1. Representative recordings of the response to the antigen, ovalbumin 0.1 mg in the Control (left) and the combined TCV-309 and ONO-1078 (right) groups.

in the Pdo-to-Phv gradient, indicating a greater increase in Rpre (4.2-fold baseline) than Rpost. At the same time the liver weight showed a gradual increase, reaching the peak of 2.1 ± 0.5 g/10 g liver weight at 10 min. Concomitant with venoconstriction, the bile flow rate decreased to 57% of the baseline of 0.03 ± 0.01 g/min/10 g liver wt.

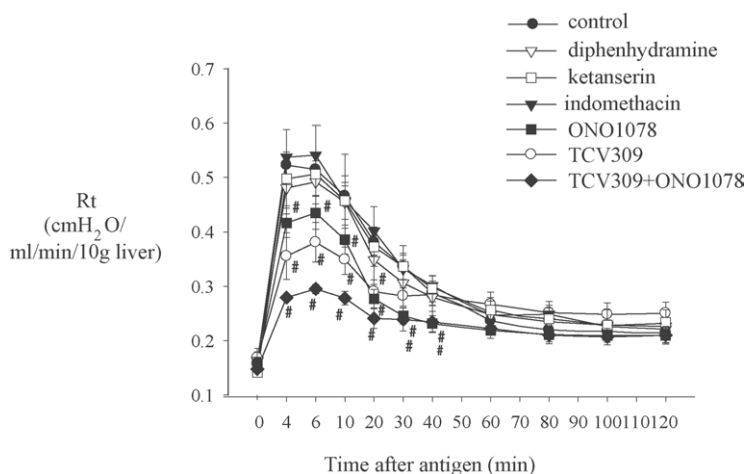


Fig. 2. Time course of total vascular resistance (Rt) in all seven groups. Values are means $\pm$ S.E.; # $P < 0.05$ vs. the Control group. All values of Rt after antigen in each group were significantly different from the corresponding baseline values.

3.2. Effects of diphenhydramine, ketanserin, indomethacin, TCV-309, ONO-1078, and combined TCV-309 and ONO-1078 on antigen-induced hepatic segmental vasoconstriction and hepatic weight gain

Fig. 2 shows the time course of changes in Rt after antigen in all groups studied. In the control sensitized group, Rt increased from the baseline levels of 0.16 ± 0.01 to the peak levels of 0.52 ± 0.02 cmH₂O/ml/min/10 g liver wt. at 4 min after antigen, followed by a gradual return towards the baseline. Pretreatment with TCV-309, ONO-1078, or the combination of TCV-309 and ONO-1078 (TCV-309 + ONO-1078), but not indomethacin, ketanserin, or diphenhydramine, significantly attenuated this increase in Rt. The order of the degrees of the inhibition was the combined TCV-309 and ONO-1078 > TCV-309 > ONO-1078, as shown in Fig. 2. Actually the peak levels of Rt after antigen decreased to 54, 71, and 79% of that of the Control group in the TCV-309 + ONO-1078, TCV-309, and ONO-1078 group, respectively. Thus the inhibitory effect of the combination of TCV-309 and ONO-1078 seemed to be exerted in an additive, rather than synergistical, manner of an inhibitory effect of each drug. Fig. 3 shows the results for Rpre and Rpost. Anaphylaxis-

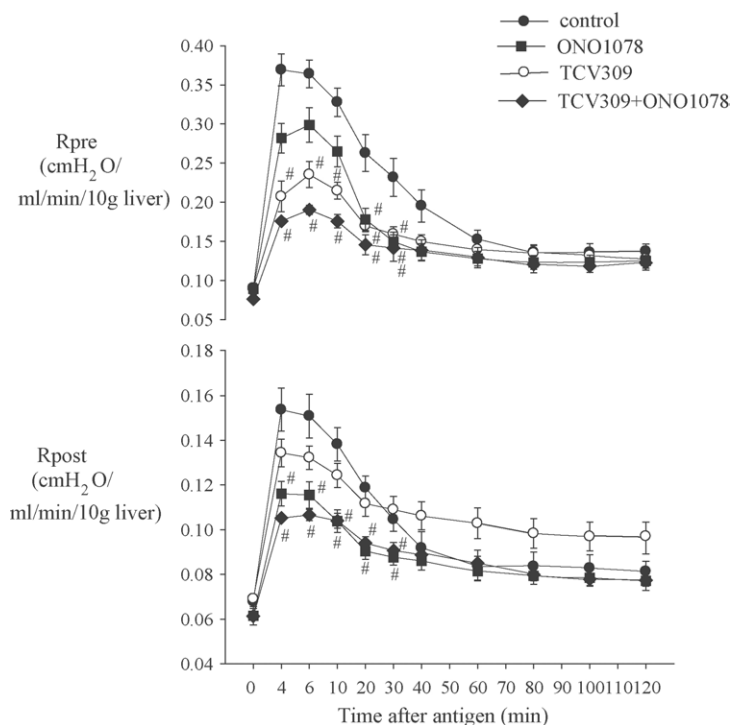


Fig. 3. Time course of the pre-sinusoidal resistance (Rpre) and post-sinusoidal resistance (Rpost) in the Control, the TCV-309, the ONO-1078, and the combined TCV-309 and ONO-1078 groups. Values are means $\pm$ S.E.; # $P < 0.05$ vs. the Control group. All values of Rpre and Rpost after antigen in each group were significantly different from the corresponding baseline values.

induced increases in R_{pre} and R_{post} were significantly attenuated by TCV-309 (by 44%) and ONO-1078 (by 25%), respectively. Combined TCV-309 and ONO-1078 pretreatment exerted additive inhibitory effects on anaphylactic segmental venoconstriction, with pre- and post-sinusoidal vessel constriction decreased by 49 and 27%, respectively. Fig. 1 (right) shows a representative example of the response in the combined TCV-309 and ONO-1078 group.

Fig. 4 shows Pdo and liver weight changes in all groups studied. In the Control group the sinusoidal pressure of Pdo increased significantly from the baseline of 3.3 ± 0.1 cmH₂O to the peak of 7.2 ± 0.4 cmH₂O at 4 min after the antigen. Pretreatment with ONO-1078 and combined TCV-309 and ONO-1078 significantly attenuated the increase in Pdo after antigen, with the peak value of Pdo at 4 min in the ONO-1078 and the combined groups being 5.6 ± 0.2 and 5.2 ± 0.1 cmH₂O, respectively. Anaphylactic hepatic weight gain was observed in all groups but not in the ONO-1078, TCV-309, or the combined TCV-309 and ONO-1078 groups. In the ONO-1078 and the combined groups, the liver weight after antigen significantly decreased to the nadir of -1.0 ± 0.1 and -1.3 ± 0.1 g/10 g liver wt., respectively. In the TCV-309 group, the liver weight tended to decrease but not significantly after antigen.

4. Discussion

In the present study, we determined the chemical mediators responsible for anaphylactic hepatic segmental venoconstriction in isolated sensitized guinea pig livers perfused at a constant flow. The hepatic vascular resistances were assigned to the R_{pre} and R_{post} by measuring the sinusoidal pressure with the vascular occlusion methods [7]. We found that hepatic anaphylactic venoconstriction was characterized by predominant pre-sinusoidal constriction and liver weight gain. This finding was consistent with our previous findings [5] although the method of sensitization of the guinea pigs with ovalbumin was different: the antigen was injected intraperitoneally in the previous study [5], while subcutaneously in the present study. The main finding of the present study was that anaphylaxis-induced pre-sinusoidal constriction was significantly inhibited by a PAF-receptor antagonist, TCV-309, and post-sinusoidal constriction by a CysLT₁ receptor antagonist, ONO-1078. The combined TCV-309 and ONO-1078 pretreatment exerted additive inhibitory effects on this segmental venoconstriction. On the other hand, inhibition of the receptor of histamine or serotonin, or inhibition of cyclooxygenase, alone did not affect the hepatic anaphylactic response. Another major finding was that anaphylactic hepatic weight gain was converted to weight loss when post-sinusoidal constriction was attenuated by ONO-1078 and combined TCV-309 and ONO-1078.

In the present study, pretreatment with TCV-309, a PAF antagonist, most intensely inhibited the increase in R_t after antigen (by 29%). This finding suggests that PAF is the primary mediator of the hepatic anaphylactic venoconstriction in guinea pigs. This hepatic venoconstriction was characterized by predominant pre-sinusoidal constriction rather than post-sinusoidal constriction. Actually, TCV-309 significantly attenuated this anaphylactic pre-sinusoidal constriction, but not the post-sinusoidal constriction, as shown in Fig. 3. With respect to the effects of PAF on the segmental hepatic vascular resistances of guinea pigs,

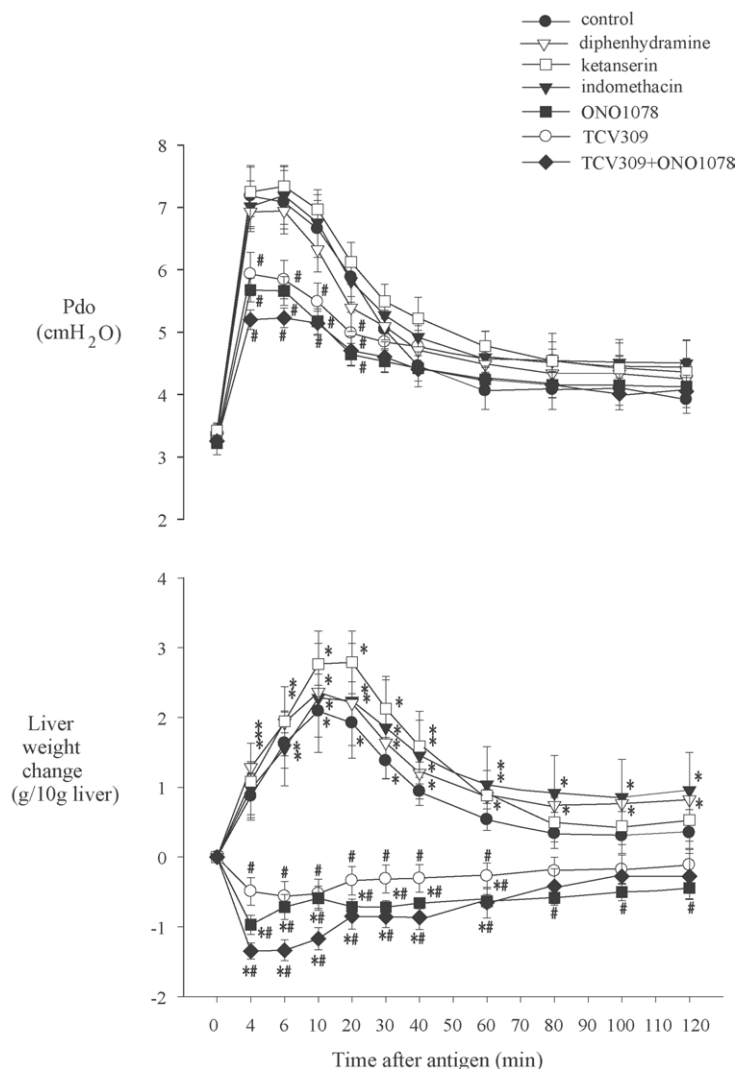


Fig. 4. Time course of the double occlusion pressure (Pdo) and the liver weight change in all seven groups. Values are means $\pm$ S.E.; * $P < 0.05$ vs. baseline. The asterisks were not given in Pdo after antigen, because all these values were significantly different from the corresponding baseline values. # $P < 0.05$ vs. the Control group.

we have recently reported that PAF injected into the isolated guinea pig livers perfused in the same way as in the present study caused predominant pre-sinusoidal constriction with liver weight gain, the response similar to the anaphylactic response [16]. The crucial role of PAF was also reported in guinea pig anaphylaxis in vivo and in isolated lung parenchymal strips in vitro [23]. Darius et al. [23] demonstrated that the histamine- and leukotriene-independent component of anaphylactic hypotension and bronchoconstriction

in guinea pigs is mediated by PAF. These findings are consistent with the present results on the hepatic anaphylaxis in guinea pigs.

Cys-LTs are demonstrated to be as another main mediator in the guinea pig hepatic vascular anaphylaxis since pretreatment with ONO-1078 significantly attenuated the increase in Rt after antigen (by 21%). In contrast to TCV-309, which selectively inhibited the pre-sinusoidal constriction, ONO-1078, selective CysLT₁ antagonist, significantly inhibited anaphylactic post-sinusoidal constriction, but not the pre-sinusoidal constriction. Although Cys-LTs constrict hepatic vessels [17,18], it is not known which vascular segment of hepatic vessels is preferentially constricted by Cys-LTs. The present study suggests that Cys-LTs contract significantly the post-sinusoidal vessels of guinea pig livers via the CysLT₁ receptor, and substantially contribute to anaphylactic hepatic venoconstriction of this animal. It has been known that the vasoactive effects of Cys-LTs are mediated by at least 2 specific receptors, CysLT₁ and Cys-LT receptor 2 (CysLT₂) [24,25]. However there are only a limited number of studies that revealed the roles of these specific receptors in hepatic circulation [26]. Graupera et al. [26] reported using intact perfused rat livers that CysLT₁ receptor blockade mimics the effect on portal perfusion pressure caused by 5-lipoxygenase inhibition, suggesting that regulation of normal vascular tone by Cys-LTs is mediated by the activation of CysLT₁ receptors. We here show that anaphylactic post-sinusoidal constriction in guinea pigs is mediated by the activation of CysLT₁ receptors. However, the roles of CysLT₂ receptor and a third class of Cys-LT receptor could not be examined due to the lack of specific antagonists [24,25]. In future, further studies are required in this respect.

Combined TCV-309 and ONO-1078 pretreatment inhibited the anaphylaxis-induced increases in Rt, Rpre, and Rpost by 46, 49, and 27%, respectively. This suggests that PAF and Cys-LTs exert the hepatic venoconstrictive actions in not synergistical but additive manner.

In the present study, diphenhydramine, a histamine H₁-receptor antagonist, did not attenuate the venoconstriction induced by ovalbumin antigen. Histamine is known to be released during systemic anaphylaxis in humans [27] and in animals [12,28,29]. Actually, Selig et al. [13] showed, by using isolated perfused lungs of guinea pigs that were sensitized with ovalbumin, that the histamine H₁-receptor antagonist prevented the antigen-induced changes in pulmonary hemodynamics, intratracheal pressure, and edematogenic responses. Organ dependent difference in responsiveness to antigen may account for the difference in the effect of histamine antagonist between lungs and livers. Indeed, we have recently shown that in guinea pigs, the hepatic vascular response to histamine was relatively weak and short-lasting as observed in isolated perfused livers: even at high concentration of 100 μ M, Rt increased only to 1.7-fold baseline, and this response lasted only 3 min [15]. Another explanation why a histamine antagonist did not prevent anaphylactic response is that histamine is released only early stage of anaphylaxis in guinea pigs [23,30]. Mest et al. [30] reported by examining the time course changes in chemical mediators during guinea pig anaphylaxis that histamine and serotonin were released immediately after antigen, reaching peak levels at 20–30 s, and then the release decreased rapidly within 60 s. Thus, when the maximum venoconstriction occurred in the present perfused guinea pig livers, histamine might have disappeared in perfusing blood. Failure of the histamine antagonist to prevent the physiological changes after the antigen administration in the present study suggests a minor role

of histamine in anaphylactic vasoconstriction in guinea pig livers. Finally, a more plausible explanation for the absence of the effect of diphenhydramine (histamine inhibitor), and also ketanserin (serotonin inhibitor) or indomethacin (cyclooxygenase inhibitor) on vasoconstriction is that more than one mediators are released at supramaximal concentrations, and thus inhibition of a single mediator has no effect on the magnitude of the constriction. For instance, even if the response to histamine, serotonin or TxA_2 is completely blocked, the response to PAF and Cys-LTs still remain intact, and no change in the constriction is observed.

In this study, liver weight gain was observed after antigen in the Control group but not in either TCV-309, ONO-1078, or the combined ONO-1078 and TCV-309 group. Moreover, the latter two groups showed the liver weight loss response. The weight gain in the Control group might be caused by post-sinusoidal venoconstriction as evidenced by a substantial increase in Rpost. This post-sinusoidal contraction could induce the upstream sinusoidal engorgement and increased extravascular fluid filtration caused by an increase in Pdo, the sinusoidal hydrostatic pressure. The absence of liver weight gain after antigen in the TCV-309, ONO-1078, or the combined groups may be due to the attenuation of post-sinusoidal constriction. Actually in these three groups, the sinusoidal pressure of Pdo, which is the indicator of post-sinusoidal resistance as well as the determinant of transvascular fluid movement and hepatic congestion, was not so much increased. We previously reported that the magnitude of an increase in Pdo in response to PAF and the TxA_2 analogue was strongly associated with the liver weight change in the same guinea pig liver preparation [16]. The perfused liver with pre-sinusoidal venoconstriction does not show liver weight gain until post-sinusoidal vessels contract enough to elevate Pdo to more than $6.5 \text{ cmH}_2\text{O}$. In the present study, the peak Pdo in the TCV-309, ONO-1078 and the combined ONO-1078 and TCV-309 groups was less than $6.5 \text{ cmH}_2\text{O}$ (Fig. 4). On the other hand, the conversion from liver weight gain to liver weight loss after antigen in the ONO-1078 and the combined groups may be explained as follows: In these two groups, the anaphylaxis-induced post-sinusoidal constriction was substantially attenuated, but the pre-sinusoidal constriction was preserved. In the presence of pre-sinusoidal constriction, the blood volume of sinusoids, that was distal to the constricted pre-sinusoidal portal venules, could be passively reduced due to a decrease in distending pressure of the sinusoids. This reduction of blood volume may result in liver weight loss.

In summary, the chemical mediators responsible for the hepatic anaphylactic segmental venoconstriction induced by ovalbumin antigen were determined in isolated guinea pig livers. Anaphylaxis-induced pre- and post-sinusoidal constriction was inhibited markedly by the PAF inhibitor TCV-309 and the Cys-LT receptor 1 antagonist ONO-1078, respectively, whereas histamine, serotonin or cyclooxygenase inhibitors did not affect this response. We conclude that anaphylaxis-induced pre-sinusoidal constriction is mainly caused by PAF and the post-sinusoidal constriction by Cys-LTs in guinea pig livers.

Acknowledgements

The authors thank Takeda Chemical Industries (Osaka, Japan) and Ono Pharmaceutical Company (Osaka, Japan) for the gift of TCV-309 and ONO-1078, respectively.

This study was supported by a Grant for Collaborative Research from Kanazawa Medical University (C2004-1, C2005-1) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan (No. 15591665).

References

- [1] Brown AFT. Anaphylactic shock: mechanism and treatment. *J Accid Emerg Med* 1995;12:89–100.
- [2] Pavsek K, Piper PJ, Smedegard G. Anaphylatoxin-induced shock and two patterns of anaphylactic shock: hemodynamics and mediators. *Acta Physiol Scand* 1979;105:393–403.
- [3] Shibamoto T, Cui S, Ruan Z, Liu W, Takano H, Kurata Y. Hepatic venoconstriction is involved in anaphylactic hypotension in rats. *Am J Physiol Heart Circ Physiol*, May 27 [Epub], in press.
- [4] Hines KL, Fisher RA. Regulation of hepatic glycogenolysis and vasoconstriction during antigen-induced anaphylaxis. *Am J Physiol* 1992;262:G868–77.
- [5] Ruan Z, Shibamoto T, Shimo T, Tsuchida H, Koizumi T, Nishio M. NO, but not CO, attenuates anaphylaxis-induced postsinusoidal contraction and congestion in guinea pig liver. *Am J Physiol Regul Integr Comp Physiol* 2004;286:R94–100.
- [6] Weil R. Studies in anaphylaxis. XXI Anaphylaxis in dogs: a study of the liver in shock and peptone poisoning. *J Immunol* 1917;2:525–56.
- [7] Yamaguchi Y, Shibamoto T, Hayashi T, Saeki Y, Tanaka S. Hepatic vascular response to anaphylaxis in isolated canine liver. *Am J Physiol Regul Integr Comp Physiol* 1994;267:R268–74.
- [8] Essex HE. Anaphylactic and anaphylactoid reactions with special emphasis on the circulation. In: Hamilton, W.F., editor. *Handbook of physiology circulation*, Section 2, vol. III, Chapter 66, Bethesda, MD: Am. Physiol. Soc.; 1965, p. 2391–408.
- [9] Patterson R, Roberts M, Pruzansky JJ. Comparisons of reaginic antibodies from three species. *J Immunol* 1969;102:466–75.
- [10] Hagmann W, Hacker HJ, Buchholz U. Resident mast cells are the main initiators of anaphylactic leukotriene production in the liver. *Hepatology* 1992;16:1477–84.
- [11] Lieberman PL. Specific and idiopathic anaphylaxis: pathophysiology and treatment. In: Bierman CW, Pearlman DL, Shapiro CG, et al., editors. *Allergy, asthma, and immunology from infancy to adulthood*. 3rd ed. Philadelphia: WB. Saunders; 1996. p. 297–319.
- [12] Morel DR, Skoskiewicz M, Robinson DR, Bloch KJ, Hoaglin DC, Zapol WM. Leukotrienes, thromboxane A₂, and prostaglandins during systemic anaphylaxis in sheep. *Am J Physiol Heart Circ Physiol* 1991;261:H782–92.
- [13] Selig WN, Tocker JE, Tannu SA, Cerasoli F, Durham SK. Pharmacologic modulation of antigen-induced pulmonary responses in the perfused guinea pig lung. *Am Rev Respir Dis* 1993;147:262–9.
- [14] Terashita Z, Imura Y, Nishikawa K. Possible pathophysiological roles of platelet-activating factor in shock and the PAF antagonists, CV-3988 and CV-6209. In: Handley DA, Saunders RN, Houlihan WJ, Tomesch JC, editors. *Platelet-activating factor in endotoxin and immune diseases*. New York: Marcel Dekker; 1990. p. 547–75.
- [15] Shibamoto T, Narushima M, Ling YQ, et al. Different hepatic vascular response to noradrenaline and histamine between guinea pig and rat. *Acta Physiol Scand* 2004;180:255–63.
- [16] Ruan Z, Shibamoto T, Shimo T, et al. Effects of platelet-activating factor and thromboxane A₂ on isolated perfused-guinea pig liver. *Prostaglandins Other Lipid Mediat* 2004;73:73–85.
- [17] Bilzer M, Lauterburg BH. Peptidoleukotrienes increase the efflux of glutathione from perfused rat liver. *Prostaglandins Leukot Essent Fatty Acids* 1993;49:715–21.
- [18] Haussinger D, Stehle T, Gerok W. Effects of leukotrienes and the thromboxane A₂ analogue U-46619 in isolated perfused rat liver. Metabolic, hemodynamic and ion-flux responses. *Biol Chem Hoppe Seyler* 1988;369:97–107.
- [19] Terashita Z, Kawamura M, Takatani M, Tsuchima S, Imura Y, Nishikawa K. Beneficial effects of TCV-309, a novel potent and selective platelet activating factor antagonist in endotoxin and anaphylactic shock in rodents. *J Pharmacol Exp Ther* 1992;260:748–55.

- [20] Lynch KR, O'Neill GP, Liu Q, et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 1999;399:789–93.
- [21] Martin GR. Vascular receptors for 5-hydroxytryptamine: distribution, function and classification. *Pharmacol Ther* 1994;62:283–324.
- [22] vom Dahl S, Wettstein M, Gerok W, Haussinger D. Stimulation of release of prostaglandin D2 and thromboxane B2 from perfused rat liver by extracellular adenosine. *Biochem J* 1990;270:39–44.
- [23] Darius HD, Lefer DJ, Smith JB, Lefer AM. Role of platelet-activating factor-acether in mediating guinea pig anaphylaxis. *Science* 1986;232:58–60.
- [24] Hui Y, Funk CD. Cysteinyl leukotrienes receptors. *Biochem Pharmacol* 2002;64:1549–57.
- [25] Capra V. Molecular and functional aspects of human cysteinyl leukotriene receptors. *Pharmacol Res* 2004;50:1–11.
- [26] Graupera M, Garcia-Pagan J-C, Titos E, et al. 5-Lipoxygenase inhibition reduces intrahepatic vascular resistance of cirrhotic rat livers: A possible role of cysteinyl-leukotrienes. *Gastroenterology* 2002;122:387–93.
- [27] Smith PL, Sobotka AK, Bleeker ER, et al. Physiologic manifestations of human anaphylaxis. *J Clin Invest* 1980;66:1072–80.
- [28] Krell RD. Investigation of the role of histamine in antigen-induced bronchoconstriction in the ascaris-hypersensitive dog. *Br J Pharmacol* 1978;62:519–28.
- [29] Silverman HJ, Taylor WR, Smith PL, et al. Effects of antihistamines on the cardiopulmonary changes due to canine anaphylaxis. *J Appl Physiol* 1988;64:210–7.
- [30] Mest HJ, Ponicke K, Heinroth-Hoffmann I, Meissner N, Giessler C. Relevance of mediators to cardiac parameters of isolated anaphylactic guinea-pig heart. *Prostaglandins Leukot Essent Fatty Acids* 1995;53:21–9.