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Hemostatic Action of OC-108, a Novel Agent for Hemorrhoids, Is Associated With Regional Blood Flow Arrest Induced by Acute Inflammation

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Abstract. Clinically, hemorrhoidal bleeding and prolapse disappeared immediately after injection of the sclerosing agent OC-108 into submucosa of hemorrhoids. The aim of this study was to elucidate the mechanism of action responsible for the immediate hemostatic effect of OC-108 using anesthetized rats. Subcutaneous injection of OC-108 in rats decreased blood flow at the injection site within 5 min. Aluminum potassium sulfate, one of the main ingredients of OC-108, reduced the skin blood flow. However, tannic acid, another main ingredient, did not. By perfusion of OC-108 on the mesenteric surface, microcirculatory blood flow was arrested without remarkable change in blood vessel diameter, accompanied by increased vascular permeability and venous hematocrit. These results indicate that OC-108 induces regional blood flow arrest with rapid onset, this effect being attributed to the action of aluminum potassium sulfate, and that hemoconcentration due to increased vascular permeability (plasma extravasation), an acute inflammatory reaction, is involved in the mechanisms of the immediate hemostatic action of OC-108.

Keywords: OC-108, hemorrhoidal bleeding, blood flow arrest, vascular permeability, hemoconcentration

Introduction

OC-108 is a novel sclerosing agent containing aluminum potassium sulfate and tannic acid as active components. It is pharmaceutically modified from Xiaozhiling, a Chinese medicinal agent for the treatment of internal hemorrhoids (1, 2).

In clinical studies on severe internal hemorrhoids, injection of OC-108 into the submucosa of hemorrhoids proved to be highly efficacious in curing the cardinal signs of prolapse and bleeding (3, 4), and the effects were comparable to those of hemorrhoidectomy (4).

In nonclinical studies with rats, OC-108 induced acute inflammation and subsequently granuloma formation with fibrosis at the injection site, as a restoration process (5, 6). In addition, in the clinical studies, hemorrhoids shrank and disappeared progressively with time during the 28 days after OC-108 injection (3, 4). These data suggested to us that hemorrhoids are sclerosed and retracted via fibrosis of the hemorrhoidal interstitium, and the bleeding is probably reduced by reinforcement around the distended hemorrhoidal veins. However, bleeding and prolapse disappeared immediately, within 3 days, after injection of OC-108 in almost all of the patients in the clinical studies (3, 4), while fibrosis has not yet been induced in the corresponding period in the nonclinical studies (5, 6). Therefore, it is difficult to explain the mode of action of OC-108 only by its tissue sclerosing effect.

In this study, we examined the pharmacologic action responsible for the immediate hemostatic effect of OC-108 in anesthetized rats.

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Materials and Methods

Animals

Male Wistar rats were obtained from Charles River Japan, Inc. (Yokohama). Animals were housed under conditions of controlled temperature (23 ± 3°C) and humidity (55 ± 10%) with 12-h illumination cycles. They were allowed to acclimate for at least 1 week before the experiments. All experiments were approved by the Animal Ethical Committee of Mitsubishi Pharma Corporation and performed in accordance with guidelines of The Japanese Pharmacological Society.

Compounds

OC-108 (4%), an undiluted solution of OC-108, was prepared at the Pharmaceutical Development Laboratories of Mitsubishi Pharma Corporation. OC-108 (2%) was prepared by diluting OC-108 (4%) with an equal volume of saline, except for the experiment investigating a concentration-response relationship. Aluminum potassium sulfate, tannic acid, and phenylephrine [(*R*)-(−)-phenylephrine HCl] were purchased from Miyazawa Pharmaceutical Co. (Tokyo), Fuji Chemical Industry Co. (Wakayama), and Wako Pure Chemical Ind. Ltd. (Osaka), respectively, and each compound was dissolved in saline. Krebs-Henseleit solution (117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2 \cdot 2H_2O$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 24.8 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11.0 mM glucose) or Tyrode's solution (136.9 mM NaCl, 5.4 mM KCl, 2.5 mM $CaCl_2 \cdot 2H_2O$, 1.0 mM $MgCl_2 \cdot 6H_2O$, 23.8 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 11.0 mM glucose) was used as a perfusion solution for mesentery.

Measurement of skin blood flow

Rats (338 – 453 g) were trimmed of their abdominal fur 1 day before measurement of skin blood flow. Animals were anesthetized with urethane (1.2 g/kg, i.p.), and restricted at dorsal position on a heat insulating pad (37°C – 38°C). Using a laser Doppler flow meter (ALF21; Advance Co., Tokyo), 4 sites that exhibit almost the same level of skin blood flow were selected as injection sites; they were in the upper right, upper left, lower right, and lower left part of abdominal skin in each rat. After stabilization of the skin blood flow, test solutions at 0.25 ml/kg were injected s.c., and the skin blood flow was measured at the same site. Simultaneously, blood pressure was monitored with a pressure transducer (DX360; Nihon Kohden, Tokyo) through a catheter inserted into a common carotid artery, and heart rate was measured with a tachometer (AT-601G, Nihon Kohden) from the blood pressure pulse. To minimize bias, the location of the test solutions for injection in each rat was allocated by the Latin square method.

Observation of mesenteric blood vessels

Rats (314 – 415 g) were anesthetized with pentobarbital sodium (25 mg/ml) by injecting properly through a catheter inserted into a cervical vein.

For the observation of mesenteric arteries and veins, rats underwent abdominal incision, and the mesentery was spread out on an observation stage (37°C), and given surface perfusion with Krebs-Henseleit solution ventilated with a mixture of gas (95% O_2 and 5% CO_2) using a cassette tube pump (SMP-21; Tokyo Rikakikai Co., Tokyo). After stabilization of the condition, the surface of the mesentery was perfused with OC-108 (2%) for 30 min or 1 mg/ml phenylephrine for 10 min; the control preparation was perfused with Krebs-Henseleit solution for 30 min. Before and after perfusion of the test solutions, mesenteric arteries and veins were photographed by a camera (FE Micro-NIKKOR 55 mm; Nikon, Tokyo), and the blood vessel diameter was measured.

For the observation of mesenteric small blood vessels, the ileal portion of the mesentery was carefully spread out on an observation stage (37°C) and given surface perfusion with Tyrode's solution ventilated with a mixture of gas (95% O_2 and 5% CO_2). After stabilization of the condition, the mesenteric surface was perfused with OC-108 (2%) or with Tyrode's solution as a control; during that period, microscopic images of the small blood vessels were recorded with a video camera (C2400; Hamamatsu Photonics, Hamamatsu). The duration of perfusion was limited to 40 min and measurement was terminated at the time blood flow was completely arrested. Diameter of the arterioles and venules at the maximal reaction was measured by use of a video manipulator (C2117, Hamamatsu Photonics); and the onset of blood flow arrest in arterioles, venules, and capillaries was measured using a timing device on the video display. The time of blood flow arrest in all the vessels was also measured as a reference.

Evaluation of vascular permeability

The surface of the ileal portion of the rat mesentery was perfused with OC-108 (2%) or Tyrode's solution under the same conditions as the experiment with the mesenteric small blood vessel observation mentioned above. Evans blue dye (Tokyo Kasei Kogyo Co., Tokyo) was injected (50 mg/kg) into a cervical vein 5 min after the perfusion of the test solutions. The animals were sacrificed by exsanguination 5 min after the Evans blue injection, and the mesentery and intestine of the perfusion site were removed, minced, and collected in test tubes. Amounts of dye extravasated into the collected

tissue were quantified according to the method of Harada et al. (7).

Determination of concentration of blood cells

An acrylic bath with a silicone plate was filled with Tyrode's solution (37°C) and ventilated with a mixture of gas (95% O_2 and 5% CO_2). The ileal portion of the mesentery was pulled out from rats (261 – 302 g) anesthetized with pentobarbital, fixed on the silicone plate with pins, and acclimated for 20 min. Then, the solution in the bath was replaced with OC-108 (2%) or with Tyrode's solution as a control. After 20 min, blood was withdrawn from a mesenteric venule, through a 31-gauge syringe needle, typically used for dentistry (AstraZeneca, London, UK), connected with a PE-10 polyethylene tube (Nippon Becton Dickinson, Tokyo), an SP31 polyethylene tube (Natsume Seisakusho, Tokyo), a glass capillary tube (VC-H075H; Terumo, Tokyo), and a vacuum tube (VT-100H, Terumo). A 20-μl aliquot of blood was taken and submitted for determination of hematocrit, red blood cells, and mean corpuscular volume with a Celltac® hematology analyzer (MEK-5258, Nihon Kohden).

Statistics

In the skin blood flow experiments, statistical differences between the saline group and each test solution group were determined by Dunnett's multiple comparison test (three-way ANOVA, factors are test solutions, animals, and injection sites). Concentration-response for OC-108 was examined by linear regression analysis. In the experiments applied to the mesentery, statistical differences between the OC-108 group and the control group for each parameter were evaluated by Student's *t*-test. Differences were assessed by a two-sided test, with an alpha level of 0.05. Statistical analyses were done with the SAS system, ver. 6.12 (SAS Institute Inc., Cary, NC, USA), and a prevalidated FORTRAN program coded by the Biometrics Section of Mitsubishi Pharma Corporation.

Results

Effect of OC-108 on skin blood flow at the injection site

OC-108 at concentrations of 0.25% – 4% significantly reduced skin blood flow at the injection site 30 and 60 min after the injection, compared with saline. The effect of OC-108 was concentration-dependent and reached plateau at 2% (Fig. 1).

Next, the onset of the effect of OC-108 on reducing skin blood flow, and identification of main components exhibiting the pharmacologic effect of OC-108, were examined. OC-108 (2%) and aluminum potassium sulfate at 20 mg/ml (equivalent to OC-108 (2%)) significantly reduced skin blood flow at 5 min and later after injection, compared with saline. The efficacy of OC-108 in decreasing skin blood flow was similar to that of aluminum potassium sulfate. On the other hand, tannic acid at 0.75 mg/ml (equivalent to OC-108 (2%)) had no effect (Fig. 2).

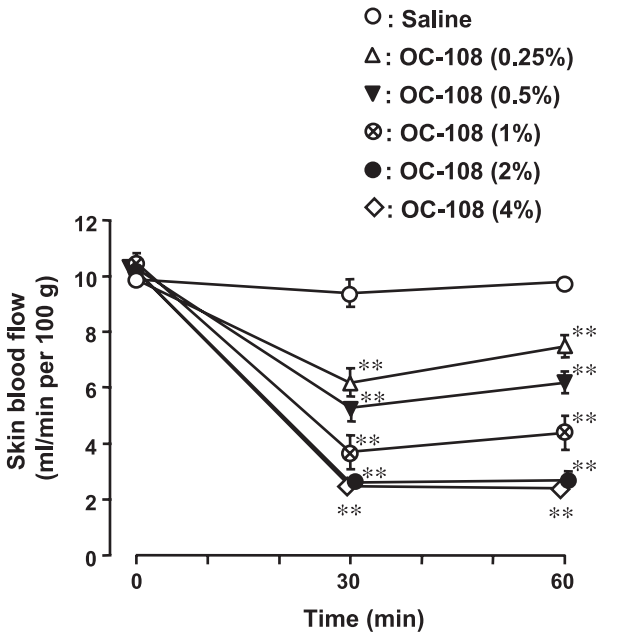

Fig. 1. Concentration response for the effect of OC-108 on skin blood flow. OC-108 (0.25 ml/kg) diluted with saline at various concentrations was injected s.c. in rat abdominal region, and the blood flow was measured at the same site. Data are expressed as means ± S.E.M. (n = 6). ***P*<0.01, compared with saline (Dunnett's multiple comparison test for three-way ANOVA).

No changes in blood pressure and heart rate were observed in any treatment group, and none of the test solutions had an effect on skin blood flow adjacent to the injection site (data not shown).

Effect of OC-108 on diameter of mesenteric arteries and veins

By perfusing the surface of the mesentery with OC-108 (2%) for 30 min, the diameter of the mesenteric arteries did not change, and there was no significant difference between the OC-108 group and the control group. In contrast, prominent constriction of mesenteric arteries was observed with perfusion of the vasoconstrictor phenylephrine for 10 min (Fig. 3). There was no change in diameter in mesenteric veins perfused with either OC-108 or phenylephrine (Fig. 3).

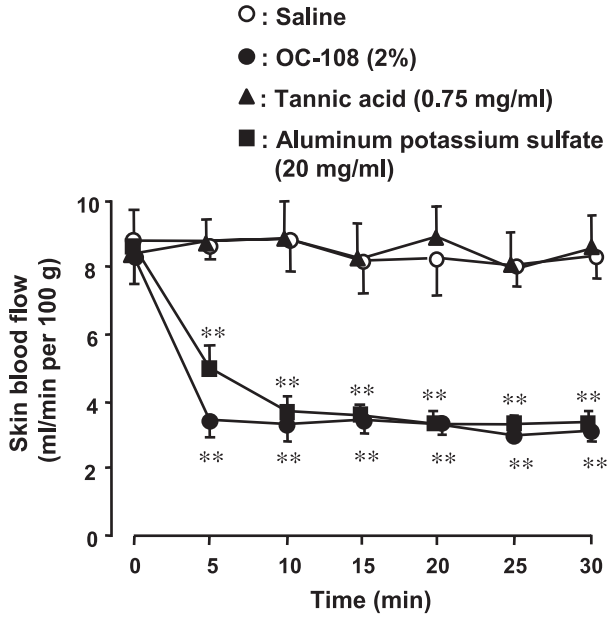

Fig. 2. Effect of OC-108, aluminum potassium sulfate, and tannic acid on the skin blood flow. Each test solution (0.25 ml/kg) was injected s.c. in rat abdominal region, and the blood flow was measured at the same site. Data are expressed as means ± S.E.M. (n = 8). ***P*<0.01, compared with saline (Dunnett's multiple comparison test for three-way ANOVA).

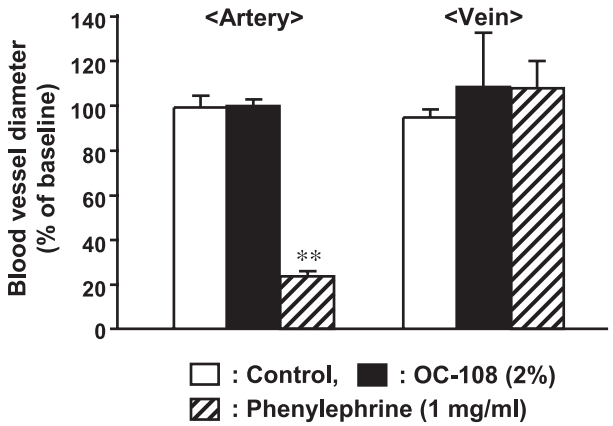

Fig. 3. Effect of OC-108 on diameter of mesenteric arteries and veins. Test solutions were perfused on the surface of the mesentery pulled out of anesthetized rats. Duration of perfusion was 30 min for OC-108 (2%) or for Krebs-Henseleit solution as a control and 10 min for phenylephrine. After the perfusion, diameter of the arteries and veins were measured. Data are normalized as % of each baseline (measured value before perfusion) and expressed as means ± S.E.M. (n = 4). ***P*<0.01, compared with the control (Student's *t*-test). Baseline values of arteries and veins were 341 ± 33 and 508 ± 27 μm in the control group, 346 ± 6 and 488 ± 43 μm in the OC-108 group, and 375 ± 7 and 524 ± 61 μm in the phenylephrine group, respectively.

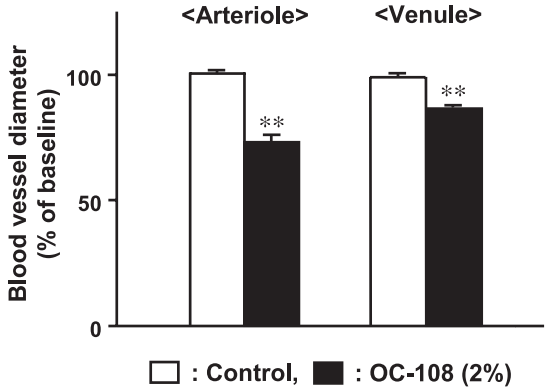

Fig. 4. Effect of OC-108 on diameter of mesenteric arterioles and venules. OC-108 (2%) or Tyrode's solution (as a control) were perfused on the surface of the mesentery pulled out of anesthetized rats. Diameters of the arterioles and venules were measured at the maximal reaction. Data are normalized as % of each baseline (measured value before perfusion), and expressed as means ± S.E.M. (n = 6). ***P*<0.01, compared with the control (Student's *t*-test). Baseline values of arterioles and venules were 21.0 ± 3.2 and 24.7 ± 2.0 μm in the control group and 19.9 ± 2.6 and 30.3 ± 3.1 μm in the OC-108 group, respectively.

Effect of OC-108 on diameter and blood flow in mesenteric small blood vessels

Diameters of mesenteric arterioles and venules following OC-108 perfusion on the surface of the mesentery at the maximal reaction showed marginal changes to 73 and 87% of the baseline level, respectively (Fig. 4), and then returned to the baseline level. However, blood flow in arterioles, venules, and capillaries began to stop 4 to 10 min after OC-108 perfusion (Fig. 5), and it was arrested completely at 10 to 11 min (data not shown). Figure 6 shows typical cases before and after OC-108 perfusion, which shows that blood flow was arrested without remarkable change in blood vessel diameter. In the control group, no change in blood vessel diameter was observed (Fig. 4), and blood flow was not stopped in all the vessels, except for 1 case out of 6 in capillaries (Fig. 5).

Effect of OC-108 on vascular permeability

Vascular permeability was determined in the mesentery from 5 to 10 min after initiation of OC-108 perfusion (duration of 5 min). OC-108 significantly increased vascular permeability by 2.5 fold, compared with the control (Fig. 7).

Effect of OC-108 on concentration of blood cells in the microcirculation

After a 20 min application of OC-108 (2%) to rat mesentery surface, blood withdrawn from a venule in the mesentery was tested hematologically. OC-108

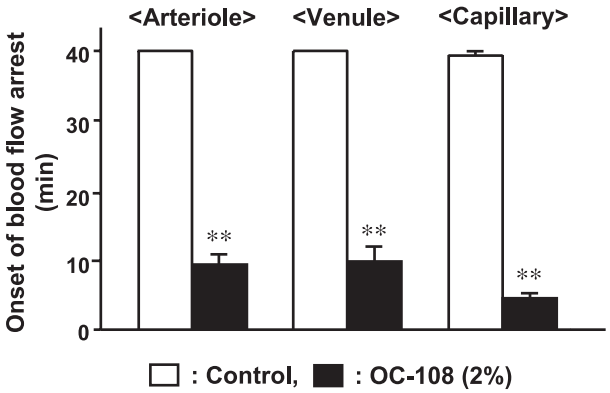

Fig. 5. Effect of OC-108 on blood flow in mesenteric small blood vessels. In the same experiment shown in Fig. 4, onset of blood flow arrest in arterioles, venules, and capillaries was measured after perfusion of test solutions on the surface of the mesentery. In case blood flow was not arrested during an observation period up to 40 min, the measurement value was defined as 40 min. Data are expressed as means ± S.E.M. (n = 6). **P<0.01, compared with the control (Student's t-test).

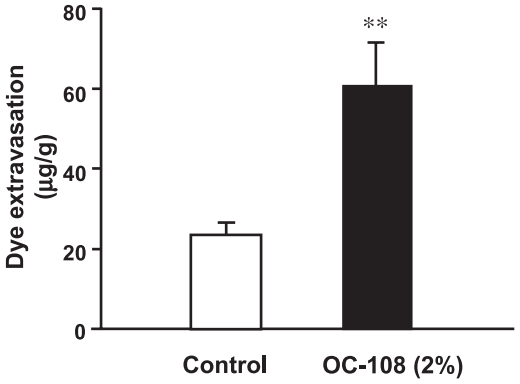

Fig. 7. Effect of OC-108 on vascular permeability. OC-108 (2%) or Tyrode's solution (as a control) was perfused on the surface of the mesentery pulled out of anesthetized rats. Evans blue dye (50 mg/kg) was injected into a cervical vein 5 min after the perfusion, and the rats were exsanguinated 5 min after the injection, the mesentery and intestine of the perfusion site collected, and amounts of dye extravasated into the tissue were quantified. Data are expressed as means ± S.E.M. (n = 6). **P<0.01, compared with the control (Student's t-test).

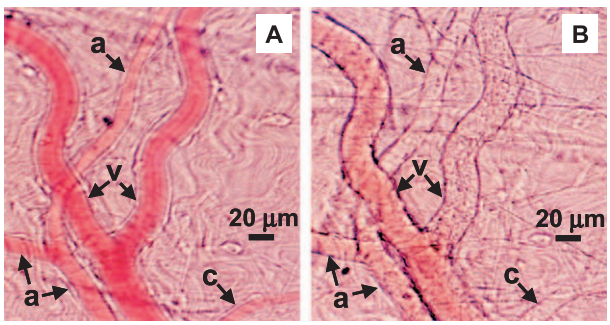

Fig. 6. Mesenteric small blood vessels before (A) and 10 min after OC-108 perfusion (B). Note blood flow arrest without conspicuous change in blood vessel diameter by OC-108 perfusion. Abbreviations: a, arteriole; v, venule; c, capillary.

significantly increased hematocrit and red blood cell counts, compared with the control. On the other hand, there was no significant difference in mean corpuscular volume between the OC-108 group and the control group (Fig. 8).

Discussion

In clinical studies, bleeding and prolapse disappeared immediately after OC-108 injection (3, 4). In this study, using anesthetized rats, we attempted to clarify the pharmacologic action responsible for the immediate hemostatic effect of OC-108.

First, the change in skin blood flow after s.c. injection

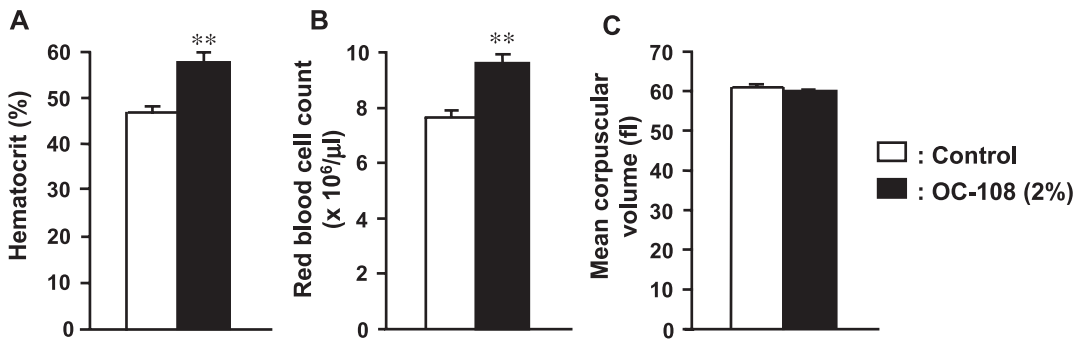

Fig. 8. Effect of OC-108 on concentration of blood cells in the microcirculation. Mesentery pulled out of anesthetized rats was immersed in OC-108 (2%) or Tyrode's solution (as a control) for 20 min. After the immersion, blood was withdrawn from a venule in the mesentery and then hematocrits (A), red blood cell counts (B), and mean corpuscular volumes (C) were measured. Data are expressed as means ± S.E.M. (n = 5). **P<0.01, compared with the control (Student's t-test).

of OC-108 at the injection site was examined. OC-108 reduced blood flow 5 min after injection and later, and the effect reached a plateau at a clinically used concentration of 2% (Figs. 1 and 2). The effect lasted at least 60 min after injection (Fig. 1), and another preliminary study suggested that the effect lasted until 48 h when OC-108 was injected s.c. in the footpad of unanesthetized rats (T. Ono et al., unpublished data). The efficacy of OC-108 in reducing skin blood flow was similar to that of an equivalent concentration of aluminum potassium sulfate (Fig. 2). The results demonstrate that OC-108 reduces tissue blood flow with rapid onset, suggesting that OC-108 rapidly reduces hemorrhoidal bleeding. These findings also demonstrate that the effect of OC-108 is attributed to aluminum potassium sulfate, the main ingredient of OC-108. The significance of tannic acid in formulating OC-108 is suggested to be an adjunct ingredient, since tannic acid reduces aluminum potassium sulfate-induced excessive leukocyte infiltration which is possibly related to tissue injury (6).

In relation to the effect of OC-108 mentioned above, we consider that reducing blood flow in hemorrhoids results in the reduction of the size of the hemorrhoids because hemorrhoids are pathologic conditions in which the venous plexus is enlarged in the anorectal region exhibiting the cardinal symptoms of bleeding and prolapse (8). Thus, immediate disappearance of prolapse after OC-108 injection in the clinical setting may be also associated with reduction of blood flow.

Next, in order to elucidate the mechanism for the decrease in blood flow, the effect of OC-108 on blood vessels was examined in the rat mesentery. By perfusion of OC-108 on the mesenteric surface, no change in diameter was observed in arteries and veins (Fig. 3). Diameter of arterioles and venules changed marginally, although arteriovenous and capillary blood flow was arrested (Figs. 4 and 5). These results indicate that constriction of blood vessels is not the main mechanism by which OC-108 decreases blood flow.

It is commonly accepted that vascular permeability is increased in acute inflammation, which causes plasma to leak out from postcapillary venules, resulting in an increase in the concentration of blood cells and the blood viscosity at the topical region, leading to a stasis of blood (9, 10). In addition, OC-108 produced acute inflammatory reactions including increased vascular permeability (6). Accordingly, we examined the effect of OC-108 on vascular permeability and blood cell concentration in venules by use of the mesentery under the same conditions as stasis induction. As a result, OC-108 increased vascular permeability and blood cell concentration in venules (Figs. 7 and 8). The data suggest that OC-108 decreases blood flow by hemostatic action caused by regional hemoconcentration due to increased vascular permeability, one of the acute inflammatory changes.

In our previous study, aluminum potassium sulfate at the concentration equivalent to OC-108, as well as OC-108, increased vascular permeability immediately after s.c. injection in mouse footpads; however, tannic acid did not (6). Similarly, in the present study, aluminum potassium sulfate, as well as OC-108, reduced rat skin blood flow immediately after injection, but tannic acid had no effect (Fig. 2). These data seem to support the idea that OC-108 decreases blood flow via increased vascular permeability as mentioned above.

Generally, a decrease in tissue blood flow is caused by constriction of peripheral blood vessels, compositional change in blood, degeneration of the interstitium, and a decrease in blood pressure. In the present study, OC-108 did not change systemic blood pressure and had no effect on skin blood flow adjacent to the injection site. The result demonstrates that a decrease in blood flow by OC-108 is confined to the injection site and not induced by decreasing blood pressure.

Aluminum potassium sulfate, which proved to be responsible for the effect of OC-108 in decreasing tissue blood flow in this study, has long been known for its astringent and styptic properties; it is used therapeutically to arrest hemorrhage by blood protein precipitation (coagulation) over the bleeding surface (11, 12). We examined the histopathologic change after injection of OC-108 in rat footpads. As a result, remarkable edema in the interstitium and stagnation in venules was observed 30 min after the injection, although thrombus formation was hardly seen during the experiment period of 3 days (T. Ono et al., unpublished data). Thus it appears unlikely that thrombus formation participates in the decrease of tissue blood flow by OC-108 in the period early after injection.

In conclusion, this study indicates that OC-108 induces regional blood flow arrest with rapid onset, which is attributed to the effect of aluminum potassium sulfate; and that hemoconcentration due to increased vascular permeability (plasma extravasation), an acute inflammatory reaction, is involved in the mechanisms of the hemostatic action of OC-108.

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