The effects of desferrioxamine on thrombus formation in injured microvessels of the rabbit ear

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Abstract: We investigated the effects of the iron chelator desferrioxamine (DFX) on thrombus formation in the arterioles and venules of the rabbit ear chamber. Thrombi were induced by irradiation with filtered light in combination with a fluorescent dye. The occlusive thrombus formation time was significantly extended by DFX. The morphological composition of thrombi in the arterioles and venules was different. In the arterioles, the thrombi consisted of platelet aggregation, but in the venules, platelets and leukocytes accumulated on the endothelium. This suggests that hydroxyl radicals may be important mediators in this model, as DFX is known as a hydroxyl radical scavenger. Furthermore, the components of thrombi in the arterioles and venules in the skin microvascular system may be different. J. Med. Invest. 46 : 200-204, 1999

Key words **:** *rabbit ear chamber, desferrioxamine, thrombus formation, microcirculation*

INTRODUCTION

Desferrioxamine (DFX) is an iron chelator used for the treatment of acute iron overload states and is known to act as a hydroxyl radical scavenger (1, 2). Recently it has also been reported that DFX protects the endothelial cells of heart and lung (3, 4) and can also shield skin flaps from necrosis (5, 6). On the other hand, it has been shown that DFX does not benefit the endothelium or improve salvage of skin necrosis (7). In flap surgery, thrombus formation is one of the causes of flap necrosis. The effect of DFX on thrombosis is, however, not clear. In this study, we used the rabbit ear chamber (REC) as a model of skin microcirculation and observed the thrombus formation resulting from endothelial damage due to dye/light reaction. The effects of DFX on this damage were also examined. Since this thrombus model is usually used for mesenteric vessels, and as there have been no reports on thrombus formation in the skin, the morphological features are also described.

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MATERIALS AND METHODS

Animals/ Rabbit Ear Chamber

A transparent round-table chamber (rabbit ear chamber : REC) was implanted in the earlobe of Japanese white rabbits weighing between 2.7 and 3.2kg (Fig.1) (8). Five to seven weeks after implantation, a complete microvascular network had formed in the chamber, and arterioles 20-30 μm in diameter and venules 45-55 μm in diameter were selected for the experiments (Fig. 2).

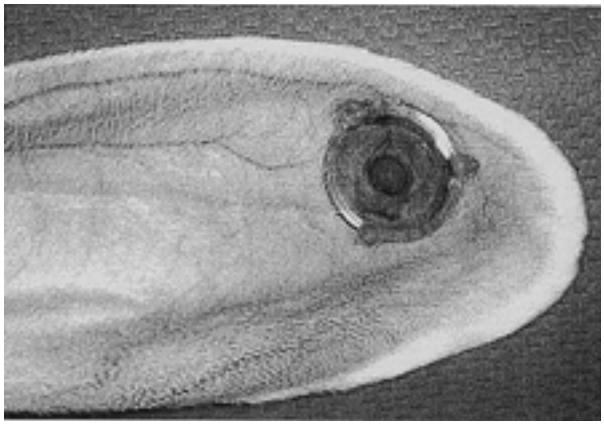

Fig.1. Rabbit ear chamber installed in a rabbit earlobe.

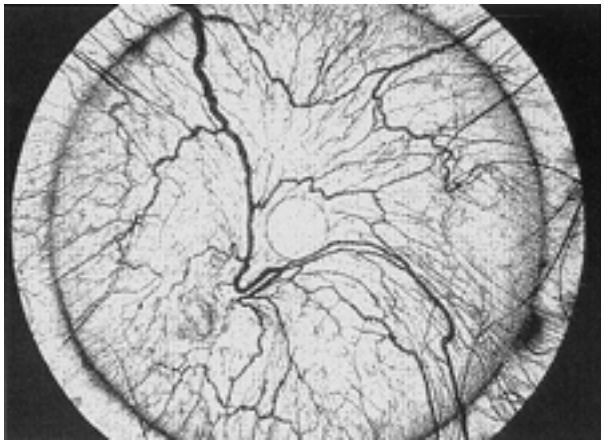

Fig. 2. A few weeks after installation, a microvascular system containing arterioles and venules is observed in the chamber.

Photochemically induced thrombosis model

No anesthesia was needed for the following studies. Thrombi were produced in the vessels with the dye/light method reported by Sato and Ohshima (9, 10). Briefly, a solution of fluorescein sodium (2.5%, 2 ml/kg ; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was injected through a vein of the contralateral ear. After confirming the appearance of fluorescence in the microvessels in the REC, filtered light (400-500 nm light passed through a fluorescein isothiocyanate excitation filter from a high pressure mercury lamp) was irradiated onto the vessels through an objective lens (x20) (Fig. 3). The diameter of the irradiated spot was 130 μm on the focal plane. The light intensity was controlled at 0.5 mW under the objective lens. Irradiated vessels were continuously monitored and recorded with an intravital microscope-television system in all cases and the time course of thrombus formation was calculated with the aid of these records. In all cases, the intervals" ti "(from irradiation to the onset of thrombus formation) and" ts "(from irradiation to complete obstruction of blood flow) were also calculated.

Experimental Groups and Administration of DFX

Thirty rabbits were prepared and divided into three groups of 10 animals each. In the control group, 10cc of saline was injected via the contralateral ear 15 minutes before aggregation was induced. In the two DFX treatment groups, 100 mg/kg of DFX (Novartis Pharma Co., Tokyo, Japan) and 10 ml of saline, or 200 mg/kg of DFX and 10 ml of saline was injected in the same way.

Statistical Analysis

The Mann Whitney U-test was used for statistical analysis of the results. P values less than 0.05 were considered statistically significant.

RESULTS

When the filtered light was irradiated onto the vessels after confirmation of fluorescence in the microvessels in the RECs, microthrombi began to stick to the wall, and continued to accumulate on the inside of the vessels. Eventually the microvessels were totally occluded by the thrombi and the blood flow was completely blocked. The composition of the thrombi in the arterioles and venules was different. Morphologically, the thrombus formation in the arterioles began with platelet aggregation (Fig. 4), but in the venules, platelets, erythrocytes and leukocytes started to gather and adhere to the endothelium from the start (Fig. 5). This difference

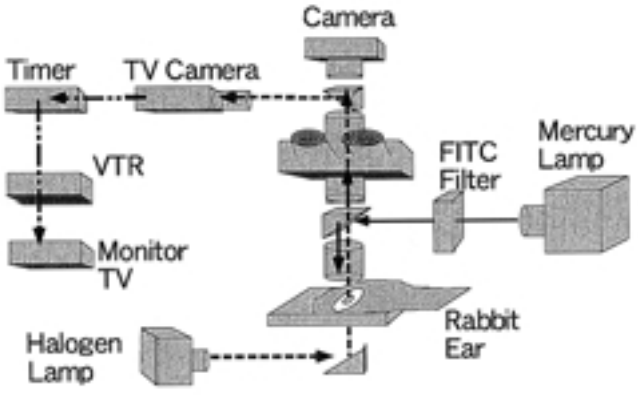

Fig. 3. Schematic diagram of the intravital microscope-television system. Solid line indicates the epi-illumination system to produce thrombi. Broken line indicates the transillumination system to observe the microvascular bed.

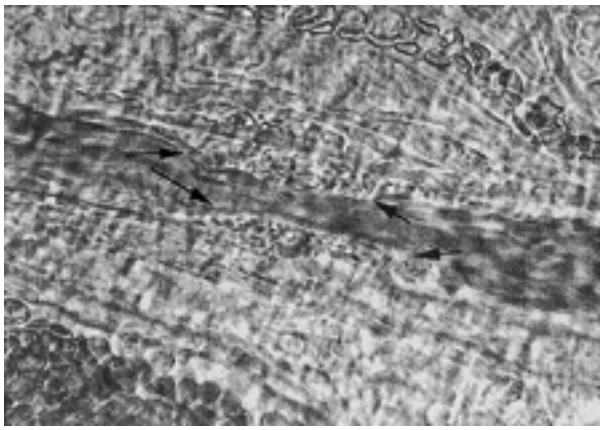

Fig. 4. Thrombus formation in the arteriole. The arrows show accumulation of platelets on the vessel wall.

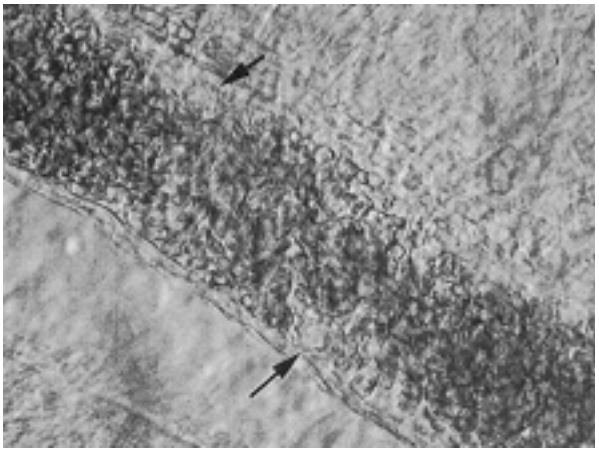

Fig. 5. Thrombus formation in the venule. The arrows show leukocytes in the thrombus.

between thrombosis in arterioles and venules was observed in all groups.

In arterioles and venules, the thrombus formation times, ti and ts, were extended as a result of DFX (Fig. 6). In the arterioles, ti and ts of the control group were 25.2±2.7 (mean±S.D.) and 84.3±4.6 sec and those of the DFX 100 mg/kg group were 36.5±5.2 ($p<0.05$) and 95.7±9.2 sec (not significant). The times of the DFX 200 mg/kg group were 45.7±6.0 ($p<0.01$) and 111.0±8.0 sec ($p<0.05$). In the venules, ti and ts of the control group were 25.0±2.0 and 104.9±5.6 sec and those of the DFX 100 mg/kg group were 38.3±7.4 (not significant) and 128.3±12.6 sec (not significant). The times of the DFX 200 mg/kg group were 43.7±7.6 ($p<0.05$) and 135.1±11.7 sec ($p<0.05$).

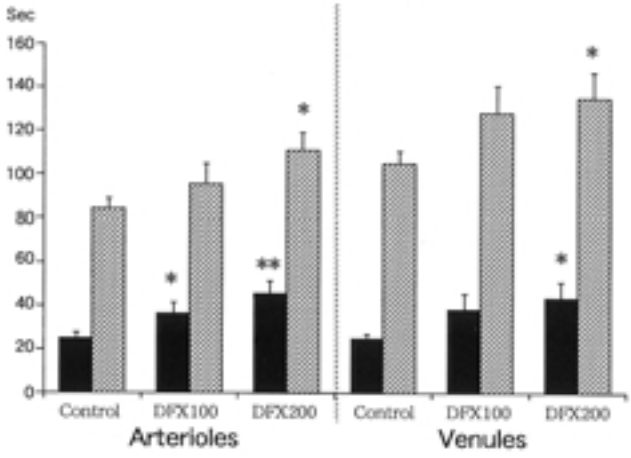

Fig. 6. Thrombus formation time. Values show mean±S.E. ■, ti (time when thrombus began to form) ; ▨, ts (time when blood flow completely stopped) : *$p<0.05$, **$p<0.01$ compared with control group.

DISCUSSION

Different methods to induce thrombosis in vivo are presently available. However, there are few reports on the application of thrombus models to the REC. Fleming and Seiler have evaluated the antithrombotic efficacy of some drugs by using a laser-induced thrombosis model in the REC (11, 12). In their model using irradiation by ruby laser to vessels, it is thought that the localized thermal effect on the red cells as a result of absorption of the ruby laser evoked the formation of a thrombus, which consisted of platelets accumulated around a core of one or two damaged red cells (13). It is, however, generally accepted that the first step of thrombogenesis is damage to endothelial cells. Therefore, their model differs from the physiological mechanism of thrombus formation.

We applied the fluorescent dye/light method, which Sato and Ohshima developed for mesenteric vessels, to the REC. It has been reported that the mechanism of thrombus formation in the *in vivo* model of the dye/light reaction does not consist of a localized thermal effect but of endothelial damage due to forms of active oxygen generated with excited dyes (14, 15). However, details of the mechanism are not clearly understood at present.

Our data showed that DFX lengthened the thrombus formation time in the arterioles and venules in a dose dependent manner. DFX is known to act as a hydroxyl radical scavenger (1, 2). With almost the same dye/light method as described here, it has been shown that, in the pial arterioles of the mouse, the hydroxyl radical scavengers dimethyl sulfoxide (DMSO) and glycerol are effective inhibitors of platelet aggregation (15). Hydroxyl radicals may therefore be important mediators of the skin microvascular responses in this model. The maximum clinical daily dose of DFX is 80 mg/kg for humans. As no statistically significant difference could be identified in the DFX 100 mg/kg group, the antithrombotic efficacy of this agent may not be very strong. However it has been reported that a drug mixture containing a small dose of DFX (20 mg/kg) improves experimental flap survival (16, 17). With that method, a mixture including a small amount of each drug can be effective and DFX can be used in a clinically viable manner.

Recently, it has been found that after a prolonged ischemic phase, reestablishment of the vascular supply causes tissue injury which is called ischemia reperfusion injury. In this injury, reactive oxygen

species such as the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH•) lead to endothelial cell injury which results in increased capillary permeability, thrombus formation, and microcirculatory failure. As the thrombi in our model may be due to endothelial injury by hydroxyl radicals, we are convinced that this model very closely resembles such clinical conditions (15, 18).

In this study, it was observed that thrombus formation in the arterioles consisted of platelet aggregation, but in the venules both platelets and leukocytes gathered and adhered to the endothelium. When the same model as used in this study was applied to mesenteric vessels, platelet aggregation was observed in the venules (9, 10). This difference in composition of the thrombus between REC and the mesenteric vessels may be caused by characteristics of the organ. In the skin microcirculation, WBC contributed to thrombus formation only in the venules but not in the arterioles. It is not clear whether the leukocyte sticking in thrombus is caused by slow blood flow velocity or by the functional features of venous endothelial cells. Further studies are needed to investigate this point.

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