

MICROVASCULAR EFFECTS OF A TOPICALLY APPLIED CATIONIC TRIPHENYLMETHANE DYE (CRYSTAL VIOLET)

Elof Eriksson and Håkan Mobacken

From the Laboratory of Experimental Biology, Department of Anatomy, and the Department of Dermatology, University of Gothenburg, Gothenburg, Sweden

Abstract. The effects of local application of crystal violet on the microcirculatory system are reported. The testing system consisted of vital microscopy of the cheek pouch of the hamster and microangiography of rabbit's ear. It appeared that the early microcirculatory disturbances caused by a dilute aqueous solution of crystal violet were transient, whereas considerable tissue toxicity was shown in the long-term experimental model. A direct cytotoxic effect is suggested.

Key words: Microcirculation; Vital microscopy; Microangiography; Crystal violet

Crystal violet, C.V. (hexamethyl-para-rosaniline chloride) is widely used in superficial pyococcal and monilial infections of the skin and mucous membranes. Some patients so treated experience severe irritation and even necrosis of the skin or mucous membranes (1). Recently it has been demonstrated in various experimental models that C.V. in concentrations considerably lower than those used clinically can induce tissue damage (8). The pathophysiological mechanisms are unknown, but the dye seems to prolong the initial phase in tissue regeneration due to an intensified and lengthened inflammatory reaction (7). A conceivable explanation for this would be primary vascular reactions to application of the dye. This hypothesis was tested in the present study by using intravital microscopy of the hamster cheek pouch preparation and microangiography of rabbit ears, where C.V. in different concentrations was applied topically.

MATERIALS

Twenty healthy hamsters weighing about 100 g were used in the study of the early microvascular changes. They were anesthetized with mebumal (Nembutal®; Abbott Lab., USA) (30 mg/kg) intramuscularly. For the analysis

of the long-term vascular effects, 8 healthy rabbits weighing about 2 kg were used.

For the cheek pouch studies, C.V. (C.I. 42555) was used in dilutions of 1:10 000 (0.01%, w/v) in isotonic saline or distilled water. Higher concentrations were not used as the depots then became non-transparent. In the microangiography experiments, C.V. 1:100 and 1:1 000 (1% and 0.1%, w/v) in distilled water were studied as well as 1:10 000 in both water and isotonic saline. We were unable to dissolve higher concentrations of C.V. in saline. Isotonic saline or distilled water was used in control experiments. All solutions were sterile.

METHODS

Hamster cheek pouch

The experimental model has previously been described by Brånemark et al. (2, 4). The cheek pouch of an anesthetized hamster was everted over a thin glass plate (Fig. 1) and observed in transillumination using a modified Leitz' Intravital Microscope equipped for microphotography. During the experiment the pouch was kept moist by irrigation with Tyrode's solution at room temperature. Animals exhibiting abnormal microvascular function 15 minutes after exposure of the cheek pouch were discarded.

The agents were then administered peripherally in the pouch in two ways (Fig. 2): (a) A deposit was produced by injection with a fine hypodermic needle of a standardized dose (0.03 ml) into the upper tissue layer of the exposed cheek pouch; (b) Using a stereomicroscope and microsurgical instruments, epithelium and superficial connective tissue were excised on one side of the pouch over an area with a diameter of about 2 mm, exposing blood vessels in the bottom of the microwound (cf. 2). Tyrode's solution was dripped onto the defect for 15 minutes. After evaluation of microvascular function, the test solution was dripped onto the defect for about 5 minutes, whereupon Tyrode's solution was again dripped on the microwound.

● On average, each pouch was used for three separate deposits or defects, about 1 cm apart. The microcirculation in the pouch as a whole was checked continuously during the experiments. The pouch was observed for 2

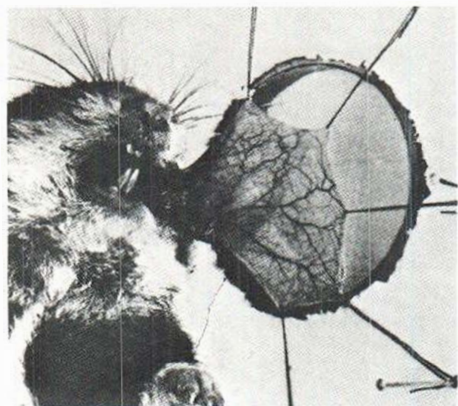


Fig. 1. Plan view of exposed cheek pouch of hamster.

hours after application of the agents. Each agent was applied to four tissue defects and six tissue deposits.

Microangiography

The method used has been described previously by Lundskog et al. (6) and Goldie et al. (5). The solutions were deposited subperichondrally on the outer side of rabbit's ear. On each ear, two deposits were made, one near the base and one near the apex. A standard dose of 0.1 ml was used. Each solution was administered twice on both sites but in separate ears. On the seventh day, the gross status of the ears was recorded followed by microangiography, as it had been found that the microvascular changes were usually most pronounced after this time interval (6). The rabbits were in this case anaesthetized with Nembutal® intravenously. Via a catheter in the aorta, they were heparinized, lidocaine (Xylocaine®; Astra, Sweden) 0.5% was administered and a barium sulphate contrast (Micropaque®; Damancy Co., England) was infused at a pressure somewhat above the systolic blood pressure. At the same time, the external jugular veins were incised in order to prevent hyperhydration. The animal died after about half an hour, but the infusion was continued for about 15 minutes. The volume of con-

trast medium was approximately 200–300 ml per animal. The ears were then cut off at their bases proximal to forcepts. A Machlett OEG-50 tube and Kodak MR plates were used for microradiography (12 kV, 15 mA, film-focus distance 12 cm, exposure time 15 minutes).

RESULTS

Hamster cheek pouch

Twenty golden hamsters were anaesthetized and one of their cheek pouches was exposed and examined in the intravital microscope (Fig. 1). In 14 of them the baseline flow conditions were judged to be normal and stable. In each of these animals three areas were studied with the solution either in a deposit or applied on a mucosal defect. The deposits and defects could be done in a standardized manner (Fig. 2). The changes seen with one type of solution—in separate deposits, for instance—seemed almost identical. The circulation remained unchanged throughout the experiments in those parts of the pouch surrounding the deposits and defects.

Saline control deposits halted the flow in all vessels in the area of the deposit for about 10 sec. Then the flow rapidly returned to control level and no further changes were seen. Saline had no discernible effect on the mucosal defects.

Distilled water control deposits caused a standstill of flow in all vessels for 10 sec. Then flow restarted in some vessels, but with reduced velocity, and in some capillaries and venules it slowly returned to normal only after 15–30 min. Within seconds after the injections, spherocytosis and even haemolysis of many red cells could be seen. During the early period after injection a number of red and white cells stuck to the walls of venules, and some

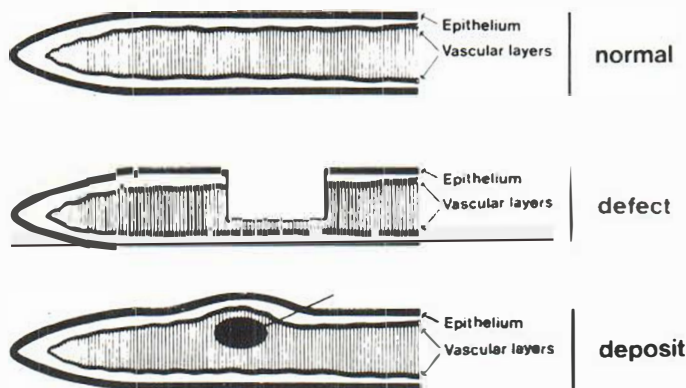


Fig. 2. Diagrammatic representation of various experimental procedures for microcirculation studies in hamster cheek pouch.

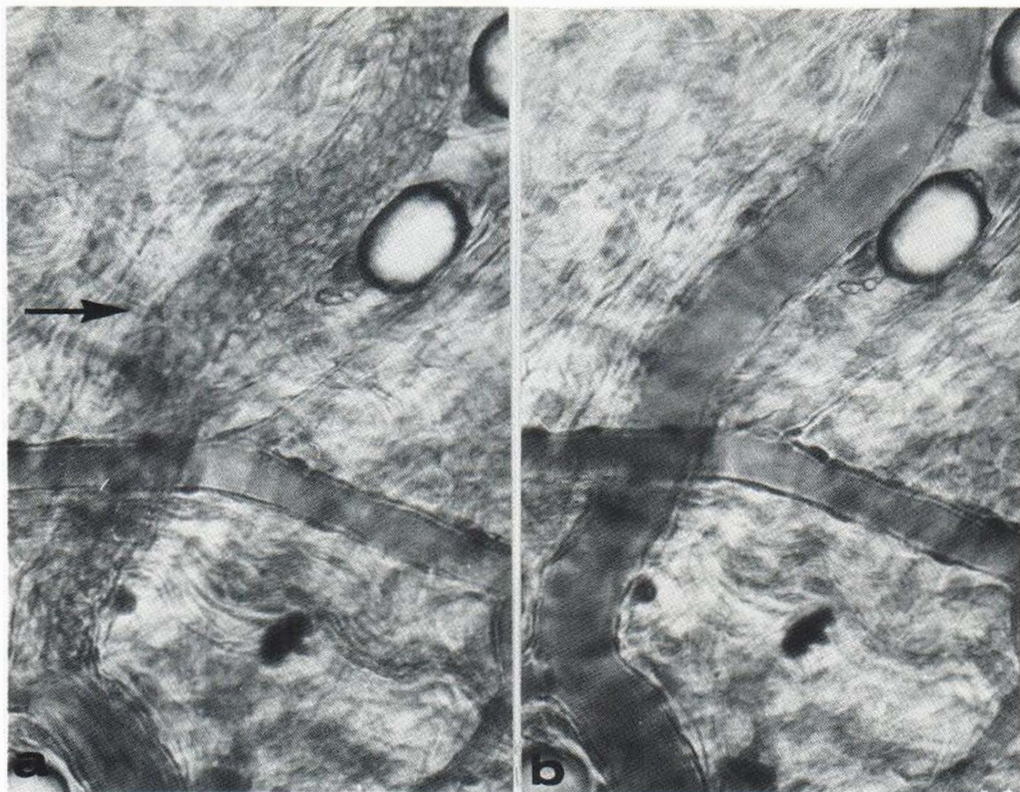


Fig. 3. Vital photomicrogram immediately after (a) application of an aqueous solution of crystal violet to exposed connective tissue in a small defect in the cheek pouch of

hamster. The haemolysis and blockage of flow was restituted following irrigation with Tyrode's solution after 5 minutes (b).

of these cells migrated through the wall of the vessel. After approximately 30 min the flow had returned to normal and remained so. Very similar changes were seen when water was dripped onto the mucosal defects.

C.V., 1:10 000 in distilled water, gave both deposits and defects a definitely blue colour (Fig. 3a) with a well defined border. The circulatory changes seen could not be separated from those caused by water alone. When the defects were washed with Tyrode after dye exposure for 5 minutes, the flow became normal within 1 minute (Fig. 3b).

C.V. in saline stained deposits and defects blue, but did not affect the circulation.

Microangiography

Six different substances were deposited subperi-chondrally in rabbit ears. The substances were:

saline and distilled water (controls). C.V. in concentrations 1:100 and 1:1000 in water, and 1:10 000 in water respectively saline.

Gross examination of the deposits revealed no changes when saline, water, or C.V. 1:10 000 in these solvents had been injected. C.V. 1:100 and 1:1 000 provoked swelling and redness.

The microangiographic method gave good filling of the microvessels, as can be judged from Fig. 4a-c. The changes seemed to vary with the concentration of C.V., but were similar regardless of the type of solvent. C.V. 1:10 000 resulted in a slight vascular proliferation, with a new network of small vessels (Fig. 4b). This was more pronounced at the higher concentration. At 1:100 the central area of the deposit was avascular and necrotic and was surrounded by a zone of intense vascular proliferation (Fig. 4c). The water and saline control deposits showed no changes (Fig. 4a).

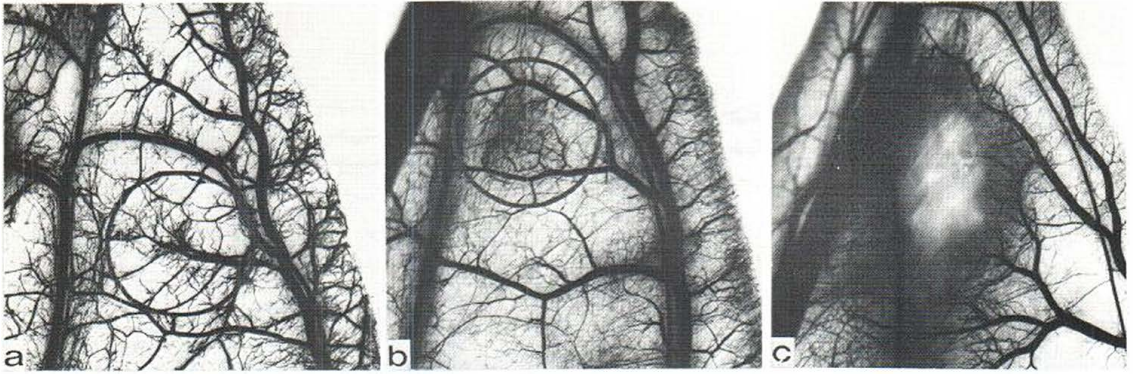


Fig. 4. The vascular system of rabbit's ear identified by microangiography on the 7th day after deposition of distilled water (a) or aqueous solutions of crystal violet 1:10000 (b) or 1:100 (c). This agent caused a rich net-

work of small vessels in the deposit area. At the highest concentration a central area of necrosis devoid of vessels can be seen.

DISCUSSION

The hamster cheek pouch preparation for intravital microscopy provides good optical resolution and the microvascular flow is stable over several hours of observation (2, 4). Our preliminary experiments showed that when C.V. in different concentrations was dripped directly on the intact mucosa and washed away with saline 30 seconds later, no circulatory changes had appeared. However, sub-mucosal deposit of a toxic substance or deposit on a mucosal defect has proved a more sensitive and reproducible model for studies on the influence on microcirculation (2, 3, 4, 9). In this series of experiments both deposits and defects were used, showing the same type of vascular changes. C.V. diluted 1:10000 had no effect on the microvasculature in the hamster cheek pouch, beyond the changes induced by the solvent. When C.V. in water was used (as with water alone) the microcirculation in the deposit or defect was brought to a standstill, but flow resumed within 15–30 minutes. Unfortunately, C.V. in dilutions of 1:100 or 1:1000 could not be used in the hamster cheek pouch model because the dye stained the tissues so darkly blue that the translucency was lost. On the other hand, it was possible by means of microangiography to distinguish between the effects of various dilutions of C.V. Quantification of the results in a strictly objective way was not within the scope of this investigation. However, with the concentrations used and judged subjectively, the different responses were clearcut. C.V. 1:1000 and

1:100 produced pronounced vascular proliferation and the higher of these two concentrations even provoked a central area of avascular necrosis.

The transient initial vascular changes observed in this study stand in contrast to the marked early disturbances in microcirculation provoked by another topical antimicrobial, dequalinium chloride (9). In clinical use, this substance has caused severe tissue damage (12). In a similar experimental model, vascular contractions were even seen which occluded the lumen of the blood vessels for several hours. The resulting insufficiency of the nutritive blood flow was considered to be a pathogenetic factor in the development of tissue injury by this agent. However, for C.V. it is obvious that the transient changes in the acute experimental model contrast to the marked changes in the tissue of the rabbit ears even though the tissue of the hamster cheek pouch is considered to be a test model more sensitive to injury than the ear of the rabbit (11). Therefore it seems reasonable to assume that the transient acute effects of C.V. on the microvascular system are not responsible for the subsequent tissue injury. The cytotoxic effect of C.V. directly on epithelial and connective tissue cells, which has been demonstrated *in vitro* (10), should be more important in this respect.

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H. Mobacken, M.D.
Department of Dermatology
Sahlgren's Hospital
S-413 45 Gothenburg
Sweden