The Effects of Saponin on Sinusoids and Capillaries in Various Organs: An Electron Microscopic Study

By

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Summary: A comparative electron microscopic study was made on the effects of saponin on the sinusoids and capillaries of various organs of rabbits and the sinusoids of the bone marrow in cats, dogs, rats and mice. Animals received an intravenous injection of saponin at a dose of 2 mg per kg of body weight and were sacrificed 6 hours after injection.

In rabbits, the sinusoidal endothelium of the bone marrow showed extensive damage, whereas the endothelial cells lining the liver sinusoids, those lining discontinuous capillaries in the small intestine and adrenal cortex, and those lining continuous capillaries in the muscles (diaphragm and biceps brachii muscle) remained unaffected. Among the various animals examined, only in the case of cats did saponin induce results similar to the marrow sinusoid damage found in rabbits.

The intravenous injection of saponin has been shown to cause rapid injury to the endothelium lining the sinusoids in the bone marrow of rabbits, resulting in normoblastemia and large scale hemorrhage in the bone marrow (Bunting, 1906; Omura and Osogoe, 1951; Argano et al., 1969; Oberling, et al., 1973; Endo, 1977; Hoshi and Weiss, 1979).

Electron microscopic studies on the effects of saponin in the early stages after injection have generally focused on the sinusoids of the bone marrow, and relatively few ultrastructural studies have been carried out on the effects of saponin on sinusoids and capillaries of the liver and other organs. Furthermore, many reports have concentrated on the effects of saponin on rabbits, and there is still a lack of clarification as to whether sinusoids in the bone marrow of other mammalian species are equally sensitive to saponin. The present study set out to help clarify these points by gathering data on the effects of saponin on sinusoids and capillaries in a number of organs over a range of mammalian species.

Materials and Methods

Animals

Animals used in the present study were as follows: albino rabbits (2–3 kg), Wistar strain rats (200–250 g), C57BL/6 strain mice (20–25 g), cats (1–2 kg) and young dogs (1–2 kg). Young dogs were used because a preliminary study showed that the bone marrow of the long bones in adult dogs had been almost totally replaced by yellow bone marrow.

Injection of Saponin

Saponin (obtained from Eastman Kodak Company, Rochester, NY) freshly dissolved
in physiological saline was intravenously injected at a dose of 2.0 mg per kg of body weight. Each group consisted of between 4–8 animals, and at least half of the animals in each group were given an injection of saponin, while the remainder were used as an untreated control. Immediately before autopsy, blood smears were prepared from each animal and stained with the May-Grunwald and Giemsa preparation.

Electron Microscopy
Animals were sacrificed 6 hours after receiving the injection of saponin. An interval of 6 hours was chosen because there were indications in a previous report that the marrow sinusoids of rabbits suffered severe damage within 6 hours from the injection of saponin (Hoshi and Weiss, 1978).

The femur and humerus bones of each animal were removed at autopsy. Bone marrow was obtained following the method of Hoshi and Weiss (1978), the bone being held vertically and split longitudinally by a razor blade tapped with a hammer. Split pieces of bone containing a thin layer of marrow were immersed in a fixative of one part Karnovsky's glutaraldehyde-paraformaldehyde mixture and one part distilled water, buffered with cacodylate. Four hours later, the tissue was immersed in 1 percent osmium tetroxide for 1 hour, and then transferred to buffer where the bone marrow was carefully lifted from the bone. The marrow was sliced parallel to the cut surface to produce a layer approximately 1 mm thick, which was then cut into smaller pieces and osmificated in 1 percent OsO₄ for 1 hour.

In addition to these bones, the liver, adrenal gland, small intestine, diaphragm and brachial muscle were also obtained from rabbits at autopsy.

Tissues obtained at autopsy were cut into layer 2 mm thick and immersed in diluted Karnovsky's fixative as described above. Four hours later, the layer of tissue were cut into smaller pieces and postfixed in 1 percent OsO₄ for 2 hours. After fixation, all pieces of marrow and tissues were dehydrated in ethanol and embedded in Araldite-Epon mixture. Section were stained with uranyl acetate and lead citrate, and observed with a JEOL 100CX electron microscope.

Results

1. Effects on sinusoids and capillaries in various organs of the rabbit

Capillaries are classified into three types; fenestrated capillaries, continuous capillaries and sinusoids. In this study, electron microscopic examinations of rabbits were carried out on sinusoids in the bone marrow and liver, fenestrated capillaries in the adrenal gland and villus of the small intestine, and continuous capillaries in the muscles.

Blood
Rabbits injected with saponin showed normoblastemia 6 hours after injection.

Bone marrow
The normal structure of the sinusoid wall in rabbit bone marrow has been described in detail in a previous report (Hoshi and Weiss, 1978). In this study, the bone marrow showed extensive hemorrhage 6 hours after injection. Sinusoids showed extensive damage, and the endothelium of the sinusoid was barely identifiable. Hematopoietic cells were found scattered among extravasated erythrocytes (Fig. 1).

Liver
In rabbits injected with saponin, the sinusoids of the hepatic lobules appeared to be intact. Endothelial cells lining the sinusoids and Kupffer cells were free from any recognizable damage, and there were no indications of cytoplasmic vacuolation or increased density in the cytoplasmic matrix (Figs. 2, 3). There was a feeling that Kupffer cells were possibly encountered in smaller numbers in treated rabbits than in the
control group, but a statistical survey of the number of Kupffer cells per unit of sinusoid wall showed that there was no significant differences (Table 1).

Other organs

There was no noticeable damage to the endothelial cells lining capillaries in the adrenal cortex, the villi of the small intestine, the diaphragm or the brachial muscle (Figs. 4, 5, 6).

II. Effect on sinusoids in the bone marrow of various mammalian species

Blood

Of the mice, rats, cats, and dogs examined in this study, only cats showed normoblastemia 6 hours after the injection of saponin.

Bone marrow of control animals

Fat cells were rarely encountered in the bone marrow of mice and rats. The hematopoietic tissue was supplied with well-developed sinusoids. The sinusoidal wall showed a trilaminar structure consisting of endothelium, basement membrane and reticular adventitial cells, and this is consistent with previous reports (Weiss, 1970, 1976; Champbell, 1972; Chamberlain et al., 1975).

In cats and dogs the morphological appearance of the bone marrow was very similar to that of rabbits. Fat cells were found relatively frequently. The spaces between fat cells were occupied by hematopoietic tissues supplied with well-developed sinusoids. The structure of the sinusoidal wall was simpler than that of mice and rats. Reticular adventitial cells were rather infrequent and beneath the sinusoidal endothelium lay an amorphous substance which seemed to be a poorly developed basement membrane lacking the tendency to form basal lamina (Fig. 7).

Bone marrow of animals injected with saponin

In cats injected with saponin, the bone marrow showed massive hemorrhage. Intact hematopoietic tissues formed narrow tissue cords between the areas filled with extravasated erythrocytes, and hematopoietic cells were found scattered infrequently among the extravasated erythrocytes (Fig. 8). Sinusoids showed extensive damage, and their endothelial cells were barely identifiable (Fig. 8).

There were no indications of hemorrhage in the bone marrow of mice, rats and dogs treated with saponin. Sinusoids remained intact, and their endothelial cells showed no recognizable signs of damage (Figs. 9, 10, 11).

Table 1. Number of Macrophages (Kupffer cells)

<table>
<thead>
<tr>
<th>N</th>
<th>Total length of sinusoid wall measured</th>
<th>No. of macrophages per 1 mm of wall</th>
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</thead>
<tbody>
<tr>
<td>Control group</td>
<td>3</td>
<td>12.81 mm</td>
</tr>
<tr>
<td>Animals injected with saponin</td>
<td>5</td>
<td>11.02 mm</td>
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A* Macrophages with identifiable nuclei
B* Identifiable macrophages.
Discussion

Previous reports based on light microscopic observations have stated that acute saponin poisoning in rabbits caused damage to endothelial cells lining the sinusoids of the bone marrow and liver as a result of the cytolytic action of the agent (Osogoe et al., 1965). The present electron microscopic observations showed that whereas saponin caused extensive damage to the endothelial cells of the marrow sinusoids in rabbits, in contrast, endothelial cells lining the hepatic sinusoids, capillaries in the adrenal cortex, small intestine and muscle remained free from recognizable damage. This suggests that the myeloid sinusoidal endothelium of rabbits may be particularly sensitive to saponin.

Saponin is known to be a hemolytic agent which causes damage to the cell membranes of erythrocytes. Although saponin intravenously injected reaches the endothelia lining blood vessels in all parts of the body, the agent preferentially damaged the sinusoidal endothelia of the bone marrow. Presumably, the cell membrane of the sinusoidal endothelial cells in the rabbit bone marrow is particularly sensitive to saponin.

Endothelial cells lining the marrow sinusoids are phagocytic. However, their phagocytic function may be only part responsible for their susceptibility to injury after an intravenous injection of saponin, since endothelial cells and Kupffer cells, both being phagocytic and lining the sinusoidal wall in the liver, remained free from noticeable damage.

It is probable that a high sensitivity to saponin is an intrinsic property of the endothelia lining the marrow sinusoids in rabbits. However, evidence against this comes from our own unpublished observations that capillaries (or sinusoids) in the yellow bone marrow of rabbits were resistant to doses of saponin which caused damage to the sinusoids in the hematopoietic marrow. It is possible that microenvironmental factors in the bone marrow may influence the properties of endothelia lining the small blood vessels supplying the marrow, and that this influence expresses itself in terms of the degree of sensitivity to saponin.

In the present study, we also examined the effect of saponin on the sinusoids in the bone marrow of some other animals. The results show that only in the case of cats did saponin induce changes similar to the normoblastemia and marrow sinusoid damage found in rabbits. These findings indicate that there are differences between species in the terms of sensitivity of the bone marrow sinusoids to saponin.

In both rabbits and cats the wall of the marrow sinusoids has a simpler structure than that of mice and rats, being almost free from adventitial support from reticular cells. However, the structural simplicity alone does not necessarily explain their relative sensitivity, since the wall of marrow sinusoids in dogs, which also has a simple structure, was not affected by similar doses of saponin. Differences between species in sensitivity of marrow sinusoids to saponin may be related more to the character of the cell membrane of the endothelial cells than to differences in the morphological structure of the sinusoidal wall.

In the field of experimental hematology, saponin-induced anemia and myelofibrosis in rabbits have been regarded as experimental models for human hemolytic anemia and myelofibrosis with myeloid metaplasia (Argano et al., 1969). The present study suggests that in addition to rabbit, cats may also be useful for such experiments.
References


Explanation of Figures

Plate I

Fig. 1. Femoral bone marrow of a rabbit sacrificed 6 hours after injection of saponin. Sinusoid appears poorly outlined as a result of severe damage sustained by sinusoidal wall. Erythroblasts and myelocytes are found intermingled with mature erythrocytes. X 3,000.

Fig. 2. Sinusoid in the liver of a rabbit after injection of saponin. The endothelium lining the sinusoidal wall remains unaffected. X 5,400. Inset, X 14,500.
Plate II

Fig. 3. Sinusoid in the liver of a rabbit after injection of saponin. Kupffer cell in the lumen of hepatic sinusoid shows no detectable morphological damage. $\times 4,600$.

Fig. 4. Capillary in adrenal cortex of a rabbit after injection of saponin. The endothelium lining the capillary wall shows no morphological damage, and exhibits occasional fenestrae closed by a diaphragm. $\times 20,000$. 
Plate III

Fig. 5. Capillary in the lamina propria from small intestine villus of a rabbit after injection of saponin. The endothelium lining the capillary is intact. × 7,000. Inset, × 40,000.

Fig. 6. Capillary in brachial muscle of a rabbit after injection of saponin. The endothelium lining the capillary is intact, and displays a number of pinocytotic pits and vesicles in the cytoplasm. × 19,000.
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Plate III
Plate IV

Fig. 7. Sinusoid in femoral bone marrow of an untreated cat. An attenuated endothelium lines the sinusoid. Note that a large part of the sinusoidal wall is devoid of adventitial support from reticular cells. X 4,400.

Fig. 8. Femoral bone marrow of a cat 6 hours after injection of saponin, showing extensive hemorrhage. The sinusoid wall shows extensive damage, and endothelia lining the wall are barely recognizable. Hematopoietic cells are found scattered among mature erythrocytes, which may represent extravasated red blood cells. X 1,800.
Plate V

Fig. 9. Sinusoid in femoral bone marrow of a dog 6 hours after injection of saponin. The sinusoidal endothelium remains free from detectable damage. Note that the sinusoidal wall generally lacks the adventitial support of reticular cells. $\times 4,200$.

Fig. 10. Sinusoid in femoral bone marrow of a rat 6 hours after injection of saponin. No damage is seen to the sinusoidal endothelium. Note that the sinusoidal wall is supported by reticular adventitial cells. $\times 4,500$. 

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Plate V
Plate VI

Fig. 11. Sinusoid in femoral bone marrow of a mouse 6 hours after injection of saponin. The sinusoidal wall remains intact and receives adventitial support from reticular cells. $\times 5,400$. 