

Toxic Effect of Patent Blue Violet on Rat Lymph Node Lymphocytes

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Summary

Histological examination of rat knee nodes 24 and 48 hours after ligation of the efferent lymph vessel visualized by Patent Blue Violet revealed many dead lymphocytes within the node. These alterations were also observed in non-ligated animals. It could be shown that the cell death was caused by Patent Blue Violet.

Introduction

Patent Blue Violet (PBV) is widely used to visualize lymph vessels for lymphography and for experimental purposes in man and animal (2, 3). The side effects reported are allergic reactions (3). This report shows that the dye might cause grave histological alterations of the nodes regional to the injected area. This was observed during a study of the effect of the rate of lymph flow on the cellularity of lymph nodes in rats.

It has previously been reported that ligation of the afferent lymph vessels of the knee node causes a marked sinus lymphocytosis, probably due to reduced outflow of lymph and recirculating cells from the node (1). Experiments were performed to study the effect of ligation on the efferent lymph vessel. In these experiments a marked cell death was observed within the node, which also was depleted of lymphocytes. Additional experiments revealed that this finding was caused by PBV.

Materials and Methods

The experiments were performed in random bred female Wistar rats weighing 180-300 gm. The right knee node was studied, using the left knee node as control. Ligation of the lymph vessels was performed in nembutal narcosis (i.p. 60 mg/100 gm) except group 5 where ether was used. The lymph vessels were exposed either in the popliteal or in the inguinal region after visualization with about 0.02 ml PBV injected subcutaneously on the dorsum of the paw. The vessels were ligated with ophthalmic nylon sutures 9-0 using a dissecting microscope. The animals were sacrificed at 24 hours except some rats in groups 1 and 2.

The animals can be divided into five groups:

Group 1. Ligation of efferent lymph vessels in the right popliteal region. Sham operation on the left side. PBV injected on both sides.

Group 2. As group 1 except that the ligation was performed in the inguinal region.

Group 3. As group 2 except that ligation was performed without using PBV for visualization of the lymph vessels.

Group 4. PBV injected in the right foot, saline in the left foot. Lymph vessels were not exposed or ligated.

Group 5. As group 4. Animals narcotized with ether instead of nembutal. PBV injected in the right foot.

Results

Group 1. This was the preliminary study which was performed on 20 animals. The animals were sacrificed at 12, 24 and 48 hours. In this group the blockage was tested by injection of PBV immediately before sacrifice. In about half of the animals the test indicated that full blockage of the efferent vessels was attained and only these were examined further.

At 12 hours the histological section revealed large amounts of cellular debris throughout the whole cortex of the node with a picture similar to that seen after local irradiation of the lymph nodes (4). The marginal as well as intermediate sinuses were dilated. At 24 hours the cellular debris had diminished somewhat. At 48 hours serial sections of the nodes showed a marked edema with dilatation of the marginal and intermediate sinuses. In some of the nodes there were practically no lymphocytes in the sinus and also few lymphocytes in the parenchyma except in limited areas at the periphery of the cortex (Fig. 1). The medullary cords were collapsed and contained practically no plasma cells.

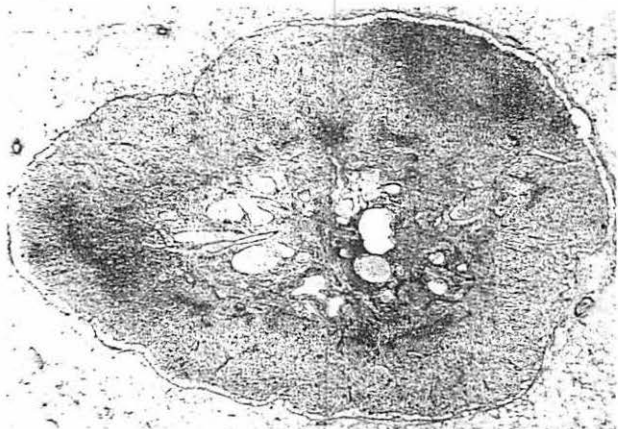


Fig. 1. Rat knee node 48 hours after ligation of the efferent lymph vessel in the popliteal region. Dilated marginal sinus. Parenchyma largely depleted of lymphocytes. Hematoxylin-eosin. 12 x.

Group 2. In this group 6 animals were studied. Three of them were sacrificed 6 hours after ligation, the others at 24 hours. The sinuses were dilated in two of the nodes, but there was no marked edema. At 6 hours some pycnotic lymphocytes were found in the knee nodes on both sides without any marked difference between right and left side.

At 24 hours there were numerous dead cells on the ligated side (Fig. 2a). On the control side the lymph node was completely normal in one animal (Fig. 2b), in two animals there was some increase of dead cells, but they were much fewer on the ligated side.

Group 3. Three animals were studied. All sacrificed at 24 hours after ligation of the lymph vessels on the right side. The knee nodes were histologically normal on both sides without any increase of pycnotic cells.

Group 4. Three animals were studied in this group. All sacrificed at 24 hours. There was no edema of the node. Numerous dead cells were found in the right knee node in all animals (Fig. 3a). The left knee node was normal in appearance without any increase of pycnotic cells (Fig. 3b).

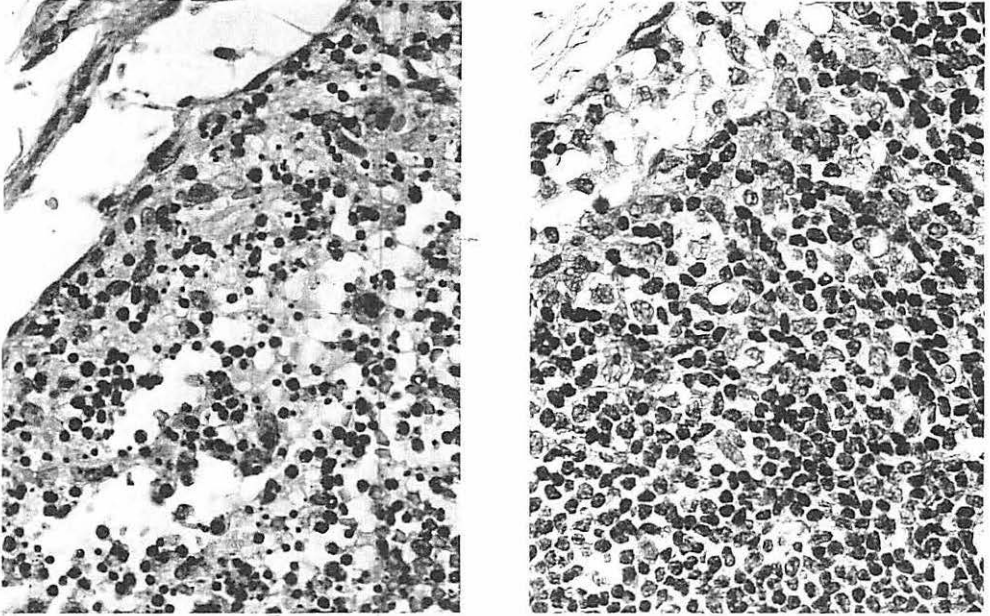


Fig. 2 a and b. Right (a) and left (b) knee nodes from rat 24 hours after ligation of the efferent lymph vessels in the right inguinal region. Nembutal narcosis. PBV injected on both sides. On the right side dilated, empty marginal sinus. Parenchyma depleted of lymphocytes. Numerous dead cells. Left knee node normal appearance. Hematoxylin-eosin. 230 x.

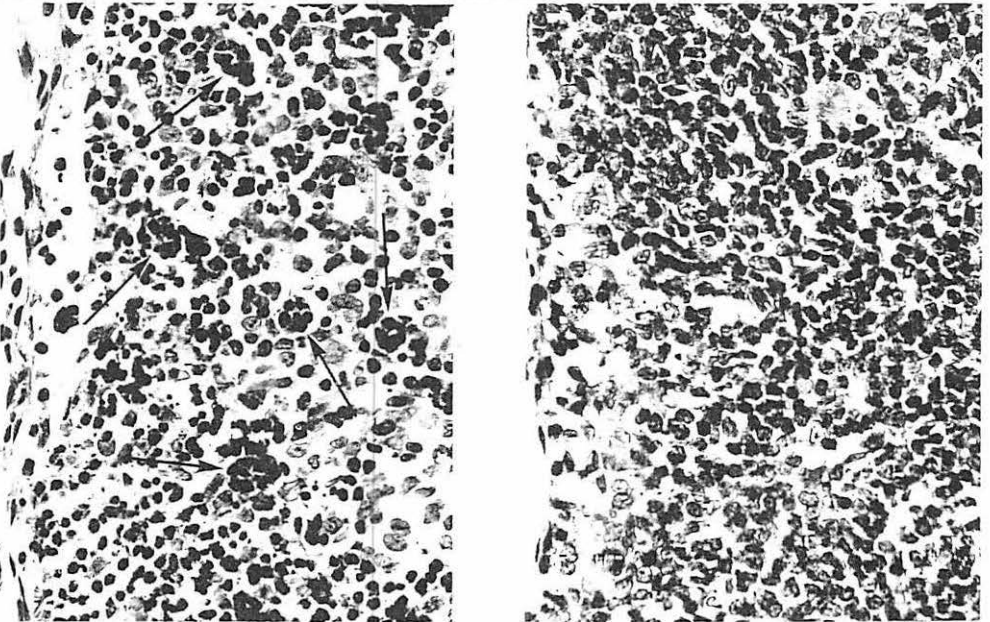


Fig. 3 a and b. Right (a) and left (b) knee nodes from rat immobilized in nembutal narcosis. PBV 0.02 ml injected in right paw, saline in the left. Numerous dead lymphocytes on the right side. Many of them phagocytized (arrows). Normal lymph node on the left side. Hematoxylin-eosin. 230 x.

Group 5. Three animals were sacrificed after 24 hours. There was some increase of dead cells on the right side. The left knee nodes were normal in appearance without any increase of pycnotic cells.

Discussion

Ligation of the efferent lymph vessels close to the knee node after visualization with PBV causes extensive cell death in the node which is depleted of lymphoid cells at 24 and 48 hours. Our first conclusion was that the surgical procedure close to the node might have damaged its blood supply. Therefore the experiment was performed with ligation of the lymph vessel at a distance from the node, in the inguinal area (group 2). In these experiments complete lymph blockage could not be obtained for more than 24 hours. The edema in the node was not so marked as after ligation close to the node, but the same degree of cell pycnosis was observed at 24 hours. At 6 hours some cellular debris was also observed on the non-ligated side, but this had disappeared at 24 hours, probably because of a better lymph flow. Cell death was not observed in animals where the lymph vessels were ligated without injection of PBV (group 3), which indicated that the dye was the main cause of the observed alterations. That the cell death could be caused by the stress following the narcosis, which lasted for 4-6 hours, was eliminated in group 4 where animals were narcotized and injected with PBV on one side only. Dead cells were found only on the dye-injected side. Our conclusion is therefore that PBV is toxic to the lymph node lymphocytes and causes cell death. This is marked and easily observed when there is a prolonged exposure to the dye because of reduced lymph flow caused either by ligation (group 1 and 2) or nembutal narcosis with long lasting immobilization (group 4). However, even a shorter contact with the dye as in group 5 with no ligation and under brief ether narcosis, revealed significant alterations on the side where the dye had been injected.

This toxic effect might be of importance for the evaluation of lymphocyte number and function in experiments where PBV has been used for localization of lymph vessels.

References

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