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Dactinomycin-induced veno-occlusive disease in rats. The hepatoprotective action of amifostine. Evaluation in a light and electron microscope

Halina Borowska[1], Ewa Arciszewska[1], Piotr Czauderna[2], Bogusław Sawicki[1]

[1]*Department of Histology and Embryology, Medical University, Białystok, Poland*
[2]*Paediatric Surgery Department, Medical University, Gdańsk, Poland*

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The purpose of the study was to draw upan experimental model of hepatic veno-occlusive disease (VOD) induced by dactinomycin (ACT) and to investigate the possible hepatoprotective effects of Ethyol (amifostine). Pathological changes corresponding to a VOD picture of varying intensification were found in the liver samples obtained from all the rats receiving ACT. Amifostine appears to act protectively to liver changes caused by dactinomycin.

Key words: veno-occlusive liver disease (VOD), ethyol (amifostine), dactinomycin (ACT)

INTRODUCTION

A new complication following chemotherapy has been observed in recent years during bone marrow transplantation [1] and treatment for nephroblastoma [2, 5], namely liver veno-occlusive disease or VOD. This term was established by Bras in 1954. Veno-occlusive liver disease was first reported from the populations of Central America and Asia who drank herbal tea which contained vegetal alkaloids [1]. It can also be induced by drugs, including antineoplastic antibiotics such as ACT used to treat nephroblastoma. The clinical criteria of VOD have been established [4]. These are hepatomegaly, ascites and/or weight increment, jaundice caused by hepatocyte necrosic and damage to the venous endothelium and intrahepatic sinuses [6].

MATERIAL AND METHODS

The study used 40 male Wistar rats divided into a control group and two experimental groups. The animals of the experimental group (group I) received dactinomycin (ACT) intraperitoneally 3 times (d 1–3), each separated by a week, in a dose of 15 mcg/kg b.w. Group II rats were given ACT + amifostine 15 min before the last ACT dose. The control animals received 0.9% solution of physiological saline. Liver specimens were collected in narcosis with ketamine on day 7 after the last ACT injection. The material was fixed in 10% formalin and in Gandre's fluid. Paraffin sections were stained with haematoxylin and eosin (H + E). The PAS method of staining for argyrophilic fibres according to Gomori and the electron microscope technique were used.

RESULTS AND DISCUSSION

Liver preparation of the control animals showed a normal histological pattern. The cytoplasm of multilateral hepatocytes stained with haematoxylin and eosin contained fine eosinophilic granules (Fig. 1). Staining with the PAS method showed a high but

Address for correspondence: Halina Borowska, Department of Histology and Embryology, Medical University, ul. Kilińskiego 1, 15–089 Białystok, Poland, e-mail: histolog@amb.edu.pl

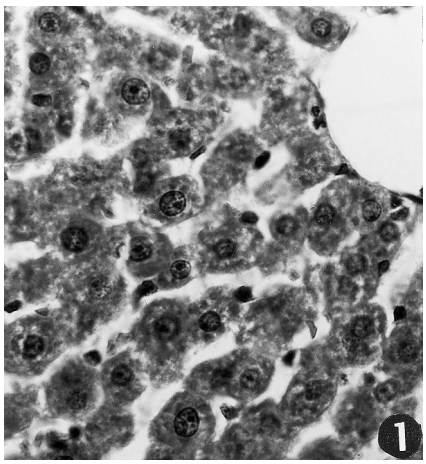

Figure 1. Control group. Rat liver specimen. H+E staining; × 400.

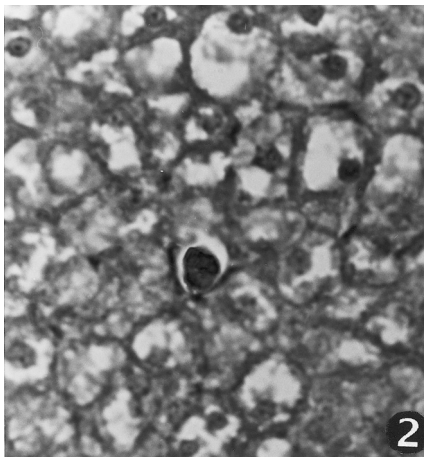

Figure 2. Experimental group II. Vacuolar degeneration of hepatocytes. Morphological differentiation of cell nuclei; × 400.

varied glycogen content in the cells. A small number of argyrophilic fibres were observed in the wall of the central veins and sinusoids when using the Gomori method. The liver preparations obtained from the animals receiving dactinomycin showed the following changes: hepatic trabecular diastasis, vacuolar degeneration of the hepatocytes and high differentiation of the cellular nuclei — in most cases they were hyperchromatic and had irregular contours (Fig. 2). In all the experimental animals thrombotic and deliquescent necrosis was found mainly in the central part of the lobules. Moreover, the walls of the central veins were thickened. The sinuses contained numerous erythrocytes and small platelet thrombi, as well as a large number of fibroblasts around the vessels. The preparations stained with the PAS method had low glycogen

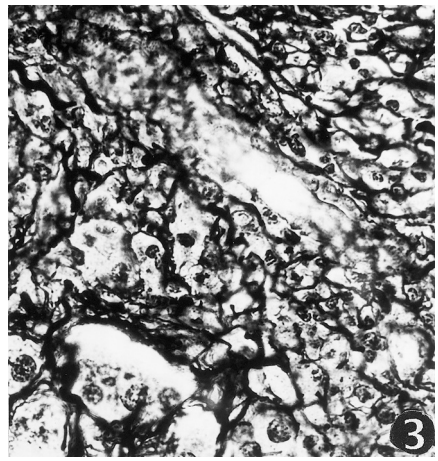

Figure 3. Experimental group I. A large number of argyrophilic fibres. Staining with the Gomori method; × 400.

content or none at all in the necrotic cells. The number of argyrophilic fibres increased, which was revealed by the Gomori method (Fig. 3). These morphological changes correspond to veno-occlusive liver disease and are similar to those described by other authors [4, 6]. The VOD symptoms were confirmed by electron microscopy. Damage to the mitochondria included swelling, the occurrence of megamitochondria, dilation of the rough endoplasmic reticulum channels and accumulation of collagen fibres (Fig. 4). The liver of animals receiving ACT with amifostine showed much less intensified pathomorphological changes. The morphological picture in the light and electron microscope resembled that of the control rats (Fig. 5). Amifostine has a wide protective spectrum [3]. Moreover, it reaches a high level of concentration in hepatic tissue [3, 7]. Its protective action against the harmful effects of chemotherapy has been proved [7]. The neutral pH of the cells promotes greater activity by the alkaline phosphatase and amifostin transport to the cell. The active metabolite of amifostine prevents the binding of cytostatics with cellular DNA, thus stabilising its chemical bindings and preventing it from destruction. It also reduces cell apoptosis induced by chemical substances. Irrespective of the protective mechanism it can be concluded that amifostine exerts a protective effect against hepatotoxic ACT action.

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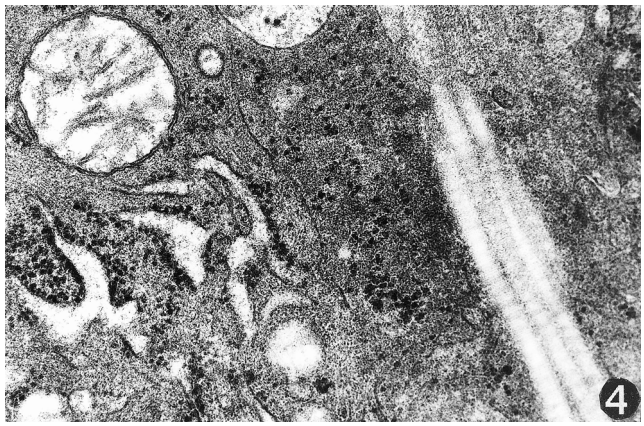

Figure 4. Experimental group I. Fragments of hepatocytes. The cytoplasm shows swelling of the mitochondria and dilated channels of the rough endoplasmic reticulum. Collagen fibrils in Disse's space. TEM; × 12000.

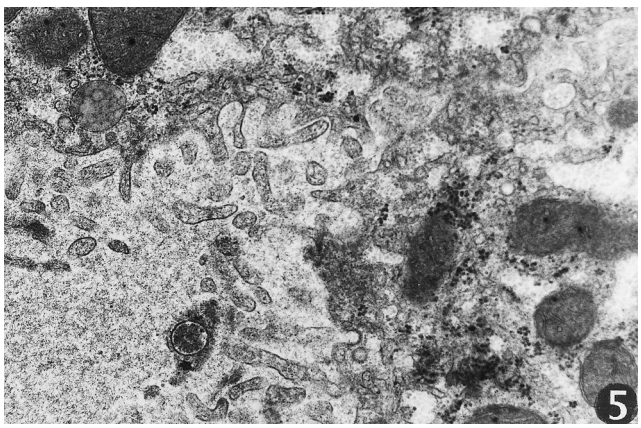

Figure 5. Experimental group II. Fragments of hepatocytes. The endothelium, microvilli and Disse's space show no significant changes. TEM; × 12000.

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