



The Effect of Cernitins on Galactosamine-Induced Hepatic Injury in Rat

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Cernitins correspond to microbiologically fermented pollen extract. Cernitin T60 contains mainly water soluble, while Cernitin GBX mainly fat-soluble substances. The aim of the present work was to examine the effect of Cernitins on the d-galactosamine-induced liver damage in rat. It has been shown, that galactosamine administration to rat resembles viral hepatitis both biochemically and histologically. Our studies proved that Cernitin T60 given orally or intraperitoneally inhibited or counteracted the elevation of amino transferases activity and the inflammatory process, necrosis and steatosis of the liver cells. The protective effect of Cernitin GBX on the liver parenchyma was only slightly expressed. It is concluded; that application of pollen extracts in-patients with liver diseases should be considered.

Cernitins are obtained from the pollen raw materials (AB Cernelle, Vegeholm Sweden). Cernitin T60 contains mainly water soluble, while Cernitin GBX mainly fat-soluble substances. The chemical composition of pollen has been subjected to several investigations. Numerous chemical substances have been identified and isolated from pollen: 21 amino acid (including 10 essential amino acids), all known vitamins, enzymes, coenzymes, steroids, minerals, and trace elements, as well as streptolysin inhibitory factor.

Previously we have demonstrated the protective effect of linden pollen and Cernitins against fatty infiltration, carbon tetrachloride, and ethionine-induced liver damage. However, hepatic impairment as the result of carbon tetrachloride and ethionine poisoning in laboratory animals does not necessarily represent an adequate experimental model of liver disease in humans. So, we decided to examine the effect of Cernitins in the galactosamine model in rats. Galactosamine administration induces an inflammatory response in the liver that in some aspects resembles the reaction seen clinically in viral hepatitis.

Materials and Methods

Ten groups of ten Wistar male rats weighing 190-250 g were used. Animals of group 1 served as the controls. Rats of group 2 received galactosamine. The remaining groups were treated with Cernitins for five days and challenged with galactosamine; four groups were given Cernitin T60: 50mg/kg per day (p.d.), orally (p.o) by intubation (group 3), 200mg/kg p.d., p.o. (group 6). The further four groups received Cernitin GBX: 50mg/kg p.d., p.o. (group 7), 200 mg/kg p.d. / p.o. (group 8), 50 mg/kg p.d., i.p. (group 9) and 200mg/kg p.d., i.p. (group 10). The animals were challenged with d-galactosamine hydrochloride on the third day at the dose of 400 mg/kg i.p. three times within 24 h. They were fasted for 16 h prior to autopsy.

D-galactosamine hydrochloride was purchased from E. Merck (Darmstadt) and Cernitins were kindly delivered by AB Cernelle (Vegeholm, Sweden).

After a mild ether anesthesia, the thorax of the animals was opened and blood was drawn from the ascending aorta. In the blood serum the following biochemical parameters were determined: alanine aminotransferase (A1AT) and aspartate aminotransferase (AspAT) activity according to Reitman and Frankel, alkaline phosphatase activity according to the method of Bodansky and bilirubin level by the method of Malloy and Evelyn. The results were analyzed by Duncan's test.

Specimens for histopathological studies were always taken from the same place of the liver. For routine microscopic investigations they were stained with hematoxylin and eosin (HE), for the lipids presence with Sudan black and for collagen fibers with Van Gieson's mixture.

Results

D-Galactosamine treatment of rats resulted in a marked increase of enzymes activity and bilirubin concentration in the blood serum of animals (Table 1). When compared to the control group, the challenging dose of galactosamine resulted in a 47-fold increase in A1AT activity and 17-fold increase in AspAT activity; alkaline phosphatase activity was elevated by 600% in these animals. Cernitin T60 application was associated with a significant and marked drop of A1AT, AspAT, and

alkaline phosphatase activity as well as the bilirubin concentration in the blood serum as compared with group 2. In rats of group 3, i.e. receiving Cernitin T60 50mg/kg orally, AspAT and alkaline phosphatase activity and bilirubin level remained normal, while A1AT activity appeared to be close to the value established for the control group. Cernitin GBX treatment of rats injected with galactosamine caused distinctly smaller decrease of the examined biochemical parameters than in Cernitin T670 treated animals. Amino-transferases activity was markedly lower after intraperitoneal application of Cernitin GBX, as compared with oral dosing. Oral administration of Cernitin GBX 200mg/kg (group 8) did not produce significant changes in A1AT and alkaline phosphatase activity as well as in bilirubin concentration, in comparison with animals receiving galactosamine alone (group 2).

Table 1. Serum alanine aminotransferase (A1AT), aspartate aminotransferase (AspAT), alkaline phosphatase (AP) activity and total bilirubin (B0 level in rats receiving Cernitin T60, Cernitin GBX and treated with galactosamine (G). Mean \pm SE. P.o= orally, i.p. = intraperitoneally

Extensive morphological alterations resembling viral hepatitis were observed in liver sections taken from rats challenged with galactosamine (Fig. 1). Numerous and huge cellular infiltrations comprised mononuclear cells, mainly lymphocytes, and mononuclear phagocytes as well as few granulocytes, accompanied by massive parenchymal cell lysis and vacuolar degeneration, visible in portal spaces and in marginal zones of the lobules (Fig. 2). Cellular infiltrations in sinusoidal vessels surrounded the damaged parenchymal liver cells. Furthermore, fine size droplet fatty degeneration appearing in the peripheral, intermediary and central zones of the lobule was also observed (Fig. 3). A slight increase in fibrous connective tissue existed and there were islands of acute necrosis throughout the section. Protective effect of Cernitin T60 against galactosamine-induced hepatic alterations was evident (Fig. 4). There were no signs of fatty degeneration and necrosis. Cellular infiltrations were almost not visible, slight dilatation of sinusoids as well as moderate hyperemia occurred (fig. 5). Only single mononuclear leukocytes of varying size could be seen in the portal space (Fig. 6). Liver of rats treated with galactosamine did not show quite evident protective action of orally administered Cernitin GBX on the parenchymal liver cells.

Extensive leukocyte infiltration was visible (Fig. 7) and fatty degeneration of the liver cell appeared (Fig. 8). However, in rats receiving Cernitin GBX intraperitoneally a slight positive effect could be observed: cellular infiltration necrosis and fatty degeneration of the liver cell was decreased as compared with animals receiving galactosamine alone. Summary of histopathological studies is presented in Table 2.

Fig. 1. Light microscopic appearance of rat liver treated with galactosamine resembling damage evoked by viral hepatitis. Stain: HE. Magn. X 130

Fig. 2. Liver of a rat treated with galactosamine. Huge leukocyte infiltration of portal space accompanied by massive parenchymal cell lysis and vacuolar degeneration in marginal zones is clearly visible. Stain: HE. Magn. X 270.

Fig. 3. The multiple fine size droplet degeneration of parenchymal liver cells of a rat treated with galactosamine. Stain: Sudan black. Magn. X 270

Fig. 4. Liver from a rat treated with Cernitin T60, 50 mg/kg orally and challenged with galactosamine. The picture demonstrates the beneficial effect of Cernitin T60 on galactosamine-induced hepatic injury. No signs of leukocyte infiltration, no such a heavy damage of hepatocytes as in the liver of a rat receiving galactosamine alone can be seen. Stain HE. Magn. x 130

Fig. 5. Liver from a rat, which was administered Cernitin T60 200 mg/kg orally. There are no signs of significant injury of the liver cells. Dilatation of sinusoids and hyperemia occurs. Stain: HE. Magn. x 550

Fig. 6. Grouping of single mononuclear leukocytes of varying size in the portal space of liver from a rat poisoned with galactosamine and protected with Cernitin T60 (50 mg/kg i.p.). The hepatocytes do not demonstrate clear signs of degeneration. Stain: HE. Magn. x 550

Fig. 7. Liver from a rat treated with galactosamine does not show the protective action of Cernitin GBX applied orally 200mg/kg. Massive leukocyte infiltration of marginal zones of the lobules followed by degeneration or lysis of parenchymal cells is visible. Stain: HE. Magn. x 270

Fig. 8. Numerous Sudan-positive granules in the liver of a rat given Cernitin GBX (200 mg/kg orally) and galactosamine. The range of lipid degeneration is almost the same as after administration of galactosamine alone. Stain: Sudan black. Magn. x 270

Table 2. Summary of histopathological abnormalities

Discussion

The liver can be damaged and its functional properties affected in a multiple ways. The damage may be caused by the direct action of toxic substances or may result from secondary reactions. Direct action is associated with d-galactosamine. It has been shown, that the degree of severity of galactosamine-induced hepatitis is dependent on the species of animals used. Galactosamine administration to rats resembles viral hepatitis both biochemically and histologically.

Exposure of rats to three intraperitoneal doses of galactosamine, three times within 24h, caused acute hepatitis with increase of AspAT and A1AT activities and intensive histopathological abnormalities of the liver including cellular infiltration, necrosis, fatty degeneration and fibrosis. Amino-transferases, especially A1AT represent highly specific index of hepatocellular injury and are much more sensitive to minimal or moderate damage of the liver than the other hepatic function tests. The amino-transaminases activity in blood serum is parallel to the degree of liver cell injury. This was confirmed by our investigations, also in respect to prevention of the occurrence of hepatic damage. Elevation of the A1AT activity indicates, that one of the first hepatic responses to galactosamine poisoning comes from parenchymal cells and involves the activity of one or more enzymes. Galactosamine toxicity causes the appearance of specific lesions in the liver cells, one characterized by inhibition of nuclear RNA synthesis accompanied by nuclear fragmentation, the other by inhibition of protein synthesis followed by accumulation of aggregates between the stacks of rough endoplasmic reticulum. Since the administration of uridine prevents and reverses in vivo inhibition of both synthesis but not the accumulation of aggregates, it is likely that an acute deficiency of uridine triphosphate due to accumulation of stable uridine diphosphate-galactosamine is the intrinsic mechanism of toxicity.

Our studies proved that Cernitins, corresponding to microbiologically fermented pollen extracts, protected against liver injury caused by d-galactosamine. Cernitin T60 administered both orally and intraperitoneally brought about a rapid, significant reversion to normal or almost normal amino-transferases and alkaline phosphatase activity as well as elevated bilirubin level. Also the damage observed histologically disappeared in animals receiving Cernitin T60 that inhibited or counteracted the inflammatory process, necrosis and steatosis of the liver cell. The protective effect of Cernitin GBX on the liver parenchyma was only slightly expressed. Inhibition of ethionine-induced rat liver injury by pollen extracts was demonstrated by us previously. In rats treated with ethionine and receiving Cernitins we noticed the increased number of nucleoli, attributed probably to the accelerated synthesis of nuclear RNA. Thus, the positive effect of Cernitins may be in consequence due to the potentiated synthesis of proteins exhibiting protective properties against the liver cell injury.

On the other hand, the definite anti-inflammatory action of Cernitin extracts was revealed in the case of croton oil-induced oedema. In the cotton pellet test in rats Cernitin T60 showed an anti-inflammatory activity corresponding to the inflammation-inhibiting effect of phenylbutazone, but was completely devoid of toxicity. It was also possible to confirm the anti-inflammatory action when compared with very active intraperitoneally injected anti-inflammatory agents. It has been proved that the action of pollen extracts is not due to the liberation of corticosteroids.

It is undeniable that Cernitin T60 prevents not only necrosis, but also lipid accumulation. Cernitin T60 has clearly visible lipotropic effect. The mechanism of this action is unknown. It can be attributed to the supply of SH groups which come from methionine and cystine contained in pollen. However, our conclusions favor the polyfactorial basis of the effect of Cernitin T60 on the galactosamine induced liver injury.

Finally, the results of our investigation suggest the possibility of pollen extracts application in patients suffering from acute and chronic liver disease; especially that Cernitins are practically un toxic and show excellent tolerance properties.

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