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# *Histological examination of the liver after experimental administration of memantine and dexamethasone*

Memantine hydrochloride is a low affinity N-methyl-D-aspartate (NMDA) receptor antagonist (4, 8) that prevents excitatory amino acid neurotoxicity without interfering with the physiological actions of glutamate necessary for learning and memory. NMDA receptor antagonists prevent the injury of the neuron through the inhibition of the $Ca^{2+}$ influx through the NMDA-operated channel and subsequently reduction in the increase of the intracellular calcium concentration. Memantine is a potential treatment for Alzheimer's, vascular and mixed dementia. On the other hand, the side-effects of NMDA receptor antagonists are serious and need to be considered before clinical use. No publications are available describing morphology of the liver after memantine administration.

Dexamethasone is a synthetic glucocorticosteroid. The major site of its metabolism is the liver (10). Treatment with high doses of glucocorticosteroids causes liver steatosis and more recently has been related to nonalcoholic steatohepatitis (1). Glucocorticosteroids are the most widely used drugs associated usually with mild steatosis, but they also may induce acute steatohepatitis leading to acute liver failure. Dexamethasone administered in high doses causes liver steatosis due to the increased fatty acids inflow (6).

Lack of data regarding the influence of memantine on the liver inclined us to undertake this research. We decided to assess morphological structure of the liver after experimental administration of memantine and after concomitant administration of memantine and dexamethasone that was used as factor inducing liver damage. We examined liver slides stained with hematoxylin and eosin, Masson's technique and PAS method with the use of light microscope.

## MATERIAL AND METHODS

The experiments were carried out on male Albino Swiss mice weighing 24–25 g at the beginning of the experiment. Care and treatment of the animals were in accordance with the guidelines for laboratory animals of the Local Ethical Committee of the Medical University of Lublin. The animals were kept under standard laboratory conditions, with free access to granular standard diet and tap water. Their weight was monitored daily. The animals were divided into three groups (including 10 animals each). Animals of the control group received distilled water (i.p. 0.2 ml/24 h) for 21 days. Animals in experimental group I received memantine. Experimental group II animals received dexamethasone. Experimental group III animals received memantine and dexamethasone. Memantine was administered i.p. in a single dose 30 mg/kg/24 h for 21 days. Dexamethasone (Dexaven-Jelfa S.A., Poland) was administered i.p. in a single dose 16 mg/kg/24h for

16 mg/kg/24h for 21days. Twenty four hours after the last memantine or last dexamethasone injection all animals were decapitated and their livers were taken for histological examinations.

Specimens of liver fixed in 4% formalin were dehydrated in graded ethanol solutions and embedded in paraffin. Seven-μm thick paraffin slices were stained with hematoxylin and eosin (H+E), Masson's technique, PAS method and assessed using a light microscope.

## RESULTS

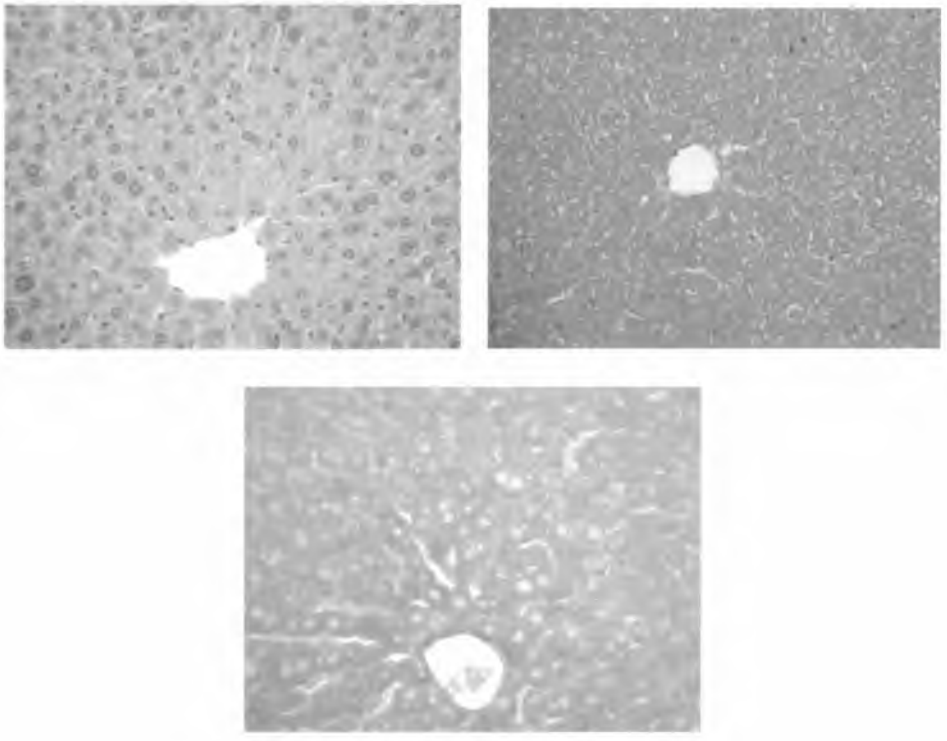

Fig. 1. Control group. Regular structure of the liver. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 400x

Liver stained with hematoxylin and eosin evidenced a regular architectonics of the liver lobules. The hepatocytes were clearly contoured and formed quite regular trabeculas. The hepatocytes nuclei were regular in shape (round or oval) with quite regularly distributed chromatin. Most hepatocytes presented one nucleus, sometimes two. Hepatocytes cytoplasm showed an affinity to acidic stains and possessed thick basophilic granules regularly located in all zones of the liver lobule. Single erythrocytes were found in the sinus lumen. The endothelial cells were flattened and Browicz-Kupffer cels were observed in the walls of the sinuses. Staining by the Masson's technique revealed a small amount of connective tissue in the vicinity of the liver triads and between liver lobules.

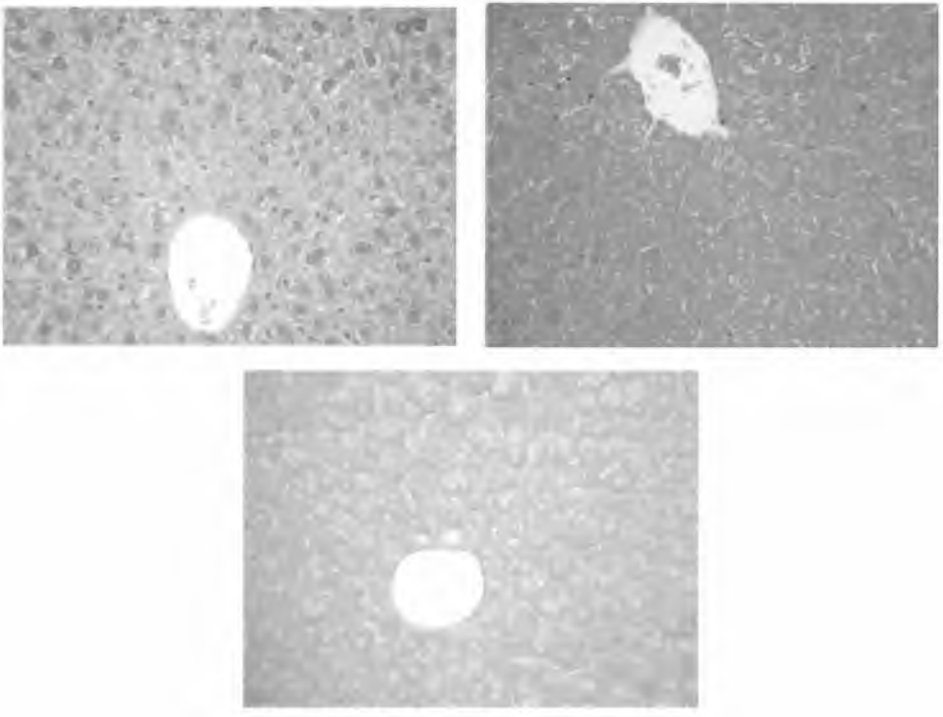

Fig. 2. Experimental group I – Memantine. Slightly increased transparence of hepatocyte cytoplasm and narrowing of the liver sinusoids are visible. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 400x

Slight histological changes were observed only in some individuals. The general architectonics of the lobules was preserved. In most hepatocytes one or two nuclei were found. The chromatin was regularly distributed. The hepatocyte cytoplasm in H+E staining showed a slightly increased transparence. Irregular transparent areas showing no affinity to acid or basic dyes were visible within some hepatocytes. The sinus lumen was slightly narrowed. The endothelial cells and Browicz-Kupffer cells were similar to the control group. Staining by the Masson's technique has shown the amount of connective tissue similar to that of the control group. Staining by the PAS method has shown a picture similar to that of the control group.

The general architectonics of the lobules was preserved. The hepatocytes nuclei were regular in shape. A numer of binuclear hepatocytes in all zones was increased in comparison with the control group. The hepatocyte cytoplasm showed smaller affinity for dyes and microvesicular changes. Numerous, no colouring vacuoles were visible in numerous hepatocytes. More numerous and enlarged Browicz-Kupffer cells were found intended to the sinus lumen. The sinus lumen was enlarged. An increased amount of connective tissue was observed in slides stained with Masson's technique. Connective tissue fibers were found mainly in the vicinity of sinusoids and around venous blood vessels. The PAS method revealed the decreased amount of glycogen and changes of glycogen localization within the hepatocyte cytoplasm in comparison with the control group.

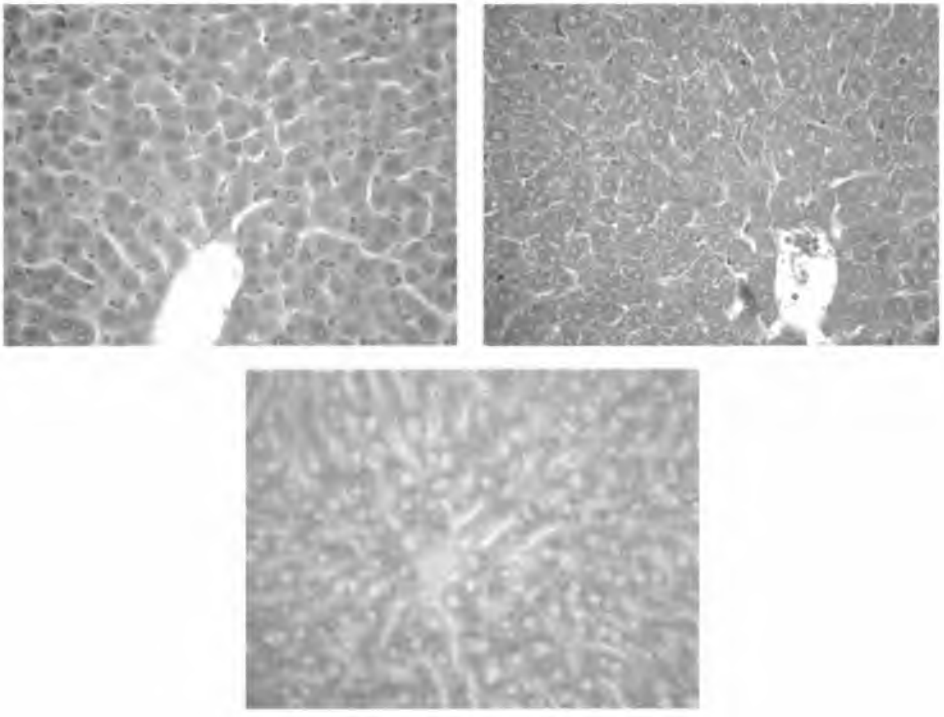

Fig. 3. Experimental group II – Dexamethasone. Liver damage induced by high doses of dexamethasone. Microvesicular steatosis, activation of Browicz-Kupffer cells, dilatation of sinusoids and perisinusoidal fibrogenesis are visible. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 400x

The picture was essentially different from the picture in the control group and experimental groups I and II. The general architectonics of the lobules was not preserved. The arrangement of hepatocytes was irregular, especially in places with large amount of connective tissue and inflammatory infiltrations. In these places hepatocytes showed far reaching morphological damage in the shape of decreased cytoplasm stainability and microgranular cytoplasm changes. Also focal necrosis of hepatocytes was observed. The sinusoidal lumen was significantly enlarged and filled with numerous red blood cells. Browicz-Kupffer cells were enlarged and much more numerous especially in necrotic areas. They formed inflammatory infiltrations in these areas. In slides stained with Masson's technique the amount of connective tissue was significantly increased and large areas of blue connective tissue were observed. Browicz-Kupffer cells formed infiltrations in these areas. In other regions hepatocytes were arranged in irregular trabeculas. They had numerous fat droplets in the cytoplasm. Their nuclei were round in shape and different in size. A numer of binuclear hepatocytes in all zones was increased in comparison with the control group. Connective tissue fibers were visible in the vicinity of sinusoides and blood vessels. The PAS method revealed a decreased amount of glycogen within hepatocyte cytoplasm and changes of its localization within the liver cells.

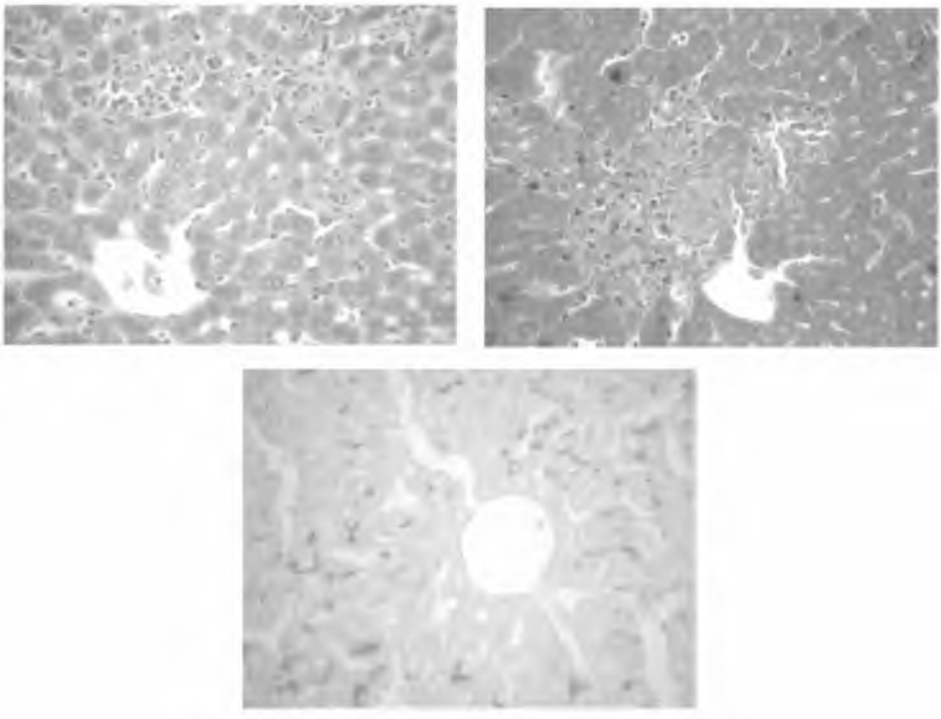

Fig. 4. Experimental group III – Memantine+Dexamethasone. Memantine intensifies liver damage induced by dexamethasone. Focal hepatocyte necrosis, inflamatory infiltrations and distinct perisinusoidal and perivenular fibrosis are visible. The upper panel presents the H+E staining, the middle panel presents staining with Masson's technique and the lower panel presents the PAS method. Magn. 400x

## DISCUSSION

The morphological changes revealed under the light microscope in the animals treated with memantine alone were characterized by a slightly increased transparence of the hepatocyte cytoplasm and a slight narrowing of the sinus lumen. Such a picture may be a result of vacuolar degeneration or accumulation of fats or carbohydrates within the hepatocyte cytoplasm. Examinations on the ultrastructural level are required to determine which of these processes is responsible for observed changes. Similar changes in the hepatocyte cytoplasm but more intensive were observed in our previous experiment regarding the influence of another the NMDA receptor antagonist – MK-801 on the liver morphology (11). Narrowing of the sinus lumen may indicate injury of the vascular pole of hepatocyte or endothelial cell damage.

Histological changes observed after administration of dexamethasone were characterized by significantly smaller hepatocyte cytoplasm stainability, the presence of numerous fat vacuoles in hepatocyte cytoplasm, more frequent occurrence of binuclear hepatocytes, sinusoidal dilatation and an activation of Browicz-Kupffer cells. The staining with Masson's technique revealed an increased amount of connective tissue especially in the vicinity of sinusoids and veins. The PAS method has shown changes in the glycogen localization.

Morphological picture of hepatocytes after dexamethasone administration indicates liver steatosis. Liver steatosis, also called fatty liver refers to accumulation of fat in the parenchymal cell of the liver. Liver steatosis is divided into two types, namely macrovacuolar and

the liver. Liver steatosis is divided into two types, namely macrovacuolar and microvesicular steatosis which differ in their histological and clinical aspects, mechanisms and prognosis (2).

Macrovacuolar steatosis is the most usual form. In this condition, hepatocytes contain a single, large vacuole of fat (mainly triglycerides) which fills up and rounds the hepatocyte displacing the nucleus to the periphery of the cell. In the absence of other liver lesions, macrovesicular steatosis, by itself, is a relatively benign condition. The most frequent causes of macrovacuolar steatosis in humans are alcohol abuse, obesity, diabetes and some dyslipemias.

In microvesicular steatosis, in contrast, hepatocytes are filled out by numerous small lipid vesicles, which leave the nucleus in the center of the cell. Microvesicular steatosis may be associated with other liver lesions such as necrosis, cholestasis or fibrosis. Even in the absence of these associated liver lesions, extensive microvesicular steatosis is by itself a serious condition.

Morphological changes observed in hepatocytes after administration of dexamethasone in our experiment were characterized by the presence of numerous fat vacuoles in hepatocyte cytoplasm, which indicates that dexamethasone induces microvesicular liver steatosis in our experimental model.

Liver steatosis occurs when fat homeostasis becomes unbalanced. Normally, triglycerides, the main components of fat in the liver, are derived from the esterification of free fatty acids that accumulate within the liver. These free fatty acids accumulate through two major pathways. They are transported to the liver packaged with albumin via gut absorption or lipolysis of adipose tissue, or they are synthesized within the liver through lipogenesis. Fatty acids that accumulate in the liver can then either undergo oxidation within hepatocyte mitochondria, peroxisomes or microsomes, or be esterified into triglycerides. The triglycerides are then secreted as very low-density lipoproteins (VLDLs), through exocytosis (3, 5).

The microvesicular and macrovesicular steatosis are induced by different mechanism. It is thought that mechanism leading to the microvesicular steatosis is impaired mitochondrial β-oxidation of natural fatty acids (2). When β-oxidation is severely impaired, fatty acids, which are poorly oxigenized by mitochondria are mainly esterified into triglycerides, but there is a residual increase in non-esterified fatty acids. Triglycerides (possibly emulsified by a rim of non-esterified fatty acids) accumulate as small vesicles. In contrast, the mechanisms leading to macrovesicular steatosis may involve various combinations of (a) increased mobilization of fatty acids from adipose tissue, (b) increased synthesis of fatty acids in the liver, (c) icreased estrification of fatty acids into triglycerides and/or decreased egress of triglycerides from the liver (2).

The increased number of binuclear hepatocytes observed in dexamethasone treated animals is a sign of liver regeneration evoked by pathogenic stimulus. N a g y et al. demonstrated that although dexamethasone prevents hepatocyte proliferation and stem cell activation, regenerative properties of the liver are preserved mainly through hepatocytic hypertrophy. Following the withdrawal of dexamethasone the enlarged hepatocytes enter the cell cycle and normal liver structure is reestablished (9). The more numerous and swollen Browicz-Kupffer cells are macrophages evolving in the phagocytosis. These cells play an important role in the pathogenesis of inflamatory liver diseases leading to fibrosis (7). Browicz-Kupffer cells secrete proinflammatory cytokines, IL-1β, IL-6 and TNF-α that stimulate stellate cells that are primarily responsible for fibrogenesis (7).

The liver sinus region was involved in the dexamethsone-induced damage. We observed significant dilatation of sinusoidal capillaries and an increased amount of connective tissue in their vicinity. Our results indicate that prolonged administration of high dexamethasone doses causes perisinusoidal fibrogenesis. This is consistent with Melgert's observations that show accelerated fibrogenesis under the influence of dexamethasone (7). Extracellular matrix components are produced mainly by hepatic stellate cells which are activated by hepatic steatosis through an increase in lipid peroxidation in hepatocytes.

Liver damage observed after concomitant administration of memantine and dexamethasone was stronger than in the case of dexamethasone itself. In the group receiving memantine with dexamethasone we observed morphological changes in the shape of microvesicular steatosis of hepatocytes leading to their focal necrosis, inflammatory infiltrations, damage of the wall of blood vessels with a distinct dilatation of sinusoids and activation of Browicz-Kupffer cells. Staining

with Masson's technique revealed perisinusoidal and perivenular fibrosis. The amount of connective tissue was significantly higher in comparison with that in the group receiving dexamethasone only. The PAS method has shown a decrease in glycogen amount and changes of its arrangement in hepatocyte cytoplasm more intensive than in the case of dexamethasone itself. Comparing the influence of memantine itself and dexamethasone itself with the concomitant influence of both chemicals it may be concluded that their concomitant administration intensifies liver damage induced by dexamethasone. The intensification of dexamethasone induced liver damage was observed earlier by Wolff et al. after concomitant administration of dexamethasone and methotrexate (12). The mechanism of liver damage intensification may be connected with impaired oxidative phosphorylation in fatty hepatocytes. Harrison et al. underline that mitochondria limit the efficiency of mitochondrial ATP synthesis by uncoupling oxidative phosphorylation in fatty hepatocytes (2). This increases hepatocyte susceptibility to injury if new oxidative stress is placed on the cell and explains an intensification of liver damage under the influence of two or more damaging factors (2).

## CONCLUSIONS

1. Memantine administration in the doses corresponding to the neuroprotective doses used in human causes slight morphological changes in the liver (a slight decrease of hepatocyte cytoplasm stainability and narrowing of the sinus lumen).

2. Dexamethasone administered in high doses causes microvesicular steatosis, activation of Browicz-Kupffer cells, dilatation of sinusoids and perisinusoidal fibrogenesis.

3. Comparing the influence of memantine itself and dexamethasone itself with the concomitant influence of both chemicals it may be concluded that memantine intensifies liver damage induced by dexamethasone leading to focal liver necrosis, inflammatory infiltrations and distinct perisinusoidal and perivenular fibrosis.

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SUMMARY

The aim of the research was histological assessment of the influence of memantine hydrochloride (NMDA receptor antagonist) and dexamethasone on the liver. The experiment was carried out on adult Albino-Swiss mouse males. Memantine was administered i.p. in a single dose 30 mg/kg/24 h for 21 days, dexamethasone i.p. in a single dose 16 mg/kg/24 h for 21days. Liver slices stained with hematoxylin and eosin, Masson's technique and PAS method were assessed using light microscope. Performed experiments revealed that memantine can cause slight morphological changes of the liver in the shape of increased transparence of hepatocyte cytoplasm and narrowing of the liver sinusoids. Dexamethasone induces liver damage in the shape of microvesicular steatosis, activation of Browicz-Kupffer cells, dilatation of sinusoids and perisinusoidal fibrogenesis. Memantine intensifies liver damage induced by dexamethasone leading to focal hepatocyte necrosis, inflammatory infiltrations and distinct perisinusoidal and perivenular fibrosis.

Ocena histologiczna wątroby po doświadczalnym podaniu memantyny i deksametazonu

Celem pracy była ocena histologiczna wpływu memantyny (antagonisty receptora NMDA) oraz deksametazonu na wątrobę zwierząt doświadczalnych. Badania wykonano na dorosłych samcach myszy Albino-Swiss. Memantynę podawano i.p. w dawce 30 mg/kg/24 h przez 21 dni, deksametazon i.p. w dawce 16 mg/kg/24 h przez 21 dni. Przy pomocy mikroskopu świetlnego oceniano preparaty wątroby barwione hematoksyliną i eozyną, metodą Massona i metodą PAS. Przeprowadzone badania wykazały, że memantyna może powodować niewielkie zmiany morfologiczne wątroby w postaci większej przejrzystości cytoplazmy hepatocytów oraz zwężenia naczyń zatokowych wątroby. Deksametazon powoduje uszkodzenie wątroby w postaci stłuszczenia, pobudzenia komórek Browicza-Kupffera, poszerzenia naczyń zatokowych i okołozatokowej fibrogenezy. Memantyna nasila uszkodzenie wątroby, wywołane wysokimi dawkami deksametazonu, prowadząc do ogniskowej martwicy hepatocytów, tworzenia nacieków zapalnych i znacznego okołozatokowego i okołożylnego włóknienia.