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Department of Histology and Embryology with the Laboratory for Experimental Cytology
Department of Clinical Pharmacology, Skubiszewski Medical University of Lublin

JOANNA SEKITA-KRZAK, KRYSTYNA CZERNY,
IWONA ŻEBROWSKA-ŁUPINA, ZOFIA DANILCZUK,
MARIA STĘPNIEWSKA

Histological examination of the liver after experimental administration of MK-801 and dexamethasone

MK-801 (*Dizocilpine maleate*) is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist (3). NMDA antagonists seem to be the most promising perspective as neuroprotective agents. They are in advanced development in the treatment of stroke and traumatic brain injury (7). They 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. MK-801 has the highest affinity for the NMDA-receptor and was considered one of the most promising neuroprotectants. It is the first drug consistently shown to be neuroprotective *in vivo* (7, 11). On the other hand, the side-effects of this drug are serious and need to be considered before clinical use. Low doses are associated with altered sensory perception, dysphoria, hypertension, nystagmus, and disorientation, with progression to agitation, paranoia, hallucinations, severe motor retardation, and ultimately catatonia at higher doses (7). Safety concerns arose after the finding of neuronal vacuolation in the cingulate gyrus and retrosplenial cortex in rats treated acutely with dizocilpine and other high-affinity noncompetitive NMDA antagonists, apparently due to a drug-induced increase in neuronal metabolic activity and glucose utilization (1, 9).

The liver is responsible for concentrating and metabolizing the majority of drugs and toxins that are introduced into the body. The majority of drugs used as neuroprotectants (including MK-801) are lipophilic and water-insoluble, so they can pass the blood-brain barrier. These drugs are rendered water-soluble by hepatic metabolism. They are processed by a variety of enzymes, especially those related to the endoplasmic reticulum in order to transform the lipophilic and water-insoluble chemical into water-soluble product in preparation for its excretion in urine (6).

Because of a lack of data regarding the influence of MK-801 on the liver we decided to assess the morphological structure of the liver after experimental administration of MK-801. We examined H+E stained slides after administration of MK-801 and after concomitant administration of MK-801 and dexamethasone that was used as factor inducing liver damage. The major site of dexamethasone metabolism is the liver (8, 10). Dexamethasone administered in high doses causes liver steatosis due to the increased fatty acids inflow (5).

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 Ethics 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 17 animals each). Animals of the control group received distilled water (i.p. 0.2 ml/24 h) for 8 days. Animals in experimental group I received MK-801. Experimental group II animals received dexamethasone. Experimental group III animals received MK-801 and dexamethasone. MK-801 was administered i.p. in a single dose 0.3 mg/kg/24 h for 8 days. Dexamethasone (Dexaven-Jelfa S.A., Poland) was administered s.c. in a single dose 120 mg/kg/24 h for 8 days. Twenty-four hours after the last MK-801 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) and assessed using a light microscope.

RESULTS

The control group. 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 or more. 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 cells poorly visible (Fig. 1).

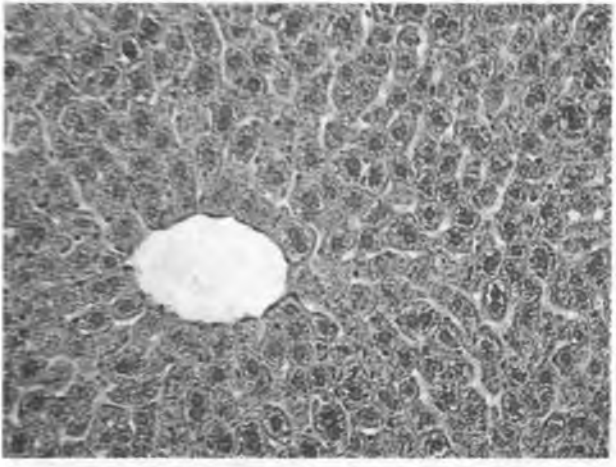

Fig. 1. Control group. Regular structure of the liver. H+E staining. Magn. 200x

Experimental group I – MK-801. 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. A higher number of binuclear hepatocytes was revealed in comparison with the control group. The chromatin was regularly distributed. The hepatocyte cytoplasm in H+E staining showed an increased transparence. Irregular transparent areas showing no affinity to acid or basic dyes

were often visible within the hepatocyte cytoplasm. The sinus lumen was distinctly narrowed. The endothelial cells were flattened and more numerous Browicz-Kupffer cells were intended to the sinus lumen (Fig. 2).

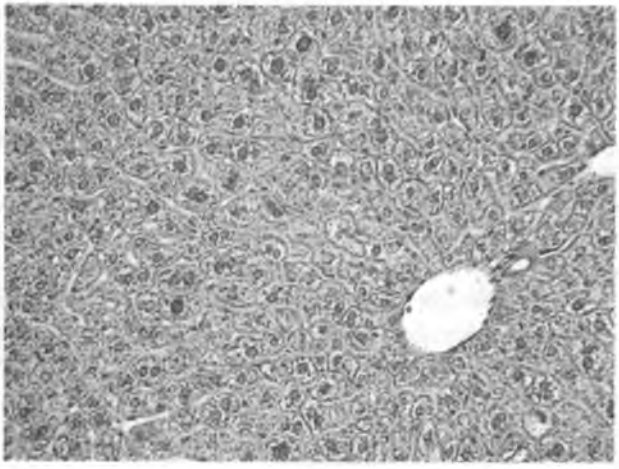

Fig. 2. Experimental group I – MK-801. Increased transparence of hepatocyte cytoplasm and narrowing of the liver sinusoids are visible. H+E staining. Magn. 200x

Experimental group II – dexamethasone. The general architectonics of the lobules was preserved. The hepatocytes nuclei were regular in shape (round) with regularly distributed chromatin. Numerous nuclei showed more condensed chromatin than in the control group. A number of binuclear hepatocytes in all zones was also increased in comparison with the control group. A clear halo was often visible around nuclei. The hepatocyte cytoplasm showed smaller affinity for dyes and was microgranular. Thick basophilic granules only rarely filled the cell interior. Single hepatocytes with more intensly acidophilic cytoplasm were scattered mainly in peripheral zone of the liver lobules. Hepatocytes mainly in peripheral zones were bigger than in the control group. More numerous and enlarged Browicz-Kupffer cells were found intended to the sinus lumen (Fig. 3).

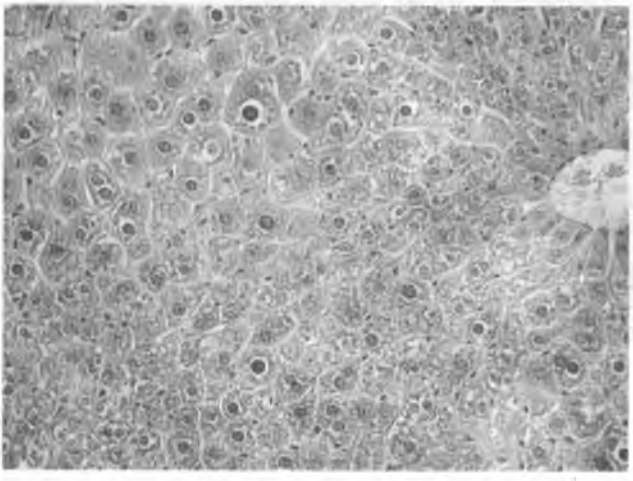

Fig. 3. Experimental group II – dexamethasone. Liver damage induced by toxic doses of dexamethasone. Increased chromatin condensation and decreased cytoplasm affinity for dyes with microgranular or homogenous appearance of the hepatocyte cytoplasm. H+E staining. Magn. 200x

Experimental group III – MK-801 + dexamethasone. 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 preserved, except regions with necrotic hepatocytes. In necrotic areas hepatocytes stained weakly pink with eosin. Their nuclei were round in shape and clear (they did not show affinity to hematoxylin). In some hepatocytes nuclei were invisible. In such regions the hepatocyte cytoplasm stained very weak pink and was homogeneous. The sinus lumen was filled with numerous red blood cells. Browicz-Kupffer cells were enlarged and much more numerous. In other regions hepatocytes were arranged in trabeculas. Their nuclei were round in shape and different in size. Numerous nuclei, especially bigger ones showed more condensed chromatin than in the control group. The number of binuclear hepatocytes in all the zones was also increased in comparison with the control group. A large number of hepatocytes had intensly acidophilic cytoplasm. These cells were much more numerous than in the case of dexamethasone itself. They formed clusters or trabeculae and were found in all the zones of liver lobules. They were more numerous in the vicinity of blood vessels. Their cytoplasm was microgranular or homogeneous. Other hepatocytes possessed microgranular, slightly basophilic cytoplasm. Numerous big fat droplets were visible in hepatocytes. Browicz-Kupffer cells were enlarged. Damaged blood vesells (central veins) with numerous red blood cells in the lumen were observed (Fig. 4).

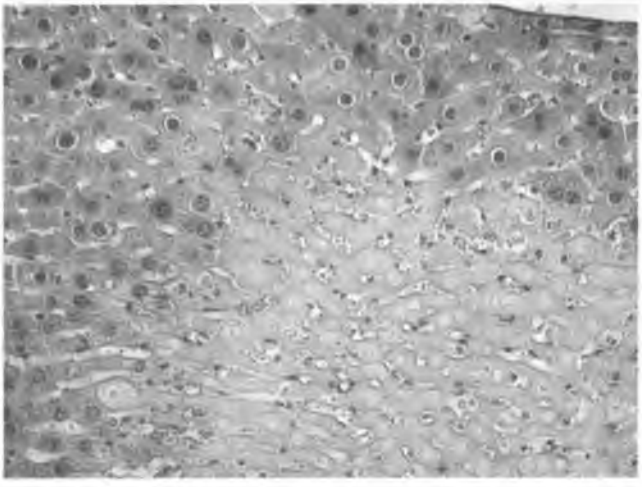

Fig. 4. Experimental group III –MK-801+dexamethasone. Hepatocytes with intensly acidophilic cytoplasm and focal liver necrosis (hepatocytes very weakly stained) are visible. H+E staining. Magn. 200x

DISCUSSION

The morphological changes revealed under the light microscope in the animals treated with MK-801 alone were characterized by an increased transparence of the hepatocyte cytoplasm, distinct narrowing of the sinus lumen and the activation of Browicz-Kupffer cells which intended into the sinus lumen. The changes observed under the light microscope show liver cell damage evoked by a pathogenic stimulus. Such a picture may be a result of vacuolar degeneration or accumulation of fats or carbohydrates within the hepatocyte cytoplasm (2). Examinations on the ultrastructural level are required to determine which of these processes is responsible for the observed changes. Vacuolation of the neuronal cytoplasm was observed in rats treated acutely with MK-801 (9). Vacuoles appeared to be enlarged neuronal ultrastructural elements (1). The more frequent occurrence of binuclear hepatocytes

after administration of MK-801 is regarded as one of the characteristic features of liver regeneration (4). The liver sinus region was also the place where the MK-801-induced changes appeared. Observed narrowing of the sinus lumen may indicate injury of the vascular pole of hepatocyte or endothelial cell damage. The more numerous and swollen Browicz-Kupffer cells are of great significance in the protective reactions of the organism. Browicz-Kupffer cells are macrophages evolving in the phagocytosis.

Histological changes observed after administration of toxic doses of dexamethasone were characterized by significantly smaller hepatocyte cytoplasm affinity to dyes, cytoplasm microgranulation and activation of Browicz-Kupffer cells. Dexamethasone is a synthetic glucocorticosteroid. The major site of its metabolism is the liver (8, 10). It is processed by a variety of enzymes, especially those related to the endoplasmic reticulum in order to transform the lipophilic and water-insoluble chemical into water-soluble product in preparation for its excretion in urine (6). Glucocorticoids can cause liver steatosis due to the increased fatty acids inflow (5). Toxic doses of dexamethasone used in our experiment caused evident liver damage. The increased number of binuclear hepatocytes is a sign of liver regeneration evoked by pathogenic stimulus.

The morphological changes observed in hepatocytes after concomitant administration of MK-801 and dexamethasone are characterized by: the increase of the nucleus size, a condensation of the chromatin especially in the big nuclei, a strong cytoplasm acidophilia in many hepatocytes and microgranular cytoplasm changes in others, by macrovesicular steatosis leading to focal necrosis and damage of the wall of blood vessels especially central veins with the activation of Browicz-Kupffer cells. Intensive cytoplasm acidophilia with numerous fine granules indicates damage of the rough endoplasmic reticulum and a large concentration of the mitochondria. Degranulation of the rough endoplasmic reticulum according to Popper (12) is connected with the decreased synthesis and secretion of the proteins by hepatocytes. More condensed chromatin also indicates smaller synthetic cell activity. Steatosis was more intensive in this group and led to the focal liver necrosis.

All the described changes indicate that MK-801 can induce morphological changes of the liver in the shape of decreased hepatocyte cytoplasm stainability and narrowing of the lumen of hepatic sinuses in some individuals. Comparing the influence of MK-801 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.

CONCLUSIONS

1. MK-801 administration in the dose corresponding to the neuroprotective dose used in human causes morphological changes of the liver in the shape of decreased hepatocyte cytoplasm stainability and narrowing of the sinus lumen in some individuals.

2. Dexamethasone administered in toxic doses causes morphological changes in hepatocytes characterized by increased chromatin condensation and decreased cytoplasm affinity for dyes with microgranular or homogeneous appearance of the cell cytoplasm in all individuals.

3. Comparing the influence of MK-801 itself and dexamethasone itself with the concomitant influence of both chemicals it may be concluded that MK-801 intensifies liver damage induced by dexamethasone leading to focal liver necrosis.

4. It seems needful to take subsequent examinations on the ultrastructural level.

REFERENCES

1. Auer R. N. et al.: The nature and time course of neuronal vacuolation induced by the N-methyl--D-aspartate antagonist MK-801. Acta Neuropathol. , 87, 1, 1994.
2. Groniowski J., Kruś S.: Podstawy patomorfologii. PZWL, Warszawa 1991.
3. Kostowski W., Pużyński S.: Psychofarmakologia doświadczalna i kliniczna. PZWL, Warszawa 1996.
4. Kruś S.: Patomorfologia wątroby. PZWL, Warszawa 1986.
5. Larson A. M.: Drugs and the Liver: Patterns of Hepatotoxicity. UpToDate, 2003.
6. Larson A. M.: Drugs and the Liver: Metabolism and Mechanism of Injury. UpToDate, 2003.
7. Muir K.W.: Clinical experience with excitatory amino acids antagonist drugs. Stroke, 26, 503, 1995.
8. Nieman L. K. et al.: Metabolism of Adrenal Steroids. UpToDate, 2003.
9. Olney J. W. et al.: Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science, 244, 1360, 1989.
10. Orth D. N: Structure-Function Relationships of Synthetic Glucocorticoids. UpToDate, 2003.
11. Park C. K. et al.: The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat. Ann Neurol., 24, 543, 1988.
12. Popper H.: Morphologic features of hepatocellular necrosis in human disease. In: Pathogenesis and mechanism of liver cell necrosis. Ed. D. Keppler, MPT, 2, 16, 1974.

SUMMARY

The aim of the research was histological assessment of the influence of MK-801 (NMDA receptor antagonist) and dexamethasone on the liver. The experiment was carried out on adult Albino-Swiss mouse males. MK-801 was administered in the dose of 0.3 mg/kg/24 h for 8 days, dexamethasone – in the toxic dose of 120 mg/kg/24 h. Liver slices stained with hematoxylin and eosin were examined with light microscope. The performed experiments revealed that MK-801 can cause morphological changes of the liver in the shape of increased transparence of hepatocyte cytoplasm and narrowing of the liver sinusoids. MK-801 intensifies liver damage induced by toxic doses of dexamethasone leading to focal necrosis of hepatocytes.

Ocena histologiczna wątroby po doświadczalnym podaniu MK-801 i deksametazonu

Celem pracy była ocena histologiczna wpływu MK-801 (antagonisty receptora NMDA) oraz deksametazonu na wątrobę zwierząt doświadczalnych. Badania wykonano na dorosłych samcach myszy Albino-Swiss. MK-801 podawano w dawce 0,3mg/kg/24h przez 8 dni, deksametazon w dawce toksycznej 120mg/kg/24h przez 8 dni. Przy pomocy mikroskopu świetlnego oceniano preparaty wątroby barwione hematoksyliną i eozyną. Przeprowadzone badania wykazały, że MK-801 może powodować zmiany morfologiczne wątroby w postaci większej przejrzystości cytoplazmy hepatocytów oraz zwężenia naczyń zatokowych wątroby. MK-801 nasila też uszkodzenie wątroby wywołane toksycznymi dawkami deksametazonu, prowadząc do ogniskowej martwicy hepatocytów.