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Histological examinations of the mouse gastric mucous membrane after experimental administration of MK-801 and dexamethasone

MK-801 (dizocilpine maleate) is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist with a high affinity to NMDA-receptor (4). The NMDA receptor antagonists constitute a promising perspective as neuroprotective agents. MK-801 is the first drug consistently shown to be neuroprotective *in vivo* (6,7). On the other hand, the side-effects of this chemical are serious and need to be considered before clinical use. No publications are avaliable describing the influence of MK-801 on the stomach morphology.

Dexamethasone is a synthetic glucocorticosteroid with a strong activity. Glucocorticosteroids are the most effective anti-phlogistic and immune suppressive substances known that have been used to treat various diseases for more than 50 years. Glucocorticosteroids administered in high doses make the gastric mucosa susceptible to damage and ulceration (1). Glucocorticosteroids are one of the risk factors leading to peptic ulcer disease. Administration of glucocorticosteroids to experimental animals results in gastric erosions and ulcers. It has been suggested that the mechanisms responsible for gastric mucosal damage induced by glucocorticosteroids include the inhibition of the synthesis of gastric mucus, enhancement of gastrin and parietal cell hyperplasia with augmented acid secretion, and possibly, suppression of arachidonic acid metabolism and prostaglandin synthesis. However, the true mechanisms of glucocorticosteroid-related mucosal damage are not well understood (5).

Lack of data regarding the influence of MK-801 on morphology of gastric mucous membrane inclined us to undertake this research. We decided to assess the influence of MK-801 administered separately and concomitantly with toxic doses of dexamethasone on the morphology of the gastric mucosa cells.

MATERIAL AND METHODS

The experiments were carried out on male Albino Swiss mice weighing initially 23–28 g. Care and treatment of the animals were in accordance with the guidelines for laboratory animals of the Local Ethical Committee, the Medical University of Lublin. The animals were kept under standard laboratory conditions, with free access to granular standard diet and tap water. 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 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 dose 0.3 mg/kg/24 h for 8 days. This dose corresponded to the neurprotective therapeutical dose used in human. MK-801 was administered 30 minutes prior to dexamethasone. Dexamethasone (Dexaven-Jelfa S A Poland)

was administered s.c. in a dose 120 mg/kg/24 h for 8 days. The dose of dexamethasone was toxic. Twenty-four hours after the last MK-801 or last dexamethasone injection all animals were decapitated. Specimens taken from the greater curvature of the stomach were fixed with 4% formaldehyde, dehydrated in graded ethanol solutions, cleared in xylene and embedded in paraffin. Seven-μm thick paraffin slices were stained with hematoxylin and eosin. The slides were assessed using a light microscope and pictures were taken with the use of the Carl Zeiss Jena microscope.

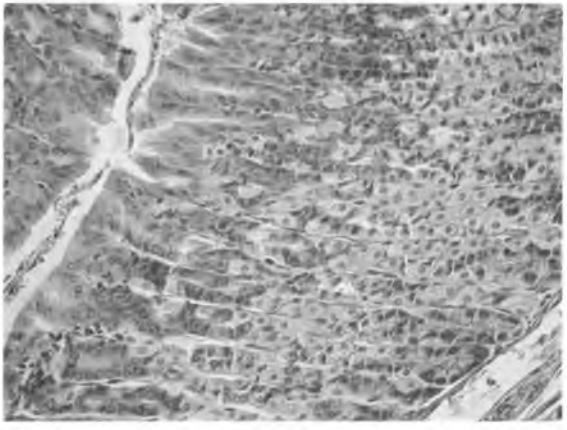

Fig. 1. The gastric mucous membrane of a mice from the control group. Basophilic chief cells are visible from the side of the muscularis mucosae. H+E staining. Magn. 200x

RESULTS AND DISCUSSION

In the group receiving MK-801 the surface mucous secreting cells were well preserved (Fig. 2). After MK-801 administration the number of chief cells and their affinity of taking stain were similar to the control animals. Parietal cells did not show morphological changes in comparison with the control group. Acidophility of the parietal cells which is connected mainly with the presence of mitochondria, was preserved after administration of MK-801.

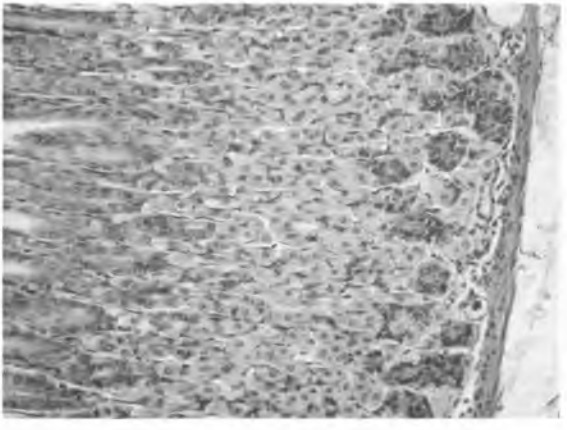

Fig. 2. The gastric mucous membrane of a mice from experimental group I (MK-801). The picture is similar to the control group. H+E staining. Magn. 200x

The most distinct changes in the group receiving dexamethasone were observed in the surface mucous secreting epthelial cells. After 8-day-long administration of toxic doses of dexamethasone these cells were damaged and the lamina propria was not covered with surface epithelium cells (Fig. 3). The surface of gastric mucosa was also devoid of mucus. It is likely that the lack of mucus layer induced by dexamethasone could be the result of down-regulation of gastric mucin gene expression in the nucleus and thereby decreased mucin biosynthesis (10). Physiologically mucus and surface epithelial cells constitute an important component of the barrier to acidic content of the stomach. Surface epithelial cells secrete mucus forming a thick layer that protects mucous membrane. Also, the tight junctions around surface cells form a part of the barrier to acid. Factors that disrupt surface epithelial cells cause gastric irritation and lead to ulceration. Our results indicate that dexamethasone administered in toxic doses causes evident damage of surface epithelial cell and weakens the defensive mechanism in the gastric mucosa. After dexamethasone administration the number of chief cells distinctly decreased. Chief cells were shrunken. They possessed pyknotic nuclei and condensed cytoplasm. These cells were evidently damaged. Such changes are typical changes indicating the decreased secretory cell activity. Parietal cells were numerous and seemed to be morphologically undamaged. Undamaged parietal cells are the source of hydrochloric acid that is the main factor responsible for damage of gastric mucosa. Previous studies demonstrated that dexamethasone may cause gastrin and parietal cell hyperplasia with augmented acid secretion (2). Lack of morphological damage to parietal cells after administration of dexamethasone seemed to be consistent with the results obtained by B a n d y o p a d h y a y et al. (1) showing that dexamethasone stimulates both basal and drug-induced gastric acid secretion. These results indicate also that dexamethasone makes the gastric mucosa susceptible to ulceration by inhibiting prostaglandin synthetase and peroxidase – two important gastroprotective enzymes. An inhibition of the activity of prostaglandin synthetase blocks the gastroprotective action of prostaglandin and an inhibition of the peroxidase elevates the endogenous H_2O_2 level generating more reactive hydroxyl radical responsible for the mucosal damage. Prostaglandin deficiency impairs gastric mucus production and epithelial cell proliferation as well as angiogenesis and thereby delay ulcer healing (6). Our study confirms the adverse effects of glucocorticosteroids on gastrointestinal tract described in many earlier reports (3, 7, 8).

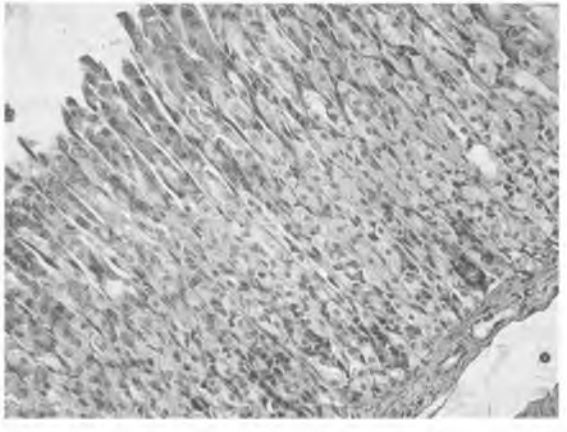

Fig. 3. The gastric mucous membrane of a mice from the experimental group II (Dexamethasone). A damage to surface epithelial cells and chief cells with the decreased number of chief cells are visible. H+E staining. Magn. 200x

The most distinct changes in the stomach mucosa we observed after the concomitant administration of MK-801 and dexamethasone (Fig. 4). Despite that they were similar to the

changes induced by dexamethasone itself, the concomitant administration of both drugs caused intensification of morphological damage to the stomach mucous membrane.

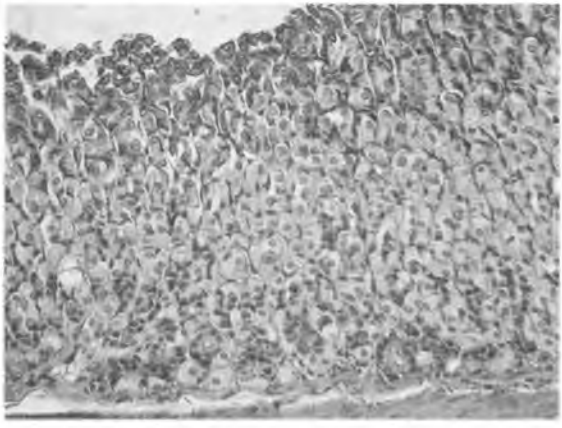

Fig. 4. The gastric mucous membrane of a mice from the experimental group III (MK-801+Dexamethasone). Distinct narrowing of mucous membrane thickness, lack of surface mucous secreting epithelial cells, atrophy of glandular cells, especially chief cells and defective arrangement of cells are visible. H+E staining. Magn. 200x

Completely damaged surface epithelial cells were observed in the distinctly thinner mucous membrane. The amount of chief cells was decreased and this cell type showed far reaching condensation of the nucleus and cytoplasm. This is an evidence of a small pepsinogen secretory activity. The arrangement of glandular cells was evidently changed. The cells were arranged irregularly. The decreased thickness of the mucous membrane and consequently the decline in the number of glandular cells are connected with degenerative processes. The flattening of gastric pits, irregular surface of the mucous membrane in many places and fibroplasia near to the muscularis mucosae also occurred. However, morphological changes (damage of the surface epithelial mucous secreting cells and reduced secretory activity of chief cells) were revealed after administration of dexamethasone itself, the concomitant administration of dexamethasone and MK-801 undoubtedly intensified damage of the mucous membrane leading to the evident atrophy of the stomach mucosa. The mechanisms that are responsible for the intensification of dexamethasone-induced gastric mucosa damage by MK-801 are unknown.

CONCLUSIONS

1. 8-day administration of MK-801 does not cause morphological changes in chief and parietal cells.

2. 8-day administration of dexamethasone in a dose of 120 mg/kg/24 h causes: damage to surface mucous secreting epthelial cells; decreased activity of chief cells; secretory activity of parietal cells seems to be preserved.

3. Concomitant administration of MK-801 and dexamethasone in the above doses intensifies toxicity and causes atrophical changes in the form of: decrease of the mucous membrane thickness; flattening of gastric pits and local damage of the mucous membrane surface; fibroplasia near to the muscularis mucosae.

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SUMMARY

The experiment was carried out on Albino Swiss mice weighting about 24–25g. Animals from experimental group I received MK-801 in a dose 0.3 mg/kg/24 h for 8 days. Experimental group II animals received dexamethasone in a dose 120 mg/kg/24 h for 8 days. Experimental group III animals received MK-801 and dexamethasone in the mentioned doses. After 24 h animals were decapitated and specimens of the stomach were taken from the greater curvature. It was stated that 8-day administration of MK-801 does not cause morphological changes of chief and parietal cells that indicates preservation of their secretory activity. Dexamethasone causes damage to surface mucous secreting epthelial cells and decreased activity of chief cells. Secretory activity of parietal cells seems to be preserved after administration of dexamethasone toxic doses. Concomitant administration of MK-801 and dexamethasone intensifies toxicity and damages the gastric mucous membrane causing its narrowing, flattening of gastric pits, damage to the surface epithelium, atrophy of secretory cells in the glands and fibroplasia near to the muscularis mucosae.

Histologiczne badania błony śluzowej żołądka myszy po doświadczalnym podawaniu MK-801 i deksametazonu

Badania wykonano na myszach Albino Swiss o masie ciała ok. 24-25 g. Zwierzęta I grupy doświadczalnej otrzymywały MK-801 w dawce 0,3 mk/kg/24 h przez 8 dni. Zwierzęta II grupy doświadczalnej – deksametazon w dawce 120 mg/kg/24 h przez 8 dni, zwierzęta III grupy doświadczalnej – MK-801 i deksametazon w wyżej wymienionych dawkach. Po 24 h zwierzęta dekapitowano i z okolicy krzywizny większej pobierano wycinki żołądka. Stwierdzono, że 8-dniowe podawanie MK-801 nie powoduje zmian morfologicznych w komórkach głównych i okładzi-

nowych, co wskazuje na zachowanie ich aktywności wydzielniczej. Deksametazon powoduje uszkodzenie powierzchniowych komórek nabłonka i zmniejszenie aktywności komórek głównych. Aktywność wydzielnicza komórek okładzinowych po podaniu toksycznych dawek deksametazonu wydaje się niezmieniona. Łączne podawanie MK-801 i deksametazonu wzmaga toksyczność i uszkadza błonę śluzową żołądka, powodując jej zwężenie, spłycenie dołeczków, uszkodzenie nabłonka powierzchniowego, zanik komórek wydzielniczych w gruczołach i fibroplazję od strony blaszki mięśniowej.