THE INFLUENCE OF GLUCOSAMINE DERIVATIVES ON APOPTOSIS OF CELLS IN EXPERIMENTS

Vetrova K.V., Davishnya N.V., Sakharova T.S., Shebeko S. K.
The National University of Pharmacy, Kharkiv, Ukraine
clinpharm@ukrfa.kharkov.ua

Today, apoptosis has an essential role in both physiological and pathological processes. In many cases, apoptosis is initiated by radiation, oxidative stress, viral infection, the influence of chemical agents including various medicines. However, in the literature there are reports on the possibility of regulation of apoptosis by various substances of synthetic and natural origin, in particular natural metabolites of the organism which include glucosamine.

The aim of our study was to investigate the effect of combinations of glucosamine derivatives with quercetin and ketoprofen on apoptosis of cells in experiments on rats.

The influence of combination of derivatives of glucosamine with quercetin on hepatocytes apoptosis was studied in doxorubicin-induced liver damage in rats. Experiment was carried out on 18 mongrel white rats weighing 200-250 g. Animals were divided into 3 groups with 6 animals: group 1 – intact, group 2 – control animals received single i.p. doxorubicin (DOX) at a dose of 10 mg/kg body weight on 8^{th} day of the experiment, 3 group were the rats that received combination aminosugars GA h/ch and N-acetylglucosamine with quercetin at a ratio of 3:1 in recolculation on GA h/ch (CA+Q) in conventionally-therapeutic dose of 82 mg/kg daily per os during 10 days. At the end of the experiment, tissue samples of liver were taken for further immunohistochemical study. Assessment of apoptosis in liver micropreparations was performed for quantification bcl-2 positive cells (containing the anti-apoptotic protein bcl-2) per 1000 hepatocytes and expressed by ‰.

It was found that administration of DOX in dose 10 mg/kg to rats leads to significant increased expression of the antiapoptotic protein bcl-2, as evidenced by increased number of bcl-2-positive cells with respect to the group of intact animals and agreed with the literature data. Although the antineoplastic antibiotic doxorubicin is inducer of apoptosis, such changes can be explained starting compensatory mechanisms of cell protection on administration of cytotoxic agent. Preventive administration of CA+Q to animals with DOX increase the number of bcl-2-positive hepatocytes and increased expression of anti-apoptotic protein bcl-2 relative to the control pathology group, and these changes were not statistically significant

difference. It was testifies to the mobilization of protective reserves of cells under the influence of CA+Q and causes anti-apoptotic effect in a cytotoxic lesion.

Also derivatives of glucosamine improves the metabolism of cartilage. These compounds are a substrate for the synthesis of glycosaminoglycans, stimulates the synthesis of proteoglycans. Despite the high chondroprotective activity of derivatives of glucosamine it should be noted the lack of efficacy of this group on anti-inflammatory and analgesic effect, which somewhat limits the possibilities of their use in patients with osteoarthritis (OA).

Today the most promising direction is the development of third generation chondroprotective drugs based on combinations derivatives of glucosamine with drugs of other groups (non-steroidal anti-inflammatory drugs (NSAIDs), vitamin, micronutrients, etc.). This can significantly extend the pharmacodynamics derivatives of glucosamine.

The resulting combination drug influences on the several pathogenic links of destructive-dystrophic lesions of cartilage. The study of the combination of glucosamine hydrochloride and ketoprofen as a topical dosage form was carried out on a model of systemic steroid osteoarthritis (SSOA) in rats. The object of the present research was glucosamine hydrochloride and ketoprofen combination in the form of a cream-gel (G\K cream-gel). Fastum gel 5%, glucosamine cream gel 5% were used as a comparison agents that were applied in a similar way with equivalent therapeutic dose of 50 mg. The 60 white male rats aged 4-5 months, weighing 250-300 g. had been used in the study.

The rats were divided into 5 experimental groups. Experimental osteoarthritis was reproduced by three times intramuscular injections in the thigh muscle of dexamethasone in a single dose of 7 mg / kg at intervals of 1 week. In the study of the ultrastructure of articular cartilage in rats standard methods of electron microscopy were used. In the study of the ultrastructure of rat articular cartilage pathology in the control pathology group were observed micropreparations expressed degenerative-dystrophic changes corresponding to the development of OA. Most micropreparations chondrocytes found in various stages of the so-called "dark-cell" death. Its main sign is full heterochromatization cell nucleus. Information from literature indicate that the described "dark" cells are exposed apoptosis. Unlike the control pathology group in ultrastructure of cartilage of animals treated with G\K cream-gel, were not observed "dark" cells, with signs of classical apoptosis.

The obtained results could detailed view of the mechanism of action of glucosamine derivatives, which are caused by the presence of a regulating effect on apoptosis.