

Morphological value of nephrotoxic effects of doxorubicin and PLGA-doxorubicin †

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Introduction. Doxorubicin (DOX) is a chemotherapy drug that causes nephrotoxicity in rodent models and, to a lesser extent, in cancer patients. Doxorubicin hydrochloride or doxorubicin - loaded poly(lactide-co-glycolide acid) (Dox-PLGA) nanoparticles at a therapeutic dose were injected intravenously male Wistar rats. PLGA is a biodegradable polymer used as highly efficient drug delivery systems. However, the therapeutic effect of Dox-PLGA is not clearly understood.

The aim of the study. To estimate a comparative assessment of the nephrotoxic effects two forms of doxorubicin.

Materials and Methods. A study was carried out on male Wistar rats weighing about 200-250 g (n=24). The animals were kept in an acclimatized room with conventional environmental conditions controlled for temperature (20 - 23 °C) and humidity with the use of a thermo-hygrometer and a photoperiod with timer, respecting the daily cycle of rodents, i.e., 12 hours/day and 12 hours/night. Food and water were available ad libitum. All experimental procedures were conducted in accordance to the Directive of the European Parliament 2010/63/EU. Morphological assessment of kidneys was performed using light and TEM microscopy.

On the 1st, 3rd and 5th day of the experiment a solution of doxorubicin hydrochloride (Teva, Israel) (n = 10) or PLGA-doxorubicin (doxorubicin in the composition particles PLGA), (LLC "Technology of medicines", Russia) (n = 10) in a therapeutic dose of 1.75 mg/kg were injected animals intravenously three times. The animals were sacrifice on the 8th and 21st days of the experiment with an overdose of 100 mg/kg of Zoletil (Virbac, France). The control group consisted of 4 intact rats.

Light Microscopy. Tissues were sectioned and fixed with 10% formalin Tissue-Tek VIP 5 Jr (Sakura, Japan). Embedded in paraffin Tissue-Tek TEC-5 (Sakura, Japan) and sectioned into 5 µm thick slices for histological examination Microm GmbH HM-340 (Thermo Scientific, Germany) hematoxylin and eosin (H&E) staining, the nucleus was colored blue with Mayer's hematoxylin, while the cytoplasm was colored red with eosin (Biovitrum, Russia). Tissues were sectioned at 5 µm and stained with periodic acid Schiff (PAS) and hematoxylin and eosin. One were observed under light microscope (Leica, Germany). The histological slides of kidney were evaluated for semiquantitative analysis. The ratio of all tubules to the number of damaged. Ultrastructural changes were studied using transmission electron microscope TEM Libra120 (Carl Zeiss, Germany). Comparison of digital data between experimental groups was performed using the Kruskal-Wallis test (ANOVA). Differences were considered statistically significant at p < 0.05.

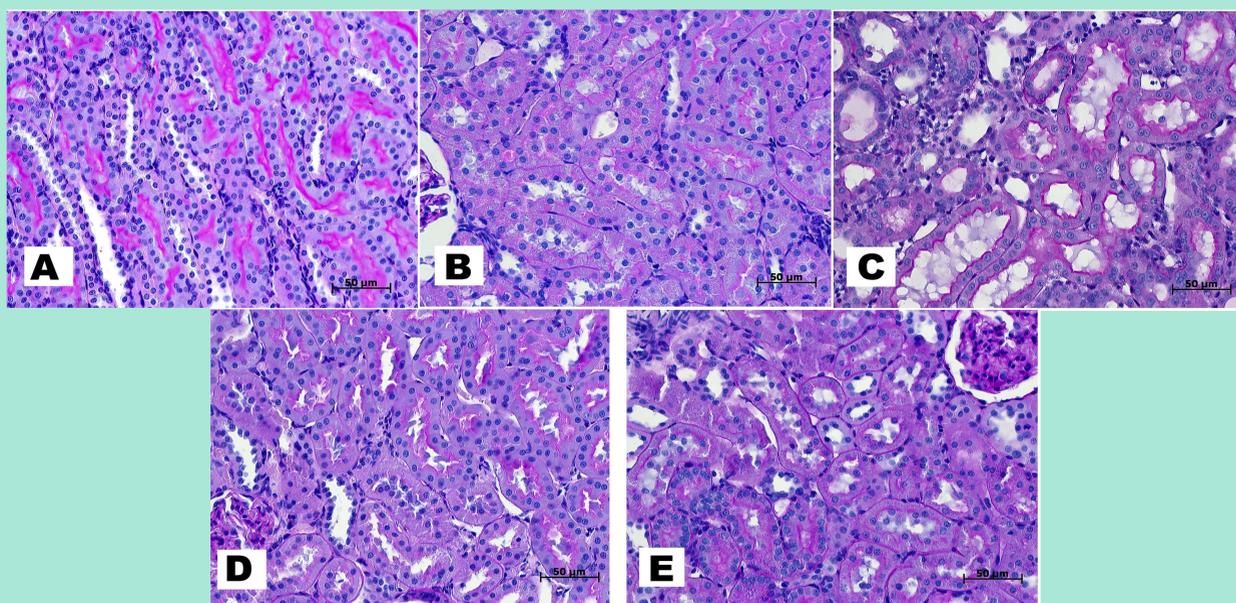


Figure 2. Proximal tubules of rats of control group (A), DOX 8 days (B), DOX 21 days (C), PLGA-doxorubicin 8 days (D), PLGA-doxorubicin 21 days (E). PAS- staining.

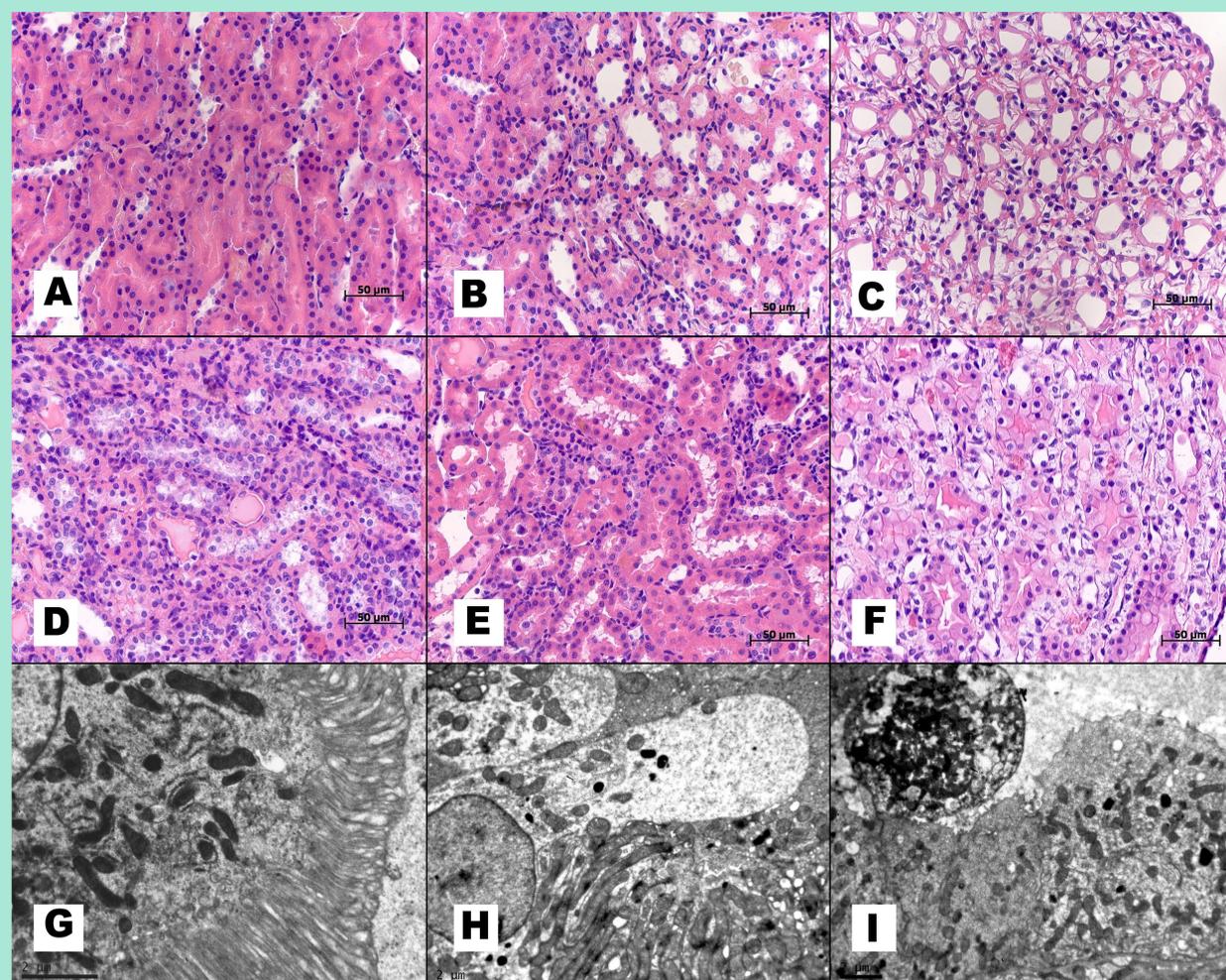


Figure 3. Proximal (left column), distal (middle column) nephron tubules and collecting ducts (right column) in control group (A-C) and after doxorubicin injection (D-I). A-F - histological sections, H&E staining; G-I - electron microscopy.

Conclusion: It was found that on the 8th day, PLGA-doxorubicin causes less pronounced degenerative changes in the epithelium of the proximal tubules. Use of the nanosomal form of PLGA-doxorubicin reduces the nephrotoxic effects of doxorubicin.

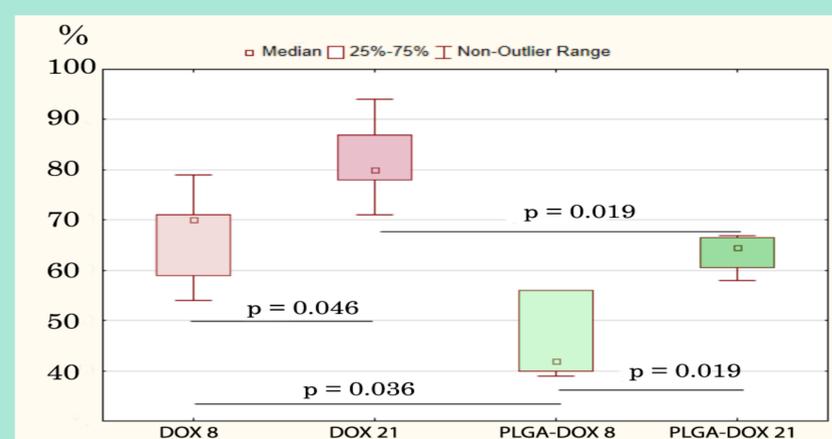


Figure 1. The percent of proximal nephron tubules with disrupted brush border in rats with doxorubicin and PLGA-doxorubicin injection on 8 and 21 days

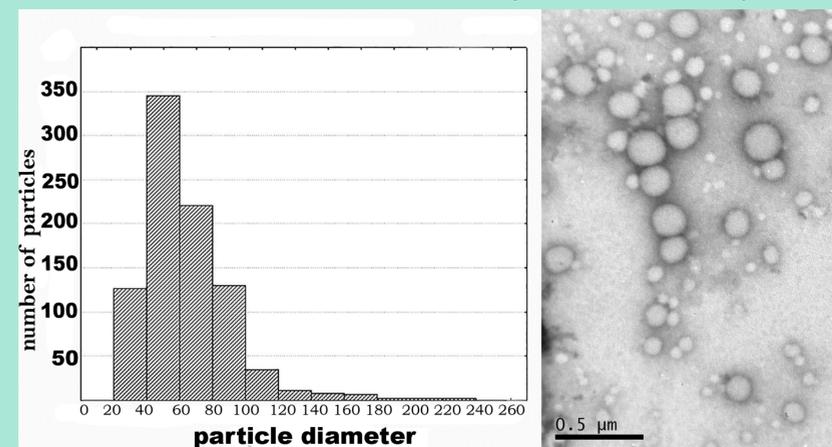


Figure 4. PLGA nanoparticles size distribution (n=886), TEM microscopy

Results and discussion. When exposed to doxorubicin and PLGA-doxorubicin in the kidneys on the 8th and 21st days of the experiment, degeneration changes in the proximal tubules with the destruction of the brush border are revealed, in the distal tubules and collecting ducts - protein casts, degeneration changes on the 21st day are more pronounced, than 8 days. During both periods of the experiment, PLGA-doxorubicin causes less pronounced degeneration changes in the epithelium than with the injection of doxorubicin, which is confirmed by the morphometric assessment (Fig 1) of the number of proximal tubules with a (Fig 2). When exposed to doxorubicin in both forms at both periods, the changes were unidirectional, most pronounced when exposed to doxorubicin hydrochloride on day 21 (Fig 3 D-I). Ultrastructural examination shows damage to the brush border of the proximal tubules, death of cells in the distal tubules and collecting tubules, filling their lumens with fibrous contents and fragments of dead cells (Fig 3 G-I).

Degeneration changes in the proximal nephron tubules with the destruction of the brush border were revealed by light and electron microscopy in the kidneys on the 8th and 21st days of the experiment after the doxorubicin hydrochloride injection and its nanosomal form Dox-PLGA (Fig 4).