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The Expression of Markers of Acute Kidney Injury Kim1 and NGAL after Administration of High Doses of Lithium Carbonate in Mice with Engrafted Skin Melanoma B16

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The expression of marker proteins of acute kidney injury after administration of high doses of lithium carbonate was assessed to evaluate the possibility of lithium use in neutron capture therapy. In mice with implanted skin melanoma B16, the expression of Kim1 (kidney injury molecule 1) and NGAL (neutrophil gelatinase-associated lipocalin) proteins in the kidneys was evaluated immunohistochemically 15, 30, 90, 180 min, and 7 days after peroral administration of lithium carbonate at single doses of 300 and 400 mg/kg. An increase in the expression of the studied proteins was found in 30 and 90 min after administration of 400 mg/kg lithium carbonate, however, 7 days after the drug administration, the expression returned to the level observed in the control group. It can be suggested that single administration of lithium carbonate in the studied doses effective for lithium neutron capture therapy will not significantly affect the renal function.

Key Words: *lithium carbonate; acute kidney injury; Kim1; NGAL; neutron capture therapy*

Boron neutron capture therapy (BNCT) is a binary radiation therapy based on the interaction of a non-radioactive ^{10}B isotope and a thermal neutron. The absorption of a neutron by boron leads to the nuclear reaction $^{10}B(n,\alpha)^{7}Li$ [1] and the release of 84% of the energy as a result of this reaction is limited by the size of one cell. Thus, the selective accumulation of boron-10 in the tumor cells and subsequent neutron irradiation should lead to the destruction of tumor cells with relatively small damage to surrounding healthy cells. Despite the promising results of BNCT clinical trials, there are a number of limitations to the widespread use of this technology in clinical practice [2,3]. Accumulation of optimal boron concentration in tumor cells, normal tissues, and blood is the one of the most important issues of BNCT [3,4]. Currently, despite the development of new tumor-targeting boron delivery agents, only second-generation boron compounds, such as boronophenylalanine and sodium borocaptate, are used in clinical trials [5,6].

The use of lithium instead of boron can be a new promising vector in the development of neutron capture therapy. Lithium has a number of physical characteristics that can provide a large thermal neutron absorption cross-section (940 barns) [1] and 100% local energy release inside the cell due to the high linear energy transfer of the reaction products. The use of most isotopes with a high thermal neutron absorption cross-section of more than 500 barns (such as ^{113}Cd, ^{135}Xe, ^{149}Sm, ^{151}Eu, Gd and some others), unlike lithium, will lead to an (n,γ) reaction and the absence of local energy release.

Lithium salts, in particular lithium carbonate (LC), are now widely used for the treatment of bipolar disorders [7,8], but they are associated with a high risk of side effects, in particular nephrotoxicity [9].

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The aim of this work was to evaluate the expression of markers of acute kidney injury after administration of high doses of LC to determine the possibility of using lithium in neutron capture therapy.

MATERIALS AND METHODS

We used mouse skin melanoma B16 cell line obtained at the Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences and C57BL/6 male mice weighing 20-22 g (age 10-12 weeks). Tumor cells (10^6) were subcutaneously implanted to mice into the inguinal area. Then, the animals were divided into groups (1 control and 10 experimental, 5 mice per group) depending on the administered dose of LC (300 or 400 mg/kg, once, in 30 μl of physiological NaCl solution *per os*) and the term of kidney sampling (15, 30, 90, and 180 min and

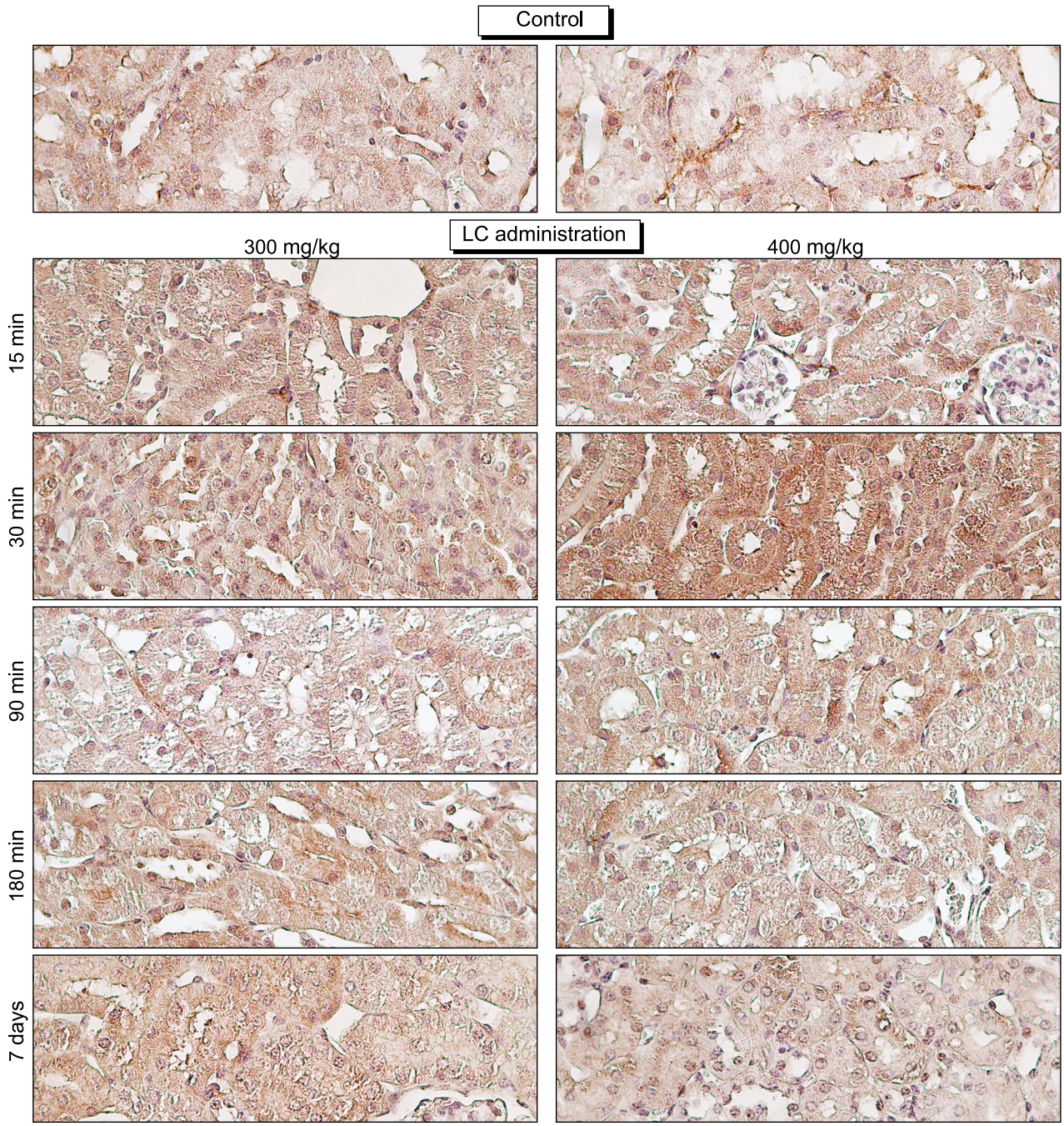

Fig. 1. Immunohistochemical staining of kidney sections for the marker of acute kidney injury Kim1 15, 30, 90, 180 min, and 7 days after peroral administration of LC at doses of 300 and 400 mg/kg, ×400.

7 days after LC administration). The mice were sacrificed by cervical dislocation and the kidneys were collected for the study. The experimental work was approved by the Ethics Committee of Research Institute of Clinical and Experimental Lymphology – a Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (No. 156, February 27, 2020).

For immunohistochemical analysis, paraffin-embedded kidney sections were dewaxed in a series of descending concentrations of alcohol), rehydrated, and heated in citrate buffer (pH 6.0) in a microwave oven for epitope retrieval. After blockade of non-specific binding, the sections were incubated with primary rabbit polyclonal antibodies to neutrophil gelatinase-associated lipocalin (NGAL; Cloud-clone corp.) and kidney injury molecule 1 (Kim1; Cloud-clone corp.) overnight at 4°C, washed with PBS 3 times for 5 min, incubated with the corresponding secondary horseradish peroxidase-conjugated goat anti-rabbit IgG antibodies (Abcam) for 1 h at 25°C. After hybridization, the sections were washed, counterstained with hematoxylin, dehydrated, and embedded in mounting medium. The images were captured with an Axio Scope.A1 microscope using an Axiocam 512 color CCD camera and ZEN 2.3 software (all – Carl Zeiss). Microscopic analysis was carried out at the Multiple-Access Center for Microscopy of Biological Subjects (Siberian Branch of the Russian Academy of Sciences).

Morphometry of digital images (10-15 visual fields in each group) obtained as a result of immunohistochemical staining was performed using ImageJ software. Stained kidney sections (10-15 fields) were assessed using an immunoreactive scale as described previously [10]. The intensity and percentage of positive staining of the cytoplasm were scored. Stained area: 0 (<1%), 1 (1-25%), 2 (>25-50%), 3 (>50-75%), and 4 (>75%); staining intensity: 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). The weighted scores for NGAL and Kim1 expression were calculated by the formula: area×intensity of staining.

The results were processed statistically using Microsoft Excel and Statistica 6.0 (StatSoft, Inc.). The mean (*M*) and standard deviation (*SD*) were calculated. The significance of differences between the studied

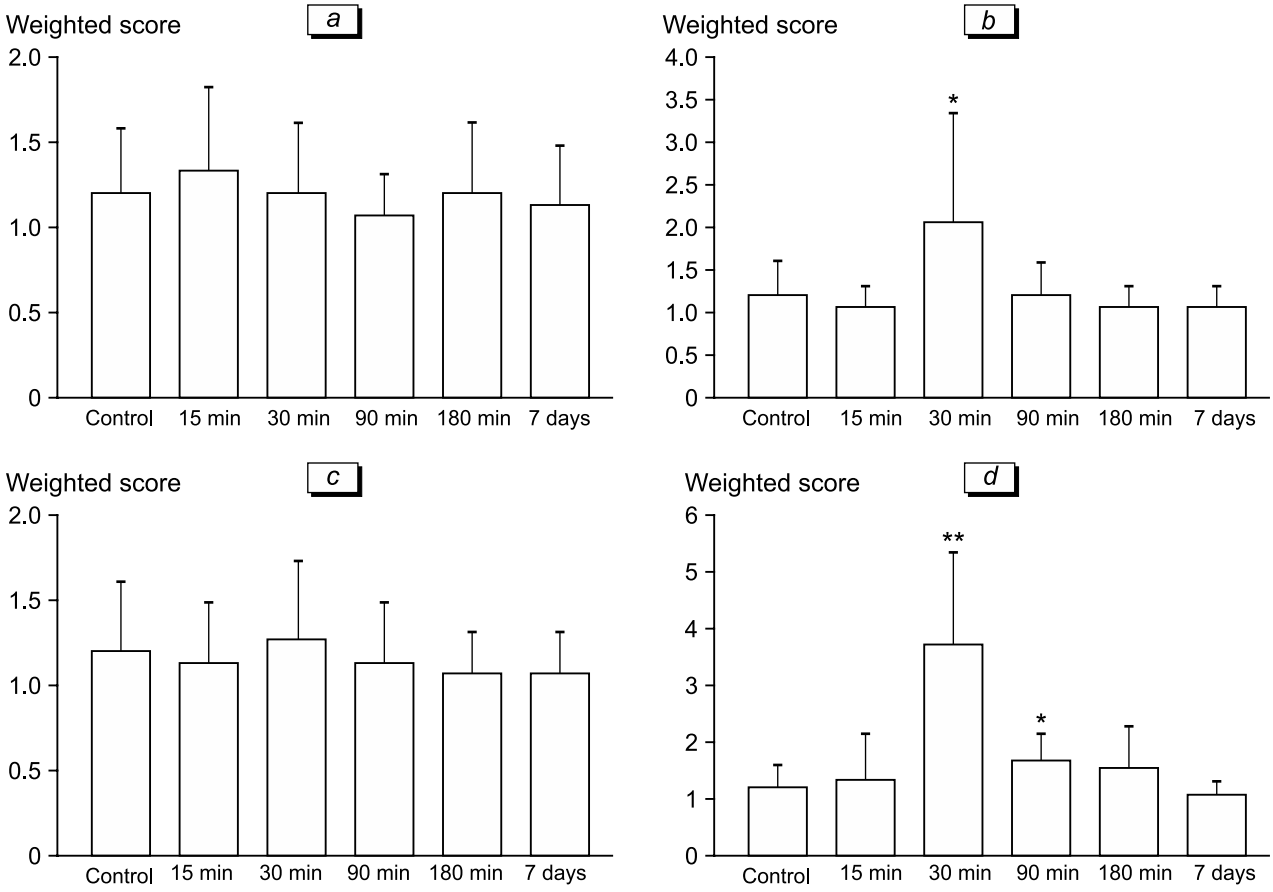

Fig. 2. The assessment of results of immunohistochemical staining of kidney sections for markers of acute kidney injury Kim1 (*a*, *b*) and NGAL (*c*, *d*) 15, 30, 90, 180 min, and 7 days after peroral administration of LC at doses of 300 and 400 mg/kg. *p<0.05, **p<0.0005 in comparison with the control group.

parameters was determined using the non-parametric Mann–Whitney *U* test at a 95% significance level ($p<0.05$).

RESULTS

Kim1 and NGAL markers were used to assess acute kidney injury after administration of high doses of LC. Kim1 is expressed by renal tubular epithelial cells upon injury and is a biomarker of nephrotoxicity [11,12]. The results of immunohistochemical staining of kidney sections for the Kim1 marker in groups are shown in Figure 1. We found no significant differences in the levels of expression of this protein in the group administered with LC at a dose of 300 mg/kg in comparison with the control group (Fig. 2, *a*). However, an increase in Kim1 expression (by 1.7 times) was noted 30 min after administration of LC at a dose of 400 mg/kg (Fig. 2, *b*). At later terms, Kim1 expression did not differ from the control group.

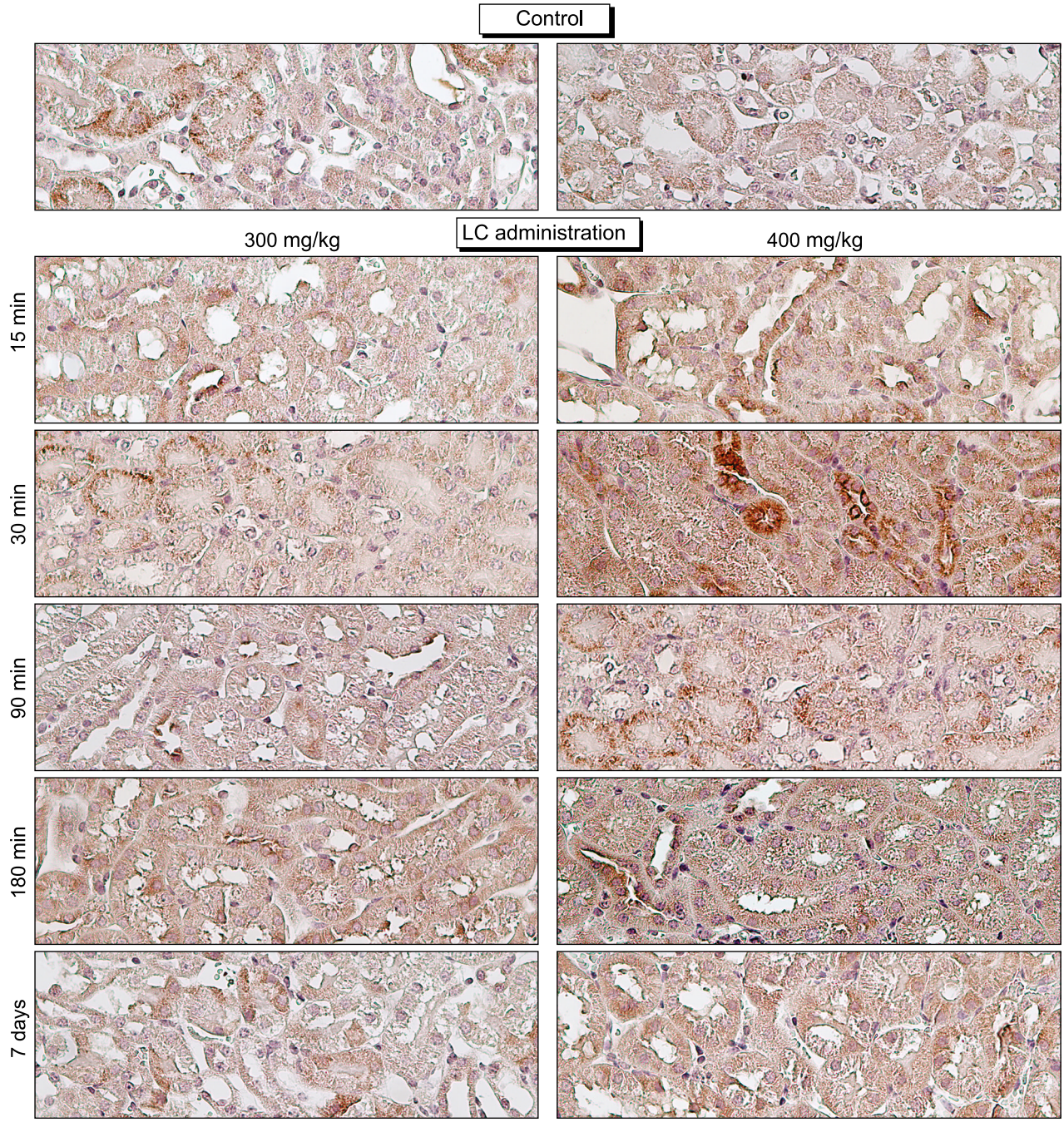

Fig. 3. Immunohistochemical staining of kidney sections for the marker of acute kidney injury NGAL 15, 30, 90, 180 min, and 7 days after peroral administration of LC at doses of 300 and 400 mg/kg, ×400.

NGAL is a member of the lipocalin superfamily proteins and is now considered as the earliest marker of acute kidney injury [12]. The results of immunohistochemical staining of kidney sections for the NGAL marker are shown in Figure 3. The expression of this protein after administration of LC at a dose of 300 mg/kg did not significantly differ from the control (Fig. 2, *c*), but increased by 3 times after 30 min and remained elevated by 1.4 times 90 min after the administration of LC at a dose of 400 mg/kg (Fig. 2, *d*). At later terms (180 min and 7 days), NGAL expression corresponded to that in the control group.

According to published reports, lithium salts are the drugs with a narrow therapeutic window; the relatively safe oral dose of lithium for humans is 450-1300 mg/day [13]. Lithium has a half-life of about 24 h, with peak levels in the range of 1-3 h after oral administration in human [7].

The kidneys are the main organ for lithium excretion, this process is highly variable and depends on age, body weight, and kidney function [13]. Among the most common and well-characterized side effects of lithium therapy is kidney injury leading to the so-called lithium-induced nephropathy.

Thus, despite the revealed increase in the expression of markers of acute kidney injury Kim1 and NGAL in mice 30 and 90 min after administration of LC at a dose of 400 mg/kg, this parameter decreased to values obtained in the control group 7 days after drug administration. Considering all of the above, as well as the lack of information on the effect of various lithium regimens (number of doses per day) on the incidence of renal failure [14], it can be suggested that single administration of LC at doses of 300 and 400 mg/kg for lithium neutron capture therapy will not significantly affect renal function.

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Conflict of interest. The authors have no conflicts of interest to declare.

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