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Effects of Lithium Nano-Scaled Particles on Local and Systemic Structural and Functional Organism Transformations Under Tumour Growth

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ABSTRACT

The results of a study of structural and metabolic changes in CBA mice with hepatocellular carcinoma caused by lithium carbonate nano-sized particles are presented. Light microscopy, electron microscopy and other biochemical methods were used to show that injection of lithium carbonate nano-sized particles to the periphery of the tumour results in enhanced destructive processes within the tumour. The number of neutrophils and macrophages in the tumour increased, whereas the density of blood vessels and haemoglobin concentration were reduced; the extent of tumour necrosis lipid peroxidation and production of nitric oxide was also increased. At the same time, the activity of antioxidant enzymes including superoxide dismutase and catalase remained the same. The introduction of lithium carbonate nano-scaled particles protects vital organs including the heart and lungs from the damaging effect of secondary products of lipid peroxidation.

Introduction

Hepatocellular carcinoma is one of the most aggressive human tumours. It is the fifth most common cancer and third highest in terms of mortality in the world (Pang and Poon, 2012; Shen and Cao, 2012). Standard medical treatments of hepatocellular cancer include surgical resection, ethanol or radiofrequency ablation (Zhang et al., 2009). Radiofrequency ablation and ethanol ablation are recognized as effective treatment for small encapsulated hepatocellular carcinomas with a diameter less than 3 cm. However, most patients have larger tumours at the moment of detection, and resection of tumours located near great vessels or the bile ducts is not performed.

It is rare for large tumours to respond to treatment with radiofrequency or chemical ablation, and it is almost impossible to secure whole ablation using these methods. During the late stage of disease, embolization (transcatheter arterial chemoembolization, TACE) can be applied, which is performed by the introduction of a chemotherapeutical drug into the hepatic artery (Tono et al., 2013). During this procedure, drugs, which block growth of blood vessels (Sorafenib, Avastin) (Lee et al., 2014), or drugs which affect the cell cycle and stimulate apoptosis of cancer cells (Doxorubicin, Cisplatin, 5-FU) (Kudo, 2012) are used. Although useful, chemotherapeutic drugs have a disadvantage: the development of side effects. Of note are the negative consequences of using cell cycle blocking drugs,

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particularly Doxorubicin, which causes numerous effects. These effects include cytotoxicity of the drug and its metabolites on liver cells (predominantly on hepatocytes), evident haemodynamic abnormalities in greater circulation (Nepomnyashchikh et al., 2006) and considerable toxic influence on other organ systems, specifically cardiovascular (Nepomnyashchikh et al., 2005).

The mechanistic effects of other drugs on tumour growth, including lithium drug, are also known. For example, lithium carbonate is used to enhance traditional thyroid cancer therapy (Tiuryaeva et al., 2010; Wolff et al., 2010) and as a drug contributing to restoration of marrow and blood constituents after chemotherapy. The following effects were noted: normalization of neutrophil content in the blood after radiotherapy and chemotherapy (Hager et al., 2001), restoration of platelet content in the blood (Hager et al., 2002), increased CD34+ cells in the blood during leukaemia (Canales et al., 1999) and enhanced cytokine production during breast cancer (Merendino et al., 1994). There are data on the use of lithium carbonate as a neuroprotective agent for cancer patients; its purpose is to increase quality of life while saving cognition, improving their emotional state (Yang et al., 2007; Khasraw et al., 2012) and preventing peripheral neuropathy development during aggressive courses of chemotherapy (Mo et al., 2012). Recent research has been conducted showing the efficiency of lithium as an agent for tumour growth suppression (Wang et al., 2008; Zhu et al., 2011). Lithium compounds are regarded as potential agents of target therapy, capable of slowing tumour growth. At the same time, with the development of nanotechnology, new, more innovative features of nanoscale structures are being revealed (Golokhvastov et al., 2013). In previous research we revealed biological effects of lithium carbonate nano-scaled particles during their introduction to intact animals (Bgatova et al., 2012). The purpose of this work was to study the influence of lithium carbonate nano-scaled particles on structural and metabolic changes in CBA mice with hepatocellular carcinoma development.

Methods

Experiments were performed on CBA line male mice from the Institute of Cytology and Genetics SB RAS. Mice weighted 18–20 g and were three months of age. Work with animals was performed according to the principles of humanity stated in directions of EC (86/609/EEC) and Declaration of Helsinki.

To model the tumour process, we used hepatocellular carcinoma-29 (H-29) cells. This tumour can cause considerable decrease in its carriers' body weight and evident symptoms of cachexia. Hepatocellular carcinoma-29 was generated and verified by employees of the Institute of Cytology and Genetics SB RAS and kindly granted for our research (Kaledin et al., 2009). H-29 cells were transferred to the abdominal cavity of CBA line mice. After 10 days, we made intake of ascitic fluid, slurried in 10-fold volume of saline and injected in 0.1 ml into intact animals' right thigh muscle. To study the influence of inorganic nano-scaled particles on tumour development we injected lithium carbonate nano-scaled particles in doses of 0.037 mg per animal once or five times after induction of tumour growth. We made an intake of material on 3, 7, 13 and 30 days after injection of tumour cells. Animals were taken out of experiment under etheric narcosis by cranio-cervical dislocation. We took five animals for each stage of the research.

We took biological samples for light optic research from thigh muscular tissue, regional inguinal lymph node, kidney, liver, hepatic lymph node, hepatocellular carcinoma-29 cells and from ascitic fluid. These samples were fixed in 10% solution of neutral formalin, dehydrated with a number of alcohols with increasing concentration and placed into paraffin. Sections 5–6 μm thick were coloured with Mayer's haematoxylin and eosin and placed into Canada balsam.

To study biological samples using the electron microscope's translucent mode, we fixed them in 1% solution of OsO_4 on phosphate buffer ($pH = 7.4$), dehydrated them using increasing concentrations of ethanol and placed into Apon. From derived blocks, we prepared semi-fine sections 1-μm thick, coloured them with toluidine blue and studied them under a light microscope, choosing the tissue areas to further study using an electron microscope. From selected material, we obtained ultrathin sections 35–45 nm thick using ultratome LKB-NOVA. We contrasted these sections with a saturated water solution of uranyl acetate and lead citrate. We then studied the sections using an electron microscope JEM 1010.

Derived microphotos were morphometrized using Image J software. Digital data were processed using generally accepted statistical methods. We calculated arithmetic mean (M), mean sample error (m) and significance level of distance between mean values (p), based on Student's test for confidence level 95% ($p < 0.05$).

Muscular tissue damage degree was estimated by intensity of lipid peroxidation processes. For determination of lipid peroxidation activity, we homogenized samples of right thigh muscular tissue in cold conditions in 2 ml of 0.85% NaCl water solution, which contained 0.1% EDTA, using a Potter homogenizer. Then, we centrifuged samples for 15 min at 4000 rpm. We determined the activity of lipid peroxidation in homogenates by determining the concentration of reaction products of thiobarbituric acid (TBA) (Volchegorsky et al., 2000). The concentration of TBA-active products was estimated at the wavelength of 532 nm and expressed in micromole/kg, considering molar extinction coefficient equal 1.56×10^5 mol^{-1} cm^{-1}. For efficiency estimation of tissue protection from products that can initiate and intensify lipid peroxidation, we studied the state of antioxidant system's enzymatic link by evaluating the level of catalase and superoxide dismutase (SOD) activity.

The function of catalase is to prevent the accumulation of hydrogen peroxide. Hydrogen peroxide is generated during dismutation of superoxide anion and aerobic oxidation of flavoproteins. SOD catalyses dismutation of superoxide radicals, thereby preventing pathogenic effects of reactive oxygen species. Enzymatic reactions can generate low levels of superoxide anion and hydrogen peroxide H_2O_2, which usually are not able to initiate directly lipid peroxidation processes. However, as a result of a numerous consecutive reactions with enzymes and metal ions of variable valence, highly reactive compounds possessing energy can be formed, which can result in C – H-bond breakage and primary lipid radicals' formation.

Catalase activity was estimated by the ability of hydrogen peroxide to make a stable dyed complex with molybdenum salts. Measurements were conducted at a wavelength of 410 nm and expressed in U/100 mg of tissue, considering hydrogen peroxides millimolar extinction coefficient equal 22.2×10^3 $mmol^{-1}$ cm^{-1}. Next, we determined SOD activity in tissue homogenates by the ability of