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A POSSIBLE MECHANISM OF ANTITUMOR ACTIVITY OF 5-(5',6'-BENZOCOUMAROYL-3')-METHYLAMINOURACIL *IN VIVO*

H.P. Kopylchuk, I.O. Shmarakov, M.M. Marchenko*
Department of Biochemistry, Y.Fedkovych Chernivtsy National University, Chernivtsy, Ukraine

Aim: **To analyze possible mechanisms of anticancer activity of hydrobromide 5-(5',6'-benzocoumaroyl-3')-methylaminouracil (BCU) *in vivo*.** ***Methods:*** **BCU was administered at the dose of 6 mg/kg for seven days to rats bearing Guerin carcinoma (Gc). Interaction of BCU with DNA isolated from Gc cells was analyzed by electrophoresis and spectrophotometry.** ***Results:*** **It has been shown that BCU administration resulted in chromatin condensation, nuclei picnosis, increase of the DNase activity and the appearance of high-molecular DNA fragments in Gc cells.** ***Conclusion:*** **BCU is shown to be able to interact directly with DNA molecules with the formation of stable complexes.**
Key Words: **hydrobromide 5-(5',6'-benzocoumaroyl-3') methylaminouracil, Guerin carcinoma, DNA.**

Modern research of novel antitumor drugs is focused on the study of molecular mechanisms of their cytostatic activity and the prevention of toxic effects. One of such substances, mechanisms of which action need a thorough research is hydrobromide 5-(5',6'-benzocoumaroyl-3')-methylaminouracil (BCU). As we have shown earlier, BCU possesses potent anticancer activity [1–3]. The structure and properties of BCU allow to predict its ability to cause degradation of DNA in tumor cells. That's why the aim of the present work was to study the effect of BCU administration on the stability of nuclear DNA of tumor cells *in vivo*.

The research was performed on 3-month old female white inbred rats (n = 150) weighting 110 to 130 g. Guerin carcinoma (Gc) was used as experimental tumor model. The strain of the tumor was kindly granted by R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine. All animal procedures were performed according to the rules of local ethic committee. Animal's euthanasia was made under light ether anesthesia.

Gc cells were routinely transplanted subcutaneously in hip area of each animal. The animals were divided into the groups: group I (experimental) – rats that received *per os* BCU at the dose of 6 mg/kg body weight every day from 7-th to 21st days after Gc transplantation; group II (control) — Gc-bearing animals without treatment.

At the 7-th, 14-th and 21-st days after Gc transplantation (responding to latent, logarithmic and exponential stages of tumor growth), the samples of tumor tissue were isolated, fixed in 10% formalin, and the slides were routinely prepared, stained by hematoxylin and eosin [4], and examined by light microscopy (magnification 100X).

Nuclear fraction of Gc cells was isolated by the method [4]. Acidic and alkaline DNases activity was determined by the degree of chromatin hydrolysis. The degree of chromatin hydrolysis was calculated by the number of polydeoxyribonucleotides (PDN) of the acid-soluble fraction, received after the incubation of the nuclear fraction in corresponding buffers for 10, 20 and 30 min at 37 °C and expressed in percentage of the total DNA in incubation mixture [3]. The significance of the differences between the data was evaluated by regression analysis [5].

DNA was isolated by the standard phenol : chloroform : isopropanol extraction method and analyzed by electrophoresis in 1% agarose gel [6]. Gels were scanned with the help of "GelDoc 2000" apparatus and analyzed using the program "Quantity One" (Bio-Rad, USA).

Complexes of BCU with high molecular DNA isolated from Gc cells at the 7th day were received *in vitro* by mixing equimolecular solutions of BCU and DNA (concentration of 10^{-4} mol/l) in 0.01 mol/l NaCl; the solution was kept at room temperature and constant stirring for 2 h. The ability of BCU to interact with the DNA was evaluated by UV-spectrophotometry at the wave length of 220–340 nm [7]. The capacity of the BCU-DNA complex was determined graphically by alteration of ΔD value dependent on the concentration of the added ligand.

The differences between the indexess were assessed using Student's *t*-criterium [5].

The study of BCU-treated Gc-bearing animals has shown that in Gc cells nuclei the increase of DNase activity and DNA fragmentation could be observed. BCU administration caused the increase of acidic and alkaline DNase activities in tumor nuclei already from the beginning of BCU administration (Fig. 1). However, its administration did not change the general tendency of increase of DNase II activity in tumor cells of the control group (Fig. 1, *a*), which is caused by intensified proliferation of tumor cells and active role of the enzyme in replication and reparation processes [3]. In contrast to that, positive correcting effect of BCU administration was manifested by the increase of alkaline DNase activity during the logarithmic period of tumor growth, which is characterized by the decrease of DNase I activity in the control group (Fig. 1, *b*).

Thus, BCU action on DNase activity resulted in the simultaneous increase of acidic and alkaline DNase

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*Correspondence: igor_shmarakov@mail.ru
Abbreviations used: BCU – hydrobromide 5-(5',6'-benzocoumaroyl-3') methylaminouracil; Gc – Guerin carcinoma; PDN – polydeoxyribonucleotide.

activities, pointing on the absence of its specificity toward mentioned enzymes. Possibly, BCU, being an antimetabolite [1], can interact with DNA causing the formation of DNA complexes or single- and double strand breaks etc.

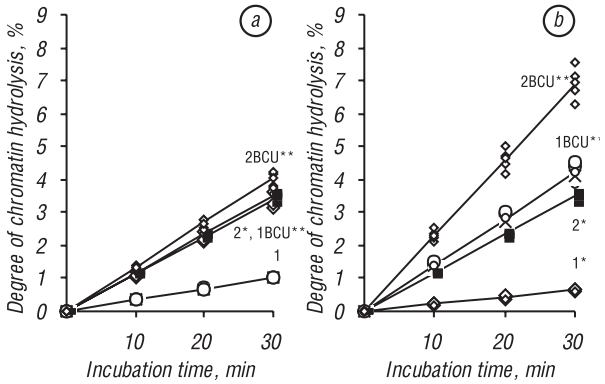

Fig. 1. PDN content in the nuclear fraction of Gc cells of experimental animals and acidic (*a*) and alkaline (*b*) DNases activity. Notes: 1, 2 — 14, 21 days after Gc transplantation accordingly; $*p < 0.05$ compared with 14th day; $**p < 0.05$ in compared with control group.

Also, it has been revealed that BCU administration leads to DNA fragmentation in Gc cells and appearance of high-molecular DNA fragments (10–50 kb) (Fig. 2), typical for apoptosis [8–9]. One could not exclude that BCU as some other anticancer drugs [10] may act through in the inhibition of topoisomerases.

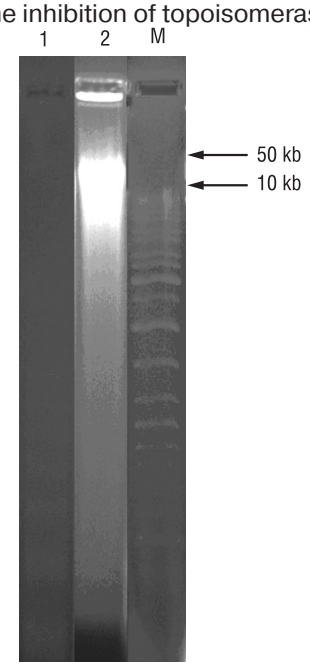

Fig. 2. Electrophoregrams of the fragmented nuclear DNA of Guerin carcinoma of control (lane 1) and experimental (lane 2) groups. M — marker preparation Gene Ruler™

Gc tissue slides examined by light microscopy have demonstrated the changes in the architectonics of tumor tissue (Fig. 3). In the tumor cells from Gc isolated at the 14-th day of the experiment of Gc-treated animals, chromatin condensation and nuclei picnosis could be seen (Fig. 3, *b*). Morphology characteristic to tumor cells was observed on the slides of Gc tissue of the control group: disproportional large-size nuclei could be viewed occupying all the inner contents of the cell, with diffuse chromatin (Fig. 3, *a*).

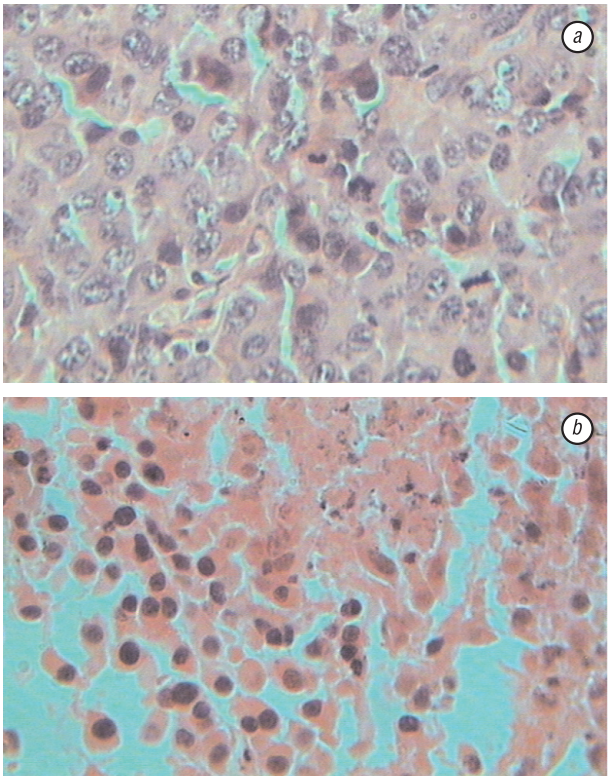

Fig. 3. Guerin carcinoma tissue slides: control (*a*) and experimental (*b*) groups

To test the assumption on a possible direct interaction of BCU with nuclear DNA, we performed a spectroscopic *in vitro* examination of mixtures of DNA and BCU. The analysis of UV-absorption spectra allows asserting the existence of interaction between BCU and DNA molecule *in vitro* by the appearance of isobestic point [11] on the diagrams of absorption spectra (Fig. 4). The existence of one isobestic point shows single type of interaction [11]. Taking into account that BCU has a flat heterocyclic structure and a non-electrophilic composition [1], the centers of its binding are DNA bases [12, 13]. For verification of this suggestion, differential spectra of the DNA and BCU solutions were studied (Fig. 5). In our experiment the differential curves are located under the abscissa and have a sinusoidal shape allowing to state that BCU interacts with the bases by intercalation, but not ruining DNA double-helical structure [14]. Also, the BCU-DNA complexes possess a significant stability and poorly dissociate (data not shown).

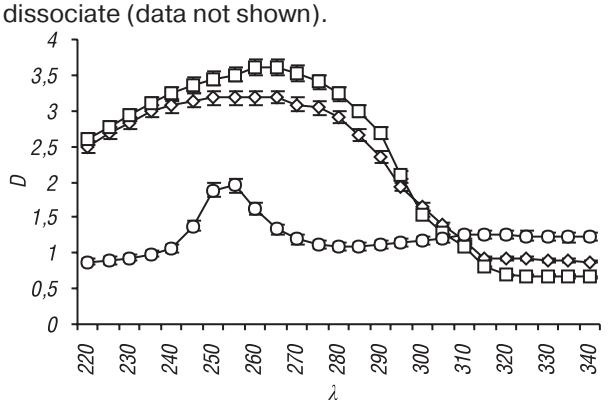

Fig. 4. UV-spectra of BCU solutions (•), Guerin carcinoma nuclear DNA (◊) and BCU-DNA equimolar mixture (■)

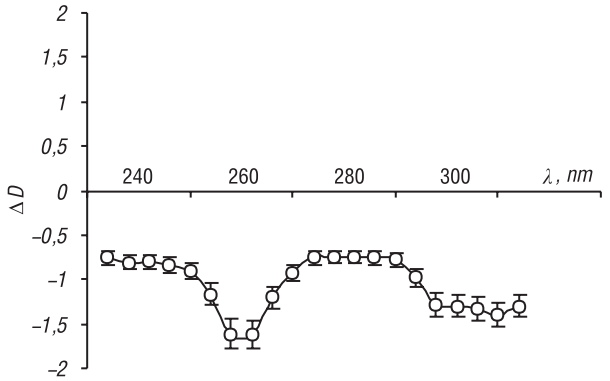

Fig. 5. Differential absorption spectra of DNA solutions in the presence of BCU

So we have shown that administration of BCU causes chromatin condensation and appearance of high-molecular DNA fragments in tumor cell nuclei.

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ВОЗМОЖНЫЙ МЕХАНИЗМ ПРОТИВООПУХОЛЕВОЙ АКТИВНОСТИ 5-(5',6'-БЕНЗОКУМАРОИЛ-3')-МЕТИЛАМИНОУРАЦИЛА *IN VIVO*

Цель: **проаналаизировать возможные механизмы противоопухолевой активности гидробромида 5-(5',6'-бензокумариол-3')-метиламиноурацила (BCU) *in vivo*. *Методы:* BCU вводили в дозе 6 мг/кг в течение 7 дней крысам с трансплантированной карциномой Герена (КГ). Взаимодействие BCU с ДНК, выделенной из клеток КГ, анализировали методами электрофореза и спектрофотометрии. *Результаты:* установлено, что введение BCU приводит к конденсации хроматина, пикнозу ядер, повышению ДНКазной активности и появлению высокомолекулярных фрагментов ДНК в клетках КГ. *Выводы:* BCU может прямо взаимодействовать с молекулами ДНК с образованием стабильных комплексов.**

Ключевые слова: **гидробромид 5-(5',6'-бензокумарил-3')-метиламиноурацила, карцинома Герена, ДНК.**