СУПРАМОЛЕКУЛЯРНЫЕ КОМПЛЕКСЫ НА ОСНОВЕ КУКУРБИТ[7]УРИЛА И СОЕДИНЕНИЙ ПЛАТИНЫ ВЛИЯЮТ НА ЭКСПРЕССИЮ МОЛЕКУЛЫ CTLA-4 НА Т-РЕГУЛЯТОРНЫХ КЛЕТКАХ

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Резюме. Опухоли занимают лидирующее место по частоте встречаемости в популяции. Не все противоопухолевые лекарственные препараты первой линии позволяют адекватно и эффективно излечивать пациентов. Для некоторых препаратов, например, цитостатиков, характерны широкий спектр побочных эффектов и резистентность опухолей к проводимой ими терапии. На сегодняшний день описаны механизмы действия таких препаратов и предполагаются наиболее вероятные причины резистентности. Для минимизации побочных эффектов и преодоления резистентности используется система доставки лекарственных препаратов на основе кукурбит[7]урила (CB[7]), которая образовывает супрамолекулярные комплексы с оксалиплатином и карбоплатином.

Важно принимать во внимание, что большой вклад вносит иммунная система, соединения платины способны оказывать иммуномодулирующее действие на иммунокомпетентные клетки и все больше данных говорит о том, что цитотоксический ответ в отношении опухолевых клеток связывают и с этими свойствами. Опухоль создает специфическое микроокружение, в котором сосредотачивается огромное количество супрессорных клеток. FoxP3$^+$Т-регуляторные клетки рекрутируются опухолью, увеличенное количество этих клеток и повышенные уровни экспрессии CTLA-4 и PD-1 способствуют прогрессированию опухолевого процесса. Данные показатели коррелируют с плохой выживаемостью пациентов. Поэтому необходимо, чтобы противоопухолевые агенты обладали влиянием на данную субпопуляцию клеток и их функциональную активность. В данном исследовании оценивалось влияние кукурбит[7]урила, соединений платины и супрамолекулярных комплексов на регуляторные Т-клетки и экспрессию молекул иммунных контрольных точек.

В исследовании использовались клетки периферической крови условно здоровых доноров (n = 8, средний возраст 29,0±2,4). Полученные стандартным путем мононуклеары инкубировали 72 часа в концентрациях 0,3 мМ и 0,1 мМ для карбоплатина и оксалиплатина соответственно, а также комплексами и CB[7] в эквивалентных дозировках, затем пробы окрашивали моноклональными антителами для определения фенотипа и экспрессии иммунных чекпоинт-молекул.

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Мы получили следующие результаты: комплекс CB[7]-карбоплатин в стимулированной и нестимулированной культурах достоверно снижал количество FoxP3+Т-регуляторных клеток по сравнению с контролем. При этом карбоплатин и комплекс CB[7]-карбоплатин снижали экспрессию CTLA-4 в нестимулированной культуре по сравнению с CB[7].

Комплексы кукурбит[7]урилов с соединениями платины являются перспективным противоопухолевым средством с иммуномодулирующими свойствами.

Ключевые слова: кукурбитурилы, макроциклические комплексы, карбоплатин, оксалиплатин, Т-регуляторные клетки, контрольные точки иммунного ответа, проточная цитометрия

CUCURBITURIL-BASED SUPRAMOLECULAR COMPLEXES WITH PLATINUM COMPOUNDS INFLUENCE EXPRESSION OF CTLA-4 ON REGULATORY T CELLS

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Abstract. Tumors are a leading pathology in the population. Chemotherapy cannot provide adequately and effectively to cure patients. Some medicine, such as cytostatic, are characterized by a wide range of side effects and resistance of solid tumors to chemotherapy by these medicines. In recent research, the mechanisms of action of cytotoxic agents have been described, and the most appropriate causes of resistance have been suggested. Drug delivery system based on Cucurbit[7]uril (CB[7]) was used to minimize side effects and overcome resistance. CB[7] has ability to form host-guest supramolecular complexes with oxaliplatin and carboplatin.

It is important to consider the immune system maintain to a great role, and platinum compounds are able to have an immunomodulatory effect on immunocompetent cells. There is convincing evidence about the cytotoxic response against tumor cells is also associated with immunomodulating properties. A specific immune microenvironment with high frequency of suppressor cells is made by tumors. FoxP3+ regulatory T cells are recruited by the tumor, an increased number of these cells and expression levels of CTLA-4 and PD-1 on them contribute to the progression of the tumor process. These markers correlate with recurrence and poor survival of the patients. Therefore, it is necessary that antitumor therapy agents have an effect on a subpopulation of regulatory T cells and their functional activity. This study evaluated the effects of cucurbit[7] uril, platinum compounds, and supramolecular complexes on FoxP3+ regulatory T cells and the expression of immune checkpoint molecules.

In this study peripheral blood cells from volunteers (n = 8, average 29.0±2.4) were used. Mononuclear cells obtained in the standard protocol were incubated for 72 h at concentrations of 0.3 and 0.1 mM for carboplatin and oxaliplatin, respectively, as well as complexes and CB[7] in equivalent dosages. Next, the samples were labeled with monoclonal antibodies to determine the phenotype and expression of immune checkpoint molecules by flow cytometry.

We obtained the following results: The CB[7]-carboplatin complex in stimulated and non-stimulated cultures significantly reduced the number of FoxP3+ regulatory T cells compared to the control. At the same time, carboplatin and the CB[7]-carboplatin complex reduced the expression of CTLA-4 in an non-stimulated culture compared to CB[7].

Complexes of Cucurbit[7]urils with platinum compounds are a perspective antitumor agent with immunomodulatory properties.

Keywords: cucurbiturils, macrocyclic complexes, carboplatin, oxaliplatin, Tregs, immune checkpoint molecules, flow cytometry

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Introduction

Nowadays tumors are the leading pathology among the population. There are many treatment regimens for different tumors, including those based on platinum agents (drugs include cisplatin, oxaliplatin, carboplatin, etc.). However, platinum agents have a lot of specific and common side effects from hematotoxicity, ototoxicity to neurotoxicity, cardiotoxicity, and there are some tumors as ovarian cancer, melanoma, and colon which resistant to platinum treatment [2, 6]. There are several mechanisms of resistance, and an important role is

played by the low accumulation of platinum in cells and detoxification components, which don't allow to bind DNA by platinum. Multiple transporters for the active transport of platinum-based antitumor agents also may be responsible for platinum resistance [13]. Therefore, it is necessary to isolate platinum from enzymes and use drug delivery system to accumulate platinum into cells. CB[7]-based drug delivery system was used for this. Cucurbiturils are nanomolecular system for binding with cations, small peptide etc. with forming host-guest complexes. CB[7] is known to protect the drugs from biodegradation [5] and to cross the cell membrane [7]. The mechanisms of cellular cytotoxicity for a large amount of antitumor drugs have been well described. Also, the effects of cytostatic against cells of the immune system have been characterized. For example, oxaliplatin selectively depleted the pool of myeloid suppressors, directed the differentiation of myeloid cells towards a mature phenotype and diminished their immunosuppressive activity [4]. Carboplatin reduced the number of cells with immunosuppressive activity and increased the level of IFNγ [12]. It should also be noted that the effect of chemotherapy has not considered as effect associated to the immunological component. Although there are a lot of data not about the direct cytotoxic effect of drugs for chemotherapy on tumors, but about a greater effect on the immune response.

It is necessary to considered that the immune system plays a key role in the development of the tumor process and its maintenance or progress tumor growth. It is known, the cells of the immune system are recruited by tumor for creation a specific immunosuppressive environment [3]. At the same time, the role of T-regulatory cells $FoxP3^+$ in tumor microenvironment have been uncertain. It is described that tumor cells recruit Tregs $FoxP3^+$ and the amount of these cells' high increases with tumor growth. Also, patients with increased levels of expression of CTLA-4 and PD-1 have a worse prognosis compare to patients with low levels of expression these molecules. High levels of expression CTLA-4 and PD-1 can promote of metastasis and malignancy [8].

CTLA-4 and PD-1 on TREG are immune response suppressor molecules influencing through different signal ways. Applying of checkpoint inhibitors improves survival with some solid tumors [10]. Since cells of the immune system contribute significantly to the antitumor response, it is necessary to study an ability of potential and well-known chemotherapy agents to influence the immune response. **The aim of the study** was to evaluate the effect of cucurbit[7]uril, platinum compounds and supramolecular complexes on regulatory T-cells and expression of immune checkpoint molecules on Tregs.

Materials and methods

Heparinized peripheral blood was isolated from 8 volunteers (average 29±2.4 years) after signing informed consent form. Peripheral blood mononuclear cells (PBMCs) were obtained by centrifugation in a density gradient ficoll-urografin according to protocol. Isolated PBMCs at a quantity of 1×10^6 cells/mL were incubated with CB[7], platinum compounds (carboplatin, oxaliplatin) and supramolecular complexes (CB[7]-carboplatin, oxaliplatin-CB[7]) using the culture medium RPMI-1640, supplemented with 10% FCS, 50 mg/mL gentamicin and 25 mg/mL thienam in 48-well plate (TPP, Switzerland) during 72 h in a standard culture conditions with 37 °C, 5% CO_2. Anti-CD3 monoclonal antibodies (aCD-3) and IL-2 were used as stimulants for cells. Carboplatin and complex CB[7]-carboplatin were added at the concentrations of 0.3 mM, while oxaliplatin and complex oxaliplatin-CB[7] at 0.1 mM. CB[7] was used as control for platinum compounds, complexes and respectively added at equivalent concentrations. Also, we used control with only culture medium as negative control.

After 72 h incubation, the treated cells were labeled by fluorochrome-conjugated antibodies to determine phenotypes cells. Regulatory T cell was determined as $CD3^+CD4^+CD25^+FoxP3^+$ (BioLegend, USA). The intracellular expression of FoxP3 was estimated according FoxP3-staining protocol using True-Nuclear™ Transcription Factor Buffer Set (BioLegend, USA). Expression of immune checkpoint molecules was performed by cell staining with fluorochrome-conjugated monoclonal antibodies specific to CD279(PD-1), CD274(PD-L1), CD152 (CTLA-4).

Samples were analyzed by flow cytometry using cytometer FACS Canto II (BD, Franklin Lakes, NJ, USA) with Diva 6.0 software (BD).

Statistical analysis was performed using GraphPad Prism software (version 9.0.0). Differences between groups were determined using non-parametric Friedman test. P value of < 0.05 was considered statistically significant. Data are presented as median ±interquartile range with $n = 8$.

Results and discussion

We evaluated the effect of complexes Cucurbit[7] uril and platinum compounds on the number of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs $FoxP3^+$). The CB[7]-carb complex was significantly reduced the amount of Tregs $FoxP3^+$ compared to the control in stimulated and non-stimulated culture. Also, the complexes CB[7] with carboplatin and oxaliplatin were significantly decrease the number of Tregs $FoxP3^+$ compared to CB[7] at an equivalent dosage in both culture (Figure 1). Significant differences in the expression of immune check-point molecules such as PD-1 and PDL-1 on Treg were not found (Figure 2). At the same time, carboplatin and the CB[7]-carb complex were reduced the expression of CTLA-4 compared to CB[7] in the non-stimulated culture. Significant difference in expression of CTLA-

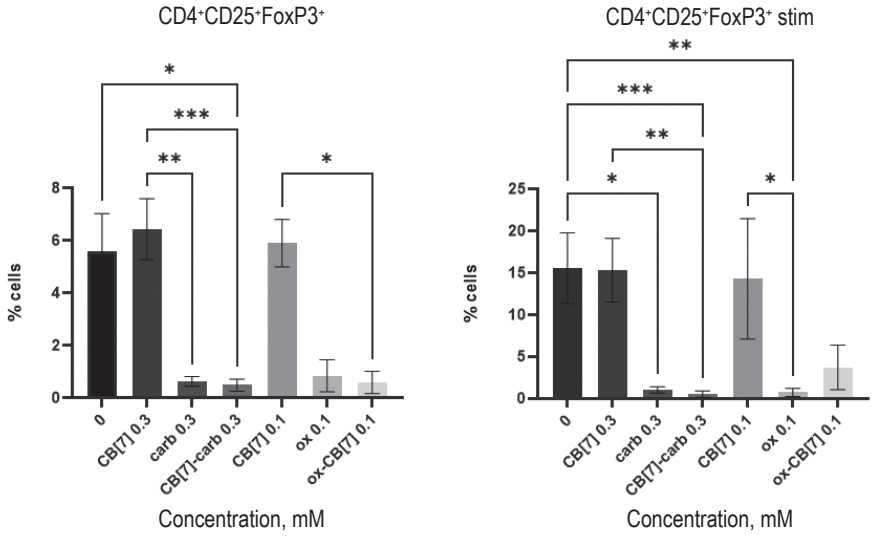

Figure 1. Number of CD4+CD25+FoxP3+ regulatory T cells after 72 h treatment with Cucucrbit[7]uril and complexes: CB[7]-carboplatin complex, oxaliplatin-CB[7] complex in stimulants (aCD3-antibody and IL-2) and non-stimulants cultures

Note. Data are presented as median ± interquartile range with n = 8. *, significant differences are $p < 0.05$ by employing one-way ANOVA, Friedman test.

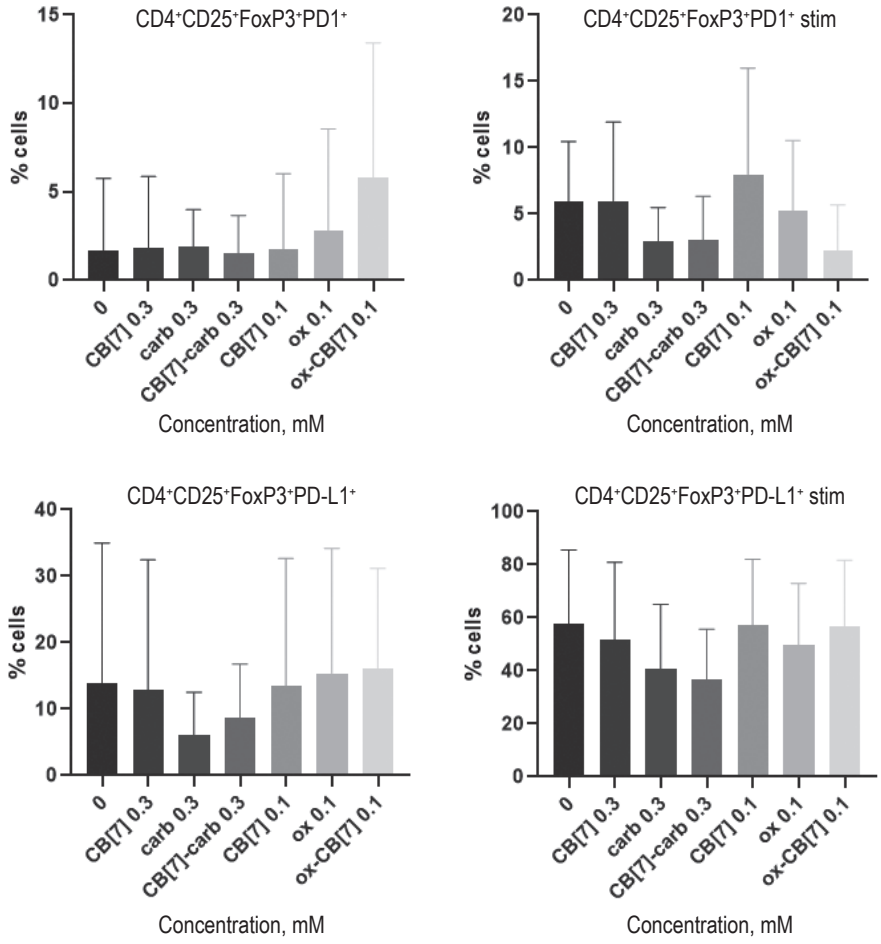

Figure 2. Expressions of PD-1 and PDL-1 immune checkpoint molecules on CD4+CD25+FoxP3+ regulatory T cells after 72 h treatment with Cucucrbit[7]uril and complexes: CB[7]-carboplatin complex, oxaliplatin-CB[7] complex in stimulants (aCD3-antibody and IL-2) and non-stimulants cultures

Note. As for Figure 1.

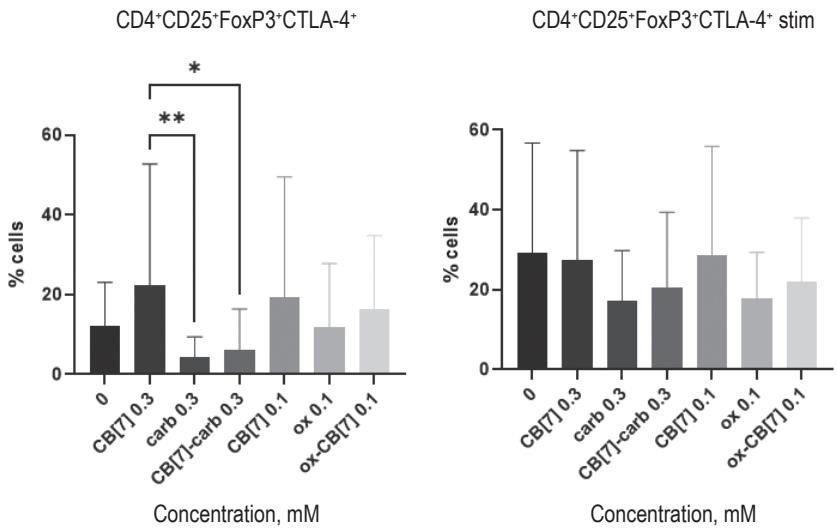

Figure 3. Expressions of CTLA-4 immune checkpoint molecules on $CD4^+CD25^+FoxP3^+$ regulatory T cells after 72 h treatment with Cucucrbit[7]uril and complexes: CB[7]-carboplatin complex, oxaliplatin-CB[7] complex in stimulants (aCD3-antibody and IL-2) and non-stimulants cultures

Note. As for Figure 1.

4 between control and treated groups were not found (Figure 3).

It is known, Tregs $FoxP3^+$ have high frequency infiltration into tumor and suppress the antitumor immune response. At the same time, a decrease in the expression of CTLA-4 and the number of Tregs $FoxP3^+$ improve the prognosis in a tumor [11]. It has also been reported that increased Treg infiltration into tumor has been associated with low common survival and relapse-free survival. Also, patients with high Treg levels had worse outcomes in tumor [9]. These results demonstrate that it is required to reduce the expression of check point molecules and the amount of Tregs in a tumor, but at the same time check point inhibitors have not to use, because it increases the risk of developing autoimmune pathologies, or their application may be effectless and non-selective, since it is necessary to observe correct balance of PD-1 expression on Tregs and $CD8^+$ [1].

Conclusions

Complexes Cucurbit[7]urils with platinum compounds are a perspective antitumor agent with immunomodulatory properties. Furthermore, applying these complexes may provide a treatment with the greatest efficiency and fewer side effects. However, further research is needed to confirm the effectiveness of this kind of therapy in patients.

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