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EVALUATING THE EFFECTS OF DISINTEGRIN ON TUMOUR GROWTH

O. M. PLATONOV, Y. D. VINNYCHUK

Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv

E-mail: *chaosplaton@gmail.com*

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Cancer is the second leading cause of death in the world after cardiovascular disease, and many types of cancer have no symptoms in the early stages, making it difficult to diagnose. At the same time, some tumors do not respond well to therapy, with its severe side effects. Tumor cells themselves can spread through the bloodstream or lymphatic system, making treatment difficult, so the search for compounds with antiproliferative and antimetastatic effects is ongoing. In our study, we tested disintegrin from *Echis multisquamatus* venom as an antitumor drug in an *in vivo* animal model to determine the degree of inhibition of Walker-256 carcinosarcoma tumor growth.

Aim. The work purposed to investigate the effect of disintegrin on the growth dynamics of Walker-256 carcinosarcoma in rats.

Materials and Methods. Crude venom of *Echis multisquamatus* was fractionated using ion-exchange chromatography followed by size-exclusion chromatography on Superdex 75 using the FPLC system (ÄKTA, GE Healthcare, USA). Analysis of the molecular weight of protein components was performed using SDS-PAGE. *The concentration of protein was measured using a spectrophotometer Optizen POP (Korea) at 280 nm.* Walker-256 carcinosarcoma cells for transplantation into laboratory animals were obtained from the National Bank of Cell Lines and Tumour Strains of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine.

Results. The two-step chromatography protocol allowed us to obtain the polypeptide from the venom of Echis multisquamatus that possessed the anti-aggregatory action. SDS-PAGE analysis confirmed the homogeneity of the obtained polypeptide with a molecular weight of 14,889 kDa. The tumor cells of Walker-256 carcinosarcoma were transplanted subcutaneously into the inguinal area of the right lower limb of 10 rats in the amount of 1.5×106 cells in 300 µl of saline (0.9% NaCl). The inhibition of tumor growth, which is one of the criteria for the effectiveness of the test substance, was observed during the period of intensive tumor growth and amounted to 18.7% on day 9 after inoculation and 36.2% on day 11.

Conclusions. The studies showed that the use of disintegrin from *Echis multisquamatus* venom led to inhibition of the growth of Walker's carcinosarcoma W-256 on the 9th to 11th day after tumor inoculation in rats. The observed inhibitory effect was moderate and less than the expected effect of antitumor agents. From the point of view of the mechanism of action of disintegrins, a reduction in the quality of cell attachment should be expected, which is not crucial in the conditions of tumor grafting.

Keywords: disintegrin, integrin receptors, cancer cells lines, cancer animal models, metastasis

The state of intercellular communication, in many cases, determines the development, tissue organization, and functioning of multicellular organisms. Therefore, one of the priorities of modern biology and medicine is to study the cellular and molecular mechanisms of intercellular interactions.

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Adhesion molecules play a key role in cell proliferation and differentiation, stabilization of intercellular contacts, as well as processes underlying intercellular signaling, being an essential intermediate between extracellular structures and the cell cytoskeleton [1, 2]. The binding of these molecules to their respective ligands initiates a number of intracellular biochemical reactions that can lead to changes in the cytoskeleton state and affect other processes [3].

The research of adhesion mechanisms in oncology is essential. Adhesion molecules are the key to cancer cells interacting with the endothelium of distant organs and non-cellular matrix elements, as a necessary property of malignant cells is a change in their morphology and locomotion [4].

The main categories of adhesion receptors include integrins, which are necessary for the dynamic regulation of adhesion and migration processes [5]. The expression of integrins on the surface of malignant cells is highly prognostic, as they are involved in almost every stage of progression, from primary tumor development to metastasis [6].

Integrins are transmembrane glycoproteins consisting of a and b-subunits that can bind to each other non-covalently in different ways to form more than 20 types of integrins. Various combinations of subunits determine the specificity of binding of the receptor's non-cellular domain to a particular ligand (most often, these are different non-cellular matrix proteins: collagens, laminin, fibronectin, etc.) [7].

Thus, the study of the complex interrelationships between adhesive and metastatic signaling networks is urgent to find new treatment approaches [3]. And, in particular, integrins are attractive targets for the development of cancer therapeutics [6, 8]. Therefore, the study of the potential of blocking integrin receptors on tumour cells, which helps to inhibit integrin-mediated interaction at different stages of the disease, may help to find new practical approaches to the treatment of malignancies. The use of experimental animal models as an object of study has excellent advantages in assessing the effectiveness of treatment, which also allows the reproducing of tumour growth in more pathophysiological conditions, ensures genomic heterogeneity of human cancer, and creates a complex tumour microenvironment [9].

The work aimed to investigate the effect of disintegrin on the growth dynamics of Walker-256 carcinosarcoma in rats.

Methods. The study of the effect of disintegrin on tumor growth *in vivo* was performed on Wistar rats aged 2.5 months and weighing 180–200 g (vivarium of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine). The rats were kept in standard vivarium conditions with natural light and a standard diet.

Walker-256 carcinosarcoma was used as a model of tumor growth. Taking into account the morphological characteristics and the course of the tumor process, this tumor can be considered an experimental model of breast cancer, which allows the reproduction of the processes that occur sequentially at all stages of growth over a short period [10]. Walker-256 carcinosarcoma cells were obtained from the National Bank of Cell Lines and Tumour Strains of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine.

To obtain isolated tumor cells, tumors were removed from animals, tissue fragments without blood vessels and necrosis were cut out, reduced to a particle size of less than 0.2 mm^3 with scissors, and incubated for 5 min at 37 °C in 0.2% trypsin solution in RPMI-1640 medium (Sigma, USA) with constant use of magnetic stirrer. The liquid was collected, filtered through 3 layers of nylon, and washed with 10% fetal calf serum. The cells were incubated with trypsin 3 times. The isolated cells were washed 3 times by centrifugation at 425 g in RPMI-1640 medium. The percentage of cell viability was counted in a Goryaev chamber by trypan blue staining. Tumor cells were transferred subcutaneously into the inguinal area of the right lower limb of 10 rats in the amount of 1.5×10^6 cells in 300 µl of physiologically buffered saline (0.9% NaCl).

The rats were divided into two groups: a control group, which received saline together with tumor cells, and an experimental group, which received cells in a 750 µg/ml disintegrin solution. On every second day, equal volumes of saline and disintegrin solution were injected into the tail vein of the control and experimental groups, respectively.

To evaluate the tumor growth, the tumor diameter was measured in two directions for each animal using a caliper every 2 days during the experiment. The tumor volume was calculated, as well as the percentage of tumor growth inhibition and the index of effectiveness of the test substance according to the formulas [11]:

$$\text{Volume (V)} = \text{length} \times (\text{width})2/2$$

$$\text{Percentage of tumour growth inhibition} = [(\text{Vcontrol} - \text{Vtest})/\text{Vcontrol}]\times100\%.$$

$$\text{Efficiency index (EI)} = \text{Vcontrol}/\text{Vtest}$$

All animal studies were carried out in accordance with the standards established by the Law of Ukraine No. 3447-IV 'On the Protection of Animals from Cruelty' and the standards established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes of 20.09.1985.

Results and Discussion. The effect of disintegrin on the growth of Walker W-256 carcinosarcoma was measured by determining the change in tumor volume in rats of the control and experimental groups on days 9, 11, and 14 after tumor implantation. At each time point of the experiment, the tumor diameter was measured in two directions, and the tumor volume, tumor growth inhibition, and the factor effectiveness index were calculated.

When comparing the results obtained, there was a tendency to reduce the tumor volume in the experimental group (especially on day 11 of tumor growth). Statistically significant differences in the mean values of tumor volumes in rats of both groups were not found, as there were substantial individual fluctuations in tumor size in animals (Table 1). However, the inhibition of tumor growth, which is one of the criteria for the effectiveness of the test substance, was recorded during the period of intensive tumor growth and amounted to 18.7% on day 9 after inoculation and 36.2% on day 11. The calculation of the efficacy index, as an indicator of the sensitivity of the tumor to the effects of the test substances, was 1.23 (day 9) and 1.56 (day 11).

Table 1. **Effect of disintegrin on the dynamics of tumor growth in rats**

Control group (n = 5)				**Experimental group (n = 5)**		
Day after tumour replantation	Length, cm	Width, cm	Volume, cm^3	Length, cm	Width, cm	Volume, cm^3
9 day	4.94±0.7	3.62±0.5	46.04±16.6	4.46±0.6	3.68±0.3	37.42±11.7
11 day	5.66±0.5	4.76±0.6	78.02±21.0 [21.3: 63.5]	4.78±0.7	4.18±0.5	49.92±19.2 [25.3: 56.2]

Conclusions. In an *in vivo* model system, it was shown that disintegrin in a dose of 100 μg/ml leads to inhibition of growth of Walker's carcinosarcoma W-256 on days 9–11 after tumor inoculation in rats.

Authors' contribution

Vinnychuk — research, conceptualization, data collection, visualization, writing original draft, review, proofreading the manuscript; Platonov — research, editing.

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