

Short communication

**THE INFLUENCE OF HETEROCYCLIC COMPOUND–PAMAM
 DENDRIMER COMPLEXES ON EVOKED ELECTRICAL RESPONSES
 IN SLICES OF HYPOXIC BRAIN TISSUE**

VLADIMIR I. POTKIN¹, DZMITRY SHCHARBIN^{2,*}, ANDREY A. DENISOV³,
 SVETLANA G. PASCHKEVICH³, MARIA BRYZSEWSKA⁴, SERGEY K.
 PETKEVICH¹, ALEXEY V. KLETSKOV¹, DMITRI O. LAPOTKO⁵,
 VLADIMIR V. KAZBANOV³, TATIANA A. GURINOVICH³
 and VLADIMIR A. KULCHITSKY³

¹Institute of Physical Organic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus, ²Institute of Biophysics and Cell Engineering of National Academy of Sciences of Belarus, Minsk, Belarus, ³Institute of Physiology of National Academy of Sciences of Belarus, Minsk, Belarus, ⁴Department of General Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland, ⁵Department of Biochemistry and Cell Biology, Rice University, Houston, Texas, USA

Abstract: We used complexes between a fourth generation polyamidoamine (PAMAM) dendrimer and one of two heterocyclic compounds – 1-(6-hydroxyhexyl)-3-(5-phenyl-isoxazole-3-yl)-urea or 5-phenyl-isoxazole-3-carboxylic acid – to reduce oxygen consumption in transverse slices of the hippocampus taken from 4-week old male rats. *In vitro* electrophysiological experiments revealed that the inhibitory effect of the hypoxic state on the evoked responses was enhanced in the presence of the complexes. The data were analyzed in terms of the potential antitumor effects of these complexes.

Keywords: Hypoxia, Dendrimer, Brain slices, Postsynaptic potential, Antitumor effects, Heterocycles, Isoxazoles

* Author for correspondence. Dr. Dzmitry Shcharbin, Institute of Biophysics and Cell Engineering of NASB, Akademicheskaja str. 27, Minsk, 220072, Belarus. Email: d.shcharbin@mail.ru, phone: (37517) 2842358, fax: (37517) 2842359

Abbreviations used: ACSF – artificial cerebrospinal fluid; EPSP – excitatory postsynaptic potential; PAMAM – polyamidoamine; PS – population spike

INTRODUCTION

Until recently, studies on hyperbaric oxygen treatment and cancer focused on whether increased oxygen levels could act as a cancer promoter [1]. On the one hand, a decrease in the extent of the vascular network limits the metastatic opportunities of surviving tumor cells under hypoxic and ischemic conditions. On the other hand, chemotherapeutic drug delivery to cancer cells is reduced, which may be one of the biggest arguments against the use of substances that inhibit blood vessel growth [2, 3].

Isoxazole derivatives and dendrimers have anticancer activity [4–5] and the latter have shown their efficacy in the field of anticancer drug delivery [4, 6–7]. Non-covalent or covalent conjugation with dendrimers can significantly improve the direct delivery of drugs, including anticancer drugs like adriamycin, doxorubicin, methotrexate, fluorouracil, or cisplatin [4, 6–7]. Moreover, modification with a dendrimer can greatly reduce the drug's side effects [8–9].

The aim of this study was to elucidate how the presence of dendrimer–heterocyclic compound complexes in brain tissue would affect the functioning of its signaling systems under hypoxic conditions. We chose hippocampal slices as the *in vitro* model to test our hypothesis. The continued presence of intercellular communication has been established both in brain slices and in cell culture [10, 11]. Hippocampal cells are involved in the control of memory and learning. They play their role by transmitting signals between the CA3 and CA1 regions. In tumor tissue, intercellular communication begins to break down early, but it is difficult to establish the functional consequences of such changes in the initial stage of the pathological process. Therefore, we evaluated the effects of heterocyclic compound–PAMAM dendrimer complexes on the intercellular communication in hippocampal slices in a state of hypoxia.

MATERIALS AND METHODS

Materials

Fourth generation PAMAM dendrimers were purchased from Sigma-Aldrich. All of the other reagents were of analytical grade and used as purchased without further purification. 5-phenyl-isoxazole-3-carboxylic acid (also referred to here as compound 1) and 1-(6-hydroxy-hexyl)-3-(5-phenyl-isoxazole-3-yl)-urea (also referred to here as compound 2) were synthesized, and their structures were verified using NMR as reported previously [10].

Complex generation

To increase the solubility of compounds 1 and 2, we synthesized complexes of each with PAMAM dendrimer. A 70-nmol suspension of PAMAM in 3 ml of normal saline was mixed with 1960 nmol of one of the heterocyclic compounds suspended in 7 ml of normal saline and stirred at 34°C for 4 h. Gradual homogenization with increasing opalescence of the mixed suspension was observed. Compound 1 reacted with the amino groups of the dendrimer surface

to form a salt-type product. Salt formation was confirmed by nephelometry when the insoluble crystals of compound 1 were fully dissolved by the PAMAM dendrimer. Compound 2 associated with the dendrimer cluster due to van der Waals interactions.

Electrophysiological experiments

We performed the electrophysiological experiments using 450- μ m thick transverse slices of the hippocampus from 4-week old male rats ($n = 13$) as described earlier [11]. The samples were taken from rats that were also used as a control group in a set of experiments carried out by another group of the Institute of Physiology of National Academy of Sciences of Belarus in order to minimize the number of animals killed for experiments. This study was approved by the Animal Care and Use Review Board at the Institute of Physiology of National Academy of Sciences of Belarus and conformed to the International Guiding Principles for Biomedical Research Involving Animals (1985) and the State Rules for Animal Accommodation.

Before the experiments, the slices were preincubated for 1 h in BSC-PT preincubator (Harvard Apparatus) in carbogen-saturated artificial cerebrospinal fluid (ACSF) at 20°C. The ACSF consisted of 124.0 mmol/l NaCl; 3.0 mmol/l KCl; 1.25 mmol/l KH_2PO_4 ; 1.2 mmol/l MgCl_2 ; 2.0 mmol/l CaCl_2 ; 26.0 mmol/l NaHCO_3 ; and 10.0 mmol/l glucose (pH 7.3–7.4). During the experiments, the slices were placed in a temperature-controlled BSC-ZT chamber (Harvard Apparatus) at 29°C and perfused with carbogen-saturated ACSF at a flow rate of 4 ml/min. Recording tungsten microelectrodes (WPI Inc.) were placed at the stratum radiatum and stratum pyramidale of the hippocampal CA1 region to monitor the excitatory postsynaptic potentials (EPSP) and population spikes (PS), respectively. Neuronal responses were evoked by electrical stimulation of presynaptic Schaffer collaterals using a stimulating microelectrode. The perfusion of the hippocampal slices in the presence of dendrimer-based complexes was performed by adding 100 $\mu\text{g/ml}$ of the PAMAM–compound 1 or PAMAM–compound 2 complexes to the ACSF.

RESULTS AND DISCUSSION

Electrophysiological experiments

After the preincubation period, the subsequent infusion of ACSF saturated with carbogen revealed a gradual stabilization of evoked electrical responses in the stratum radiatum (excitatory postsynaptic potentials, EPSP) and stratum pyramidale (population spikes, PS). The first minutes of observation are illustrated in Figs 1 and 2.

The results show that the hypoxic stimulus was accompanied by a reduction in the EPSP amplitude and a short-term increase (during the first 1 to 1.5 min) in the amplitude of PS, followed by a catastrophic fall in EPSP to its minimum at 4 to 5 min (Figs 1B and 2C). Starting from the second minute of hypoxia, the

amplitude of PS decreased rapidly, replicating the fall in EPSP amplitude. PS amplitude recovery after the cessation of hypoxia occurred 3 to 5 min later than the restoration of the amplitude of the EPSP.

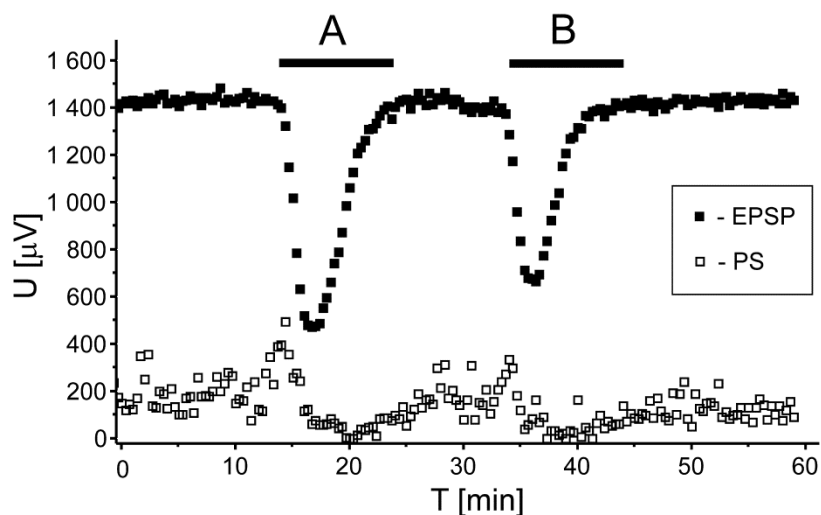


Fig. 1. Amplitude (uV) of the excitatory postsynaptic potentials (EPSP; filled squares) and population spikes (PS; open squares) in hippocampal slices after exposure to a hypoxic solution. A – Hypoxia in the presence of the PAMAM complex with 1-(6-Hydroxy-hexyl)-3-(5-phenyl-isoxazole-3-yl)-urea. B – Hypoxia alone (control).

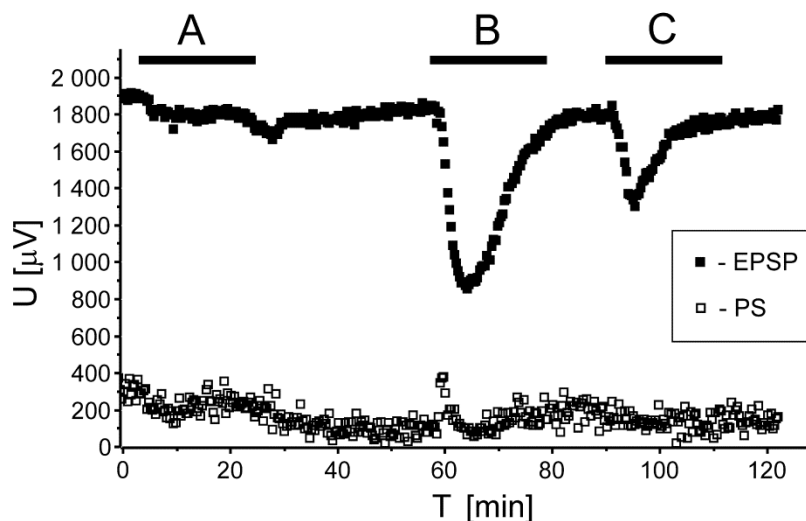


Fig. 2. The amplitude of the excitatory postsynaptic potentials (EPSP) and population spikes (PS) in hippocampal slices after 10 min of hypoxic stimulus. A – In the presence of the PAMAM complex with 5-phenyl-isoxazole-3-carboxylic acid without hypoxia. B – In the presence of PAMAM complex with 5-phenyl-isoxazole-3-carboxylic acid with hypoxia. C – Hypoxia alone (control).

The addition of the complex of the PAMAM dendrimer with compound 2 (Fig. 1A) to the hypoxic solution of ACSF increased the drop in EPSP and PS in the hippocampus (Fig. 1A). The amplitude of EPSP was reduced by 20–40% when hypoxia occurred in the presence of the complex of the PAMAM dendrimer with compound 1 (Figs 1A and 2B). The recovery of the amplitude of EPSP was almost twice as slow under such conditions. Also, during the initial period of hypoxia combined with dendrimer-based complexes, a brief upturn in PS amplitude was always present, reflecting the dissociation of the input–output relationship [11].

The decrease in the ability to form EPSP (input) in the hippocampus under hypoxic conditions in the presence of dendrimer–heterocyclic compound complexes is accompanied by an activation of processes forming PS (output). The obtained data indicate that the inhibitory effect of hypoxia on triggered responses in the hippocampus is enhanced in the presence of the studied complexes. The dendrimer–heterocyclic compound complexes were found to have no effect on the events occurring in the population of hippocampal cells during the stimulation of Schaffer collaterals.

The use of dendrimer-based complexes with compounds that amplify hypoxia has potential for situations that require the destruction of biological tissues, in particular for cancer treatment. The first step in this direction could be the combination of such complexes with substances that affect regeneration [12] or inhibit the endothelial growth factor of blood vessels. This could lead to the disruption of angiogenesis, impaired blood flow to the tumor cells, a voltage drop of oxygen in tumor tissues (hypoxia), deceleration of tumor growth, or the destruction of the tumor cells.

Acknowledgements. This study was partly financed by the 7 Framework Program of the European Union, Project IRSES No. PIRSES-GA-2012-316730 “NANOGENE”.

REFERENCES

1. Moen, I. and Stuhr, L.E.B. Hyperbaric oxygen therapy and cancer – a review. **Target Oncol.** 7 (2012) 233–242.
2. Depret, J., Teboul, J.L., Benoit, G., Mercat, A. and Richard, C. Global energetic failure in brain-dead patients. **Transplantation** 60 (1995) 966–971.
3. Pastorekova, S., Zatovicova, M. and Pastorek, J. Cancer-associated carbonic anhydrases and their inhibition. **Curr. Pharm. Design** 14 (2008) 685–698.
4. Kulchitsky, V.A., Talabaev, M.V., Chernov, A.N., Grigoriev, D.G., Demidchik, Y.E., Shcharbin, D.G., Chekan, N.M., Kazbanov, V.V., Gurinovich, T.A., Gordienko, A.I., Sergeeva, E.K., Potkin, V.I. and Kalunov, V.N. Improving the efficiency of chemotherapeutic drugs by the action on neuroepithelial tumors. In.: **Glioma – Exploring Its Biology and Practical Relevance** (Anirban Ghosh, Ed.) 21 (2011) 465–486.

5. Kamal, A., Bharathi, E.V., Reddy, J.S., Ramaiah, M.J., Dastagiri, D., Reddy, K., Viswanath, A., Reddy, T.L., Shaik, T.B., Pushpavalli, S.N. and Bhadra, M.P. Synthesis and biological evaluation of 3,5-diaryl isoxazoline/isoxazole linked 2,3-dihydroquinazolinone hybrids as anticancer agents. **Eur. J. Med. Chem.** 46 (2011) 691–703.
6. Cai, X., Hu, J., Xiao, J. and Cheng, Y. Dendrimer and cancer: a patent review (2006-present). **Expert Opin. Ther. Pat.** 23 (2013) 515–529.
7. Shcharbin, D., Dzmitruk, V., Shakhbazau, A., Goncharova, N., Seviaryn, I., Kosmacheva, S., Potapnev, M., Pedziwiatr-Werbicka, E., Bryszewska, M., Talabaev, M., Chernov, A., Kulchitsky, V., Caminade, A.-M. and Majoral, J.-P. Fourth generation phosphorus-containing dendrimers: prospective drug and gene delivery carriers. **Pharmaceutics** 3 (2011) 458–473.
8. Ziamba, B., Matuszko, G., Bryszewska, M. and Klajnert, B. Influence of dendrimers on red blood cells. **Cell. Mol. Biol. Lett.** 17 (2012) 21–35.
9. Lazniewska, J., Milowska, K., Katir, N., El Kadib, A., Bryszewska M., Majoral, J.-P. and Gabryelak, T. Viologen-phosphorus dendrimers exhibit minor toxicity against a murine neuroblastoma cell line. **Cell. Mol. Biol. Lett.** 18 (2013) 459–478.
10. Kulchitsky, V.A., Potkin, V.I., Zubenko, Yu.S., Chernov, A.N., Talabaev, M.V., Demidchik, Y.E., Petkevich, S.K., Kazbanov, V.V., Gurinovich, T.A., Roeva, M.O., Grigoriev, D.G., Kletskov, A.V. and Kalunov, V.N. Cytotoxic effects of chemotherapeutic drugs and heterocyclic compounds at application on the cells of primary culture of neuroepithelium tumors. **Med. Chem.** 8 (2012) 22–32.
11. Garkun, Y.S., Yakubovich, N.V., Denisov, A.A., Molchanov, P.G., Emel'janova, A.A., Pashkevich, S.G. and Kulchitsky, V.A. Generation of excitatory postsynaptic potentials in the hippocampus after functional modification of glycosaminoglycans. **Bull. Exp. Biol. Med.** 145 (2008) 395–397.
12. Tabata, Y. Nanomaterials of drug delivery systems for tissue regeneration. **Meth. Mol. Biol.** 300 (2005) 81–100.