Inhibition of Water Transport Across Cell Membranes by Dimephosphon

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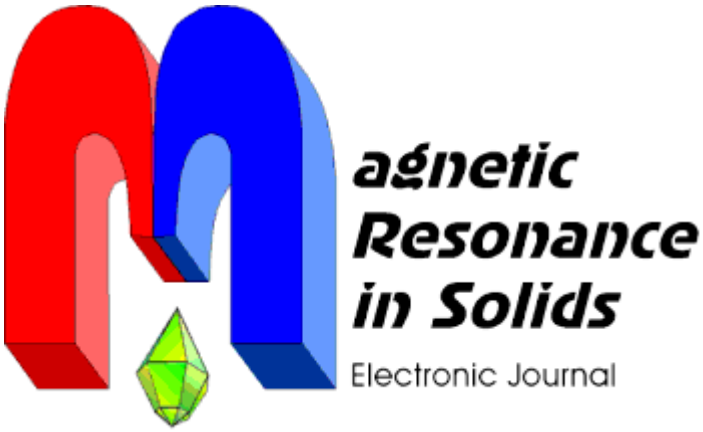

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The inhibitory effect of widely used drug dimephosphon on water transport in human red cells at 37ºC was studied in comparison to intact human erythrocytes. Intracellular water residence time characterizing water permeability of cell membrane was obtained with doping NMR technique. Inhibitory effect of dimephosphon is concentration-dependent.

Keywords: Water permeability, red blood cell membrane, NMR, drug-membrane interaction.

1. Introduction

1,1-Dimethyl-3-hydroxybutyl phosphonic acid ethers synthesized at the A.E. Arbuzov Institute of Organic and Physical Chemistry (Kazan Research Center) are organophosphorus compounds with a variety of pharmacological effects.

The dimethyl ether of 3-hydroxybutyl phosphonic acid (dimephosphon, see Fig.1) like a majority of drugs is an amphiphile one, with octanol – water partition coefficient of 1.09 ± 0.09 [1]. It is widely used in metabolic therapy with wide application spectrum, but the molecular mechanism of its medical effect is not clear yet.

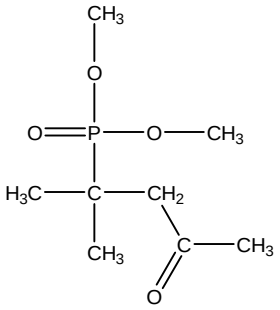

Fig.1. Structural formula of dimephosphon

Recent investigations of effect of dimephosphon and other acid derivatives on synaptic transmission in neuromuscular junction [1] suggest dual-effect model (blockade and modulation) of channel-blocking action. The modulation model of protein blocking is also associated with interactions through lipid bilayer. It's well recognized that action of membrane proteins, transporters and enzymes is strongly affected by their local environment, including membrane lipid bilayer. Therefore it seems interesting to study the effect of 1,1-Dimethyl-3-hydroxybutyl phosphonic acid derivatives (dimethyl, diethyl, diproyl and dibytil ethers) on biological membranes properties.

The red blood cell (RBC) suits ideally for investigating of many physiological processes in cells including water transport across cell membranes because of its simple structure (no internal membranes) and availability. As there are two water transport pathways: across the lipid bilayer and through transmembrane specific water-channels aquaporins, effect of pharmacological reagents on the water exchange rate in RBCs can provide more information about molecular mechanisms of water transport and drug – membrane interaction.

2. Materials and methods

Venous blood was drawn from healthy male volunteers into sample tubes with heparin (15 IU/ml). The RBCs were isolated by centrifugation and washed three times in medium S (150 mM NaCl, 5.5 mM glucose, 5 mm HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid), pH 7.4. Samples for NMR measurements were prepared by carefully mixing 0.2 ml RBC suspension, 0.1 ml doping solution (30 mM $MnC1_2$, 110 mM NaC1, pH 7.4) and 0.1 ml of medium S or pharmacological agent solution of appropriate concentration.

Studies of proton T_2 relaxation in paramagnetically doped RBC suspensions is focused on extracting the intracellular water residence time τ_a [2]. Fitting experimental relaxation data from doped cell suspensions to biexponential functions supplies the parameters from which τ_a is calculated using expression (1):

$$\tau_a = \frac{(A^2 + B^2 + 2ABC)}{(A^2 - B^2)(B + AC)} \tag{1}$$

where $A = \frac{1}{2}(\frac{1}{T_a'} - \frac{1}{T_b'})$; $B = \frac{1}{T_a} - \frac{1}{2}(\frac{1}{T_a'} + \frac{1}{T_b'})$; $C = 1 - 2P_a'; T_a', T_b', P_a'$ are apparent relaxation times and relative populations of intra- and extracellular water, measured in doped RBC suspension, correspondingly. The intrinsic relaxation time of the intracellular water T_a measurements were carried out on packed cell samples (whole blood centrifuged at 5000 g for 30 min.)

NMR relaxation data were obtained with home-built NMR spectrometer at a Larmor frequency of 19 MHz with Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. All experiments, including drug exposure were performed at 37^0 C on samples with 26-28 % hematocrit in medium S.

3. Results and discussion

No significant erythrocyte volume changes were observed. Intrinsic relaxation time of intracellular water without or with dimephosphon was typically within 140-150 ms.

All used concentrations of dimephosphon (0.0015 – 2 % v/v) caused an increase of mean residence time of intracellular water molecules τ_a above the control value 9.2 ± 0.4 ms in a concentration-dependent manner.

The relative increase of τ_a for different concentrations is represented in Fig.2. As one can see from the figure, residence time increases with the dimephosphon concentration increasing. It indicates that one (or both) of the water transport pathways are inhibited.

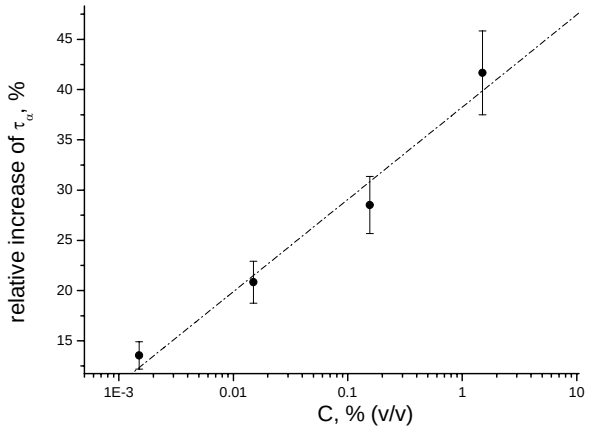

Fig.2. Relative increase of intracellular water residence time in human RBC by dimephosphon.

It is significant to note that aquaporin water channels can be specifically blocked by organomercuric SH-reagents, especially pCMB and pCMBS, reaching the maximal inhibition of water transport (increasing of τ_a at 50 %) in ≈60 min [3]. It should be noted that exposure of dimephosphon to RBC suspension produced faster water transport inhibition (in ≈ 5 min), but less efficient than pCMB.

Further experiments will be held in order to get detail understanding of the molecular mechanism of the found effect.

Acknowledgement

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