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Assessment of Biological Activity of New Compounds Designated to Act as Lysosomotropes

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Abstract

This work contains the results of studies on the influence of new lysosomotropic substances on an erythrocyte membrane. The compounds studied were hydrochlorides of N,N-dimethylglycine alkyl esters (DMG-n) and N,N-dimethylalanine alkyl esters (DMAL-n) having two different-length alkyl chains (n = 12 and 16), oxalates of dimethylaminoalaninates ($DMAL_s$-n; n = 8, 10, 12, 14 and 16) and methobromides of glycinates and alaninates (DMALM-12 and DMGM-12). They were found to hemolyze erythrocytes, to change their osmotic resistance and to influence erythrocyte membrane fluidity.

The results obtained indicate that observed changes were dependent on lipophilicities of the compounds. It was especially evident in the case of hemolytic efficiencies of the homologous series of alanine oxalates. Also, DMG-n and DMAL-n compounds significantly differed in their hemolytic properties. Again, slightly better hemolytic efficiency of DMG compounds in comparison with corresponding compounds having the same alkyl chain, DMAL, confirm such a conclusion. However, their hemolytic efficiencies were found to be moderate, which makes them potentially useful membrane modifiers. That feature is important for lysosomotropic compounds and its confirmation was the primary aim of the presented work.

It is worth mentioning that DMGM and DMALM compounds exhibited better hemolytic efficiencies than all other compounds studied – which is probably caused by the fact that they were used as bromides. Bromides are commonly found to be more active than compounds with other counterions.

Keywords: lysosomotropes, hemolytic activity, osmotic resistance, membrane fluidity

Introduction

Lysosomes are small cell organelles filled with hydrolytic enzymes for intracellular digestion and surrounded by membranes separating them from cell and thus being protected against action of cell components. Its interior acidity is kept at about pH 5, which ensures optimal activities of the enzymes. Uncontrollable damage to lysosomes may lead to cell death. On the other hand controllable modification of the lysosome membrane enables the permeation of substrates into the lysosome and the removal of decayed products from it. That feature of lysosomes is often used to introduce into an organism pharmacological compounds or such that can be converted into lysosomotropic substances [1]. Weak bases permeate especially well through the lysosome membrane. Their concentration may reach a very high level [2]. The biological activity of lysosomotropic compounds presents a large spectrum and depends mainly on their structure and basicity. The point is that some of these compounds, as weak bases, may enter into lysosomes and after protonation due to lysosomal pH, be-

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come trapped there [2]. Some of them may fulfill a therapeutic role once inside [3, 4], others may decrease the lysosome membrane barrier and enable drugs accumulated inside the lysosomes to outflow and fulfil their therapeutic role. Membrane barrier modificators should incorporate into the membrane without serious damage to it and be as low in toxicity as possible. It seems that such features facilitate the group of amino-ethyl esters of fatty acids in becoming easily degradable in the cell to nontoxic metabolites described earlier [5, 6]. It has been stated that such compounds have good growth inhibitory activity on the yeast *Saccharomyces cerevisiae*, which is on the chain length of the alkyl substituent in the acid moiety [7]. They have also a very good inhibitory activity on the yeast *S. cerevisiae* plasma membrane ATP-ase. Also, it has been found that they interact with an erythrocyte membrane and that the active specimen seems to be the protonated form of the aminoester, the unprotonated compound being rather inactive in such experiments [8].

In the present paper a new group of lysosomotropic n-alkyl esters of α-aminoacids was examined and their interaction with erythrocyte membrane and expressed as hemolytic activity was established. The compounds tested have very similar structure to those examined earlier but the structure of the ester fragment is turned over by 180^0 and the long n-alkyl substituent belongs to the alcohol fragment. It was interesting to discover how such a relatively small change in the aminoester structure may affect the activity of the compounds.

Materials and Methods

Lysosomotropic Compounds

The structures of new lysosomotropic substances are presented in Fig. 1. These were: hydrochlorides of n-alkyl N,N-dimethylglycinates (DMG-n) and N,N-dimethylalaninates (DMAL-n) synthesized in our laboratory by amination of the corresponding chloroacetates [9] or α-bromopropionates with dimethylamine in ethereal solution and transformed into hydrochlorides by passing dry hydrogen chloride through an ethereal solution of the free amino compounds. Oxalates of dimethylaminoalaninates ($DMAL_s$-n) were obtained from the free amines and oxalic acid in acetone. Free n-dodecyl glycinate and alaninate were quaternazed to the corresponding methobromides (DMGM-12 and DMALM-12 respectively) in the reaction with methyl bromide at room temperature. ^{1}H-NMR spectra (Brucker instrument 300 MHz, $CDCl_3$, HMS as an internal standard) and GLC (Shimadzu instrument GC-17A) confirmed the high purity of the synthesized compounds.

Hemolytic Studies

The experiments were conducted on fresh, heparinized pig blood. For washing the erythrocytes and in the experiments performed, an isotonic phosphate solution of pH 7.4 (131 mM NaCl, 1.79 mM KCl, 0.86 mM $MgCl_2$, 11.80 mM $Na_2HPO_4 \cdot 2H_2O$, 1.80 mM $Na_2H_2PO_4 \cdot H_2O$) was used. Upon removal from the plasma, the erythrocytes were washed four times in a phosphate buffer and then incubated in the same solution but containing proper amounts of the compounds studied. The modification was conducted at 37°C for 0.5 h, each sample containing 10 ml of erythrocyte suspension of 2% hematocrit, stirred continuously. After modification 1 ml samples were taken, centrifuged and the supernatant assayed for hemoglobin content using a spectrophotometer (Spekol 11, Carl Zeiss, Jena) at 540 nm wavelength. Hemoglobin concentration in the supernatant, expressed as a percentage of hemoglobin concentration in the supernatant of totally hemolyzed cells, was assumed as the measure of the extent of hemolysis.

Osmotic resistance was measured for erythrocyte modified for 0.5 h in solutions containing 10 μM lysosomotropic substances. Hematocrit was 2%. The modified erythrocytes were then suspended in hypotonic solutions of NaCl from 0.4% to 0.9% and hemolysis was measured as described.

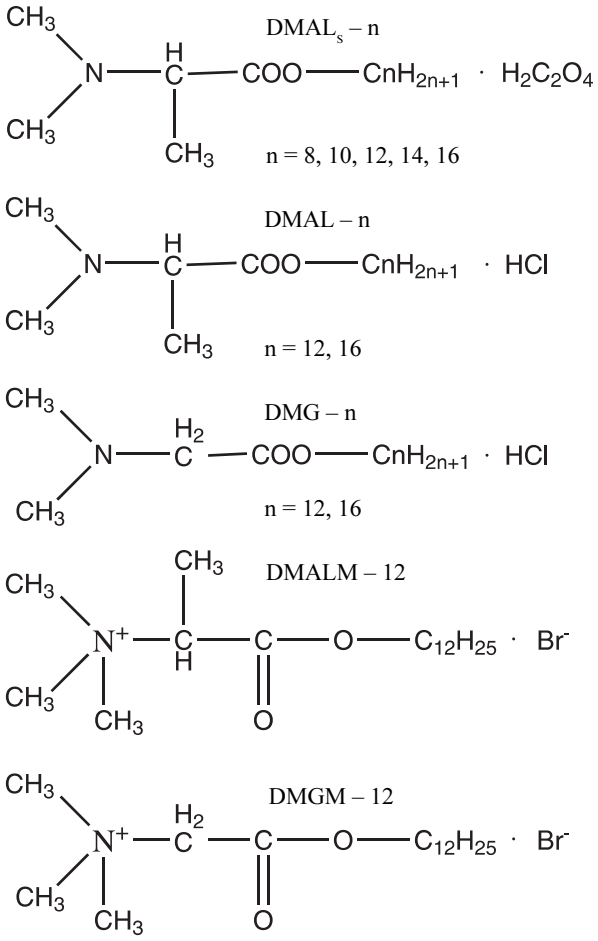

Fig. 1. The structure of new lysosomotropic compounds.

Fluorimetric Studies

TMA-DPH 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene, p-toluenesulfonate fluorescent probe was purchased from Molecular Probes Inc. (Eugene, Oregon, USA).

Fluorescence measurements were performed on erythrocyte ghosts labelled with TMA-DPH at 10 μM concentration, using a SFM spectrofluorimeter (KONTRON, Zurich, Switzerland). The concentration of compounds in samples was 25 μM. On the basis of fluorescence intensity measurements, the anisotropy coefficient was calculated [10]:

$$A = \frac{I_{II} - GI_{\perp}}{I_{II} + 2GI_{\perp}}$$

where I_{II} is the intensity of fluorescence emitted parallel to the polarization plane of the exciting light, I_T is the intensity of fluorescence emitted perpendicular to the polarisation plane, and G is the grating correction factor.

Results and Discussion

The results of the studies conducted are presented in Table 1. It contains the concentrations of lysosomotropic compounds studied inducing 50% and 100% hemolysis (C_{50} and C_{100}), the percent concentration of hypotonic sodium chloride solutions (C_{NaCl}) inducing 50% hemolysis of erythrocytes, and coefficients of anisotropy of fluorescent probe TMA-DPH used in fluidity experiments.

It was found that hemolytic activity of particular compounds of the oxalate aminoalaninate homologous series ($DMAL_s$-n) markedly increased with the alkyl chain length of the compound, i. e. with the increase of lipophilicity of the compound. The same may be said about hydrochlorides DMAL-n and DMG-n where compounds with $C_{12}H_{25}$ alkyl chains hemolyzed erythrocytes significantly weaker than those with $C_{12}H_{25}$ alkyl chains. It must be noted that the hemolytic efficiency of corresponding compounds (with the same alkyl chains) increased in sequence DMG-n > DMAL-n) > ($DMAL_s$-n). However, observed differences were not big ones. Also, no big differences were found for hemolytic efficiencies of DMGM-12 and DMALM-12 compounds, but those compounds hemolyzed erythrocyte far better than all other compounds. It is known that compounds with different counterions have a different influence on the parameters of biological and model membranes they are acting upon [11-13]. The bromide ions present in the solution enable very intensive interaction of compounds with membranes.

The hemolytic activity of compounds is accompanied by changes in the osmotic resistance of the erythrocytes. The more hemolytically active the compounds, the greater the changes in osmotic resistance, the measure of which was sodium chloride concentration inducing 50% hemolysis. Likewise, compounds of the biggest lipophilicities induce the greatest changes in erythrocyte membrane fluidity. However, changes in the anisotropy coefficients (A) did not differ very much from compound to compound.

In our earlier investigations we had found that the hemolytic activity of another group of lysosomotropic compounds depended on their basicity and increased with pK_a of the compound. [9]. This did not seem to be the only criterion determining hemolytic activities of lysosomotropic substances. DMG-12 and DMAL-12 had pK_a values 6.86 and 6.97, respectively. It means that one

Table 1. Concentrations of the compounds studied inducing 50% (C_{50}) and 100% hemolysis (C_{100}) of erythrocytes, NaCl concentrations inducing 50% hemolysis (C_{NaCl}) and coefficients of anisotropy (A).

Compound	C_{50} [mM]	C_{100} [mM]	C_{NaCl} [%]	A
$DMAL_s$-8	5.5	6.2	0.67	0.269
$DMAL_s$-10	4.9	5.6	0.67	0.259
$DMAL_s$-12	3.8	4.3	0.69	0.251
$DMAL_s$-14	2.9	3.1	0.70	0.251
$DMAL_s$-16	1.8	2.3	0.72	0.248
DMG-12	3.3	4.0	0.72	0.245
DMG-16	0.9	1.2	0.75	0.232
DMAL-12	3.6	4.2	0.70	0.250
DMAL-16	1.4	1.9	0.74	0.244
DMGM-12	0.10	0.16	0.75	0.239
DMALM-12	0.11	0.18	0.74	0.253
Control			0.65	0.270

Standard deviation for C_{50}, C_{100} and C_{NaCl} determination did not exceed 5%, and for A did not exceed 4%.

could have expected a higher activity of alanine than glycine derivatives which was not the case (see Table 1). Possibly, the reason was the wide acidic fragment of alanine constituing the obstacle for incorporation of DMALn derivatives into the erythrocyte membrane, which was manifested by lower hemolytic activity. One can thus infer that basicity of a lysosomotropic compound is only one of the properties determining its biological activity.

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