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Effect of 5-aminoorotic acid and its gallium (III) complex on the antioxidant activity of rat blood serum

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Investigating pro-oxidant and antioxidant properties of a potential medication is a matter of importance, particularly in the field of oncology. Gallium(III) salts have anticancer activity. In this investigation, the impact of a new complex of Ga(III), GaAOA, with 5-aminoorotic acid (HAOA) and HAOA itself on the antioxidant activity of rat blood serum was estimated.

Aqueous solutions of 10^{-6}, 10^{-5} and 10^{-4} M HAOA and GaAOA were left in contact with rat blood serum containing 1 mg/ml proteins. The discoloration of 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$, characteristic wavelength of 515 nm) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical-cation ($ABTS^{\bullet+}$, characteristic wavelength 660 nm) caused by HAOA and GaAOA both in absence and in presence of blood serum was measured and compared with the corresponding discoloration in presence of the blood serum alone.

In presence of HAOA and GaAOA the antioxidant activity of the rat blood serum via electron transfer and hydrogen donation increased, the effect being stronger with GaAOA compared to HAOA.

Keywords: antioxidant activity, 5-aminoorotic acid, gallium(III) complex, DPPH, ABTS, blood serum

INTRODUCTION

Strong anticancer activity while preserving healthy tissues is a desired property of any anticancer agent. Fine tuning pro-oxidant and antioxidant properties of a medication is important for both the efficacy against the disease and the patient's quality of life during treatment. Ga(III) salts are universally known for their anticancer activity [1-4]. 5-aminoorotic acid (HAOA), a derivative of the orotic acid (vitamin B_{13}), exhibited antioxidant properties [5], and its Ln(III) complexes were found to be both antioxidants and promising anticancer agents [6-8]. A new complex of Ga(III) with 5-aminoorotic acid has been synthesized and its antioxidant properties have been evaluated [9]. The *in-vitro* impact of HAOA and its complex with Ga(III) on both the 2,2-diphenyl-1-picrylhydrazyl radical and Fenton-generated hydroxyl radical ($OH^{\bullet}$) was investigated. Both demonstrated significant radical-scavenging activity.

The aim of the present investigation was to estimate the effect of GaAOA and HAOA on both total hydrogen donor and electron donor antioxidant activity in rat blood serum. In order to do that, the reactivity of rat serum toward $DPPH^{\bullet}$ (characteristic λ=517 nm) and $ABTS^{\bullet+}$ (characteristic λ= 660 nm), both in presence and absence of the investigated compounds was measured.

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EXPERIMENTAL PART

Materials of finest grade (SIGMA-ALDRITCH) were used. Standard aqueous solutions of HAOA (10^{-3} M) and GaAOA (3.10^{-4} M) were prepared using bi-distilled water. As GaAOA produced opalescence in physiological K, Na-phosphate buffer (PBS), the blood serum and the standard solutions were further diluted with bi-distilled water as well. For the purpose of the analysis, the solutions of the investigated substances were diluted to 10^{-4} – 10^{-6} M in the cuvette. The blood serum was diluted to a protein concentration of 1mg/ml and 0.04 ml of that solution were used in both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ analyses. The discoloration of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ was measured using a spectrophotometer Shimadzu 1601 equipped with a program package. The discoloration of the solutions containing blood serum in the presence of HAOA and GaAOA was presented as percentage of the corresponding discoloration in presence of the blood serum alone.

$DPPH^{\bullet}$ assay: The experiment was similar to this described in [5]. Briefly, a standard solution of $DPPH^{\bullet}$ in 96% ethanol was prepared, covered with aluminum foil and kept in dark in a freezer (-20°C). Before analysis the standard solution of $DPPH^{\bullet}$ was diluted with ethanol to give absorption (A) between 0.9 and 0.7 at 517 nm. The total volume of the solution in the cuvette was 2 ml, as follows: blank measurement – 1.96 ml ethanol and 0.04 ml sample; control measurement – 1,96 ml $DPPH^{\bullet}$ and 0,04 ml

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bi-distilled water; sample measurement – 1.96 ml DPPH$^{\bullet}$ and 0.04 ml sample.

The Radicals Scavenging Activity (RSA, %) was determined, using the formula:

$$RSI = \frac{[A_{control} - (A_{sample} - A_{blank})]}{A_{control}} * 100$$

where $A_{control}$ – relative change of A(517 nm) of pure DPPH$^{\bullet}$ solution in presence of the solvent for 5 minutes, A_{sample} – relative change of A(517 nm) in the presence of DPPH$^{\bullet}$, Blood serum and the compound investigated and A_{blank} – relative change of A(517 nm) of ethanol, containing the blood serum and the compound (if present) after 5 minutes

The final result for RSA of the blood serum in the presence of a tested compound was presented as a percentage of that for the blood serum alone.

ABTS$^{\bullet+}$ assay: A modified method of Erel [10] was used in this experiment. Briefly, 30 mM standard solution of ABTS$^{\bullet+}$ was prepared by dissolving ABTS$^{\bullet+}$ in Na-acetate buffer (pH=3.6) and adding hydrogen peroxide. After 1 hour at room temperature, the resulting standard solution was stored in a refrigerator. This standard solution was named R2 (reactant 2) The absorbance at 660 nm, A(660 nm) was selected for measuring the discoloration of the ABTS radical cation in this investigation. The medium for the spectrophotometric measurement, reagent 1 (R1), was Na-acetate buffer of pH=5.8. The total volume of the reactants in the cuvette was 1 ml, containing: for the blank measurement – 0.96 ml R1 and 0,04 ml sample; for the control measurement – 0.96 ml R1, 0.04 ml bi-distilled water and 0,04 ml R2; for the sample measurement – 0.96 ml R1, 0.04 ml sample and 0.04 ml R2.

The Radicals Scavenging Activity (RSA, %) was determined, using the formula:

$$RSI = \frac{[A_{control} - (A_{sample} - A_{blank})]}{A_{control}} * 100$$

where $A_{control}$ – relative change of A(660 nm) of pure ABTS$^{\bullet+}$ solution in presence of the solvent (R1) for 5 minutes; A_{sample} – relative change of A(660 nm) in the presence of ABTS$^{\bullet+}$, blood serum and the compound investigated; A_{blank} – relative change of A(660 nm) of R1, containing the blood serum and the compound (if present) after 5 minutes.

The final result for RSA of the blood serum in the presence of a compound is presented as a percentage of this for the blood serum alone.

For each experimental point 5 parallel measurements were performed. Then the average and standard deviation was calculated. Differences between experimental data points were statistically evaluated using One Way ANOVA with Bonferoni post-test and differences between standard deviations were estimated by Bartlett's test. Only statistically different results were discussed.

RESULTS

The activity of the blood serum alone in hydrogen donation was 31% of its activity in electron transfer.

The RSA toward DPPH and ABTS of HAOA and GaAOA alone were presented in Fig. 1.

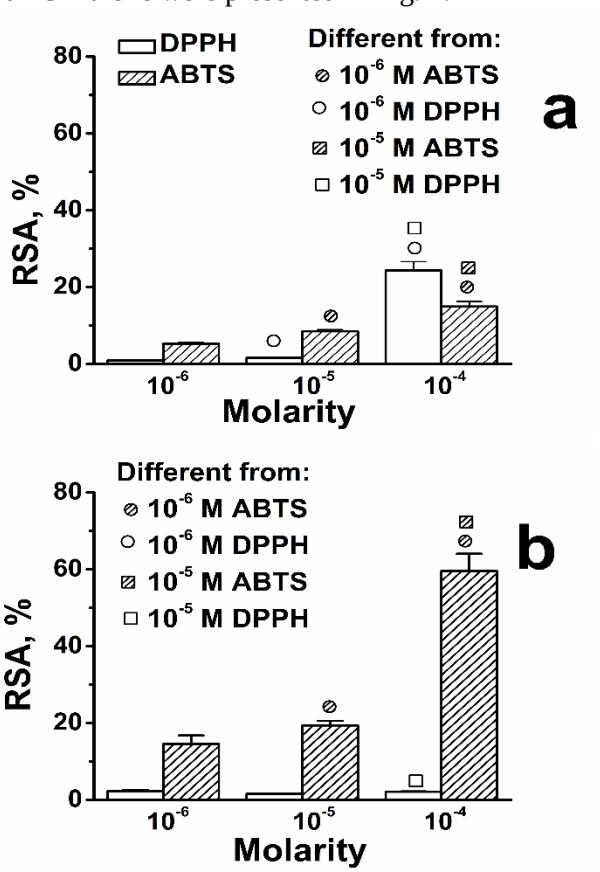

Fig. 1. Radicals scavenging activity (RSA) toward stable free radicals in the presence of HAOA (a) and GaAOA (b).

In the absence of blood serum (Fig. 1 a), the antioxidant activity of HAOA via both hydrogen and electron donation increased with the concentration. At concentrations below 10^{-4} M the electron transfer pathway prevailed. The hydrogen donation at 10^{-5} M HAOA and below this concentration was very low. Between concentrations of 10^{-4} M and 10^{-6} M the hydrogen donation activity of GaAOA (Fig.1b) was very low, similar to this of HAOA at concentrations below 10^{-4} M (Fig. 1a). The participation of GaAOA in electron transfer (Fig. 1b) was very intensive and increased with the concentration of the complex. Fig.1 suggested much higher antioxidant effect of GaAOA than this of HAOA, mainly due to participation in electron transfer reactions. Fig. 1b gave ground to propose that the active center involved in hydrogen donation in the ligand structure might be inactivated in the GaAOA complex.

The activity of the blood serum in hydrogen donation was 31% of its activity in electron transfer. The effect of a compound on a type of reaction was presented as a percentage from the involvement of the serum alone in this type reaction. The effects of HAOA and GaAOA on the participation of rat blood serum in hydrogen donation and electron transfer were illustrated in Fig.2.

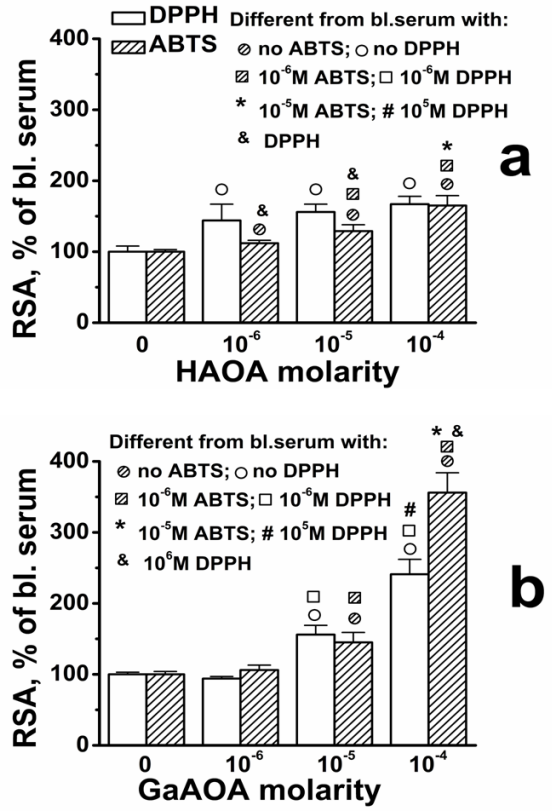

Fig. 2 . Effects of HAOA (a) and GaAOA (b) on the participation of rat blood serum in hydrogen donation and electron transfer.

Both HAOA (Fig.2 a) and GaAOA (Fig. 2b) increased the involvement of the rat blood serum in hydrogen donation and electron transfer reactions. In presence of HAOA the blood serum antioxidant activity via reactions of hydrogen donation increased to 167±11 %, while the antioxidant action trough reactions of electron transfer increased to 165±14 % (Fig. 2a). In presence of GaAOA in the blood serum the hydrogen donation increased to 241±21 % and the participation in electron donation increased to 356±28 % (Fig.2 b). Data in Fig.1a suggested a strong enhancement of the hydrogen donation and electron transfer due to GaAOA interactions with the serum.

DISCUSSION

As a result of the present investigation it was safely concluded that both HAOA and GaAOA were *in vitro* antioxidants. Both DPPH and ABTS tests demonstrate that HAOA behaves as a better proton donor rather than an electron donor (Fig. 1a), while GaAOA being a markedly better electron donor than proton donor (Fig. 1b). It might be assumed that GaAOA was more active antioxidant than HAOA (Fig. 1). HAOA with a concentration 10^{-4} M increased predominantly the proton donating antioxidant activity, while GaAOA increased mainly the electron donating antioxidant activity within the range of all concentrations tested.

Both compounds investigated increased the antioxidant activity of the rat blood serum, the effect of GaAOA being stronger than this of HAOA (Fig. 2). As Ga(III) exhibits a prooxidant effect [4,9] and the Ga(III) complex with 5-aminoorotic acid was better antioxidant than HAOA alone in the blood serum, it might be assumed that the complex did not decompose in the serum. If the interactions with the components of the serum did not result in GaAOA decomposition, there is a chance for transportation of the intact complex in the blood stream. Latter is important for a promising tumor targeting of an anticancer agent.

CONCLUSIONS

1. Both 5-aminoorotic acid and its complex with Ga(III) were *in vitro* antioxidants, due to participation in hydrogen donation and in electron transfer reactions. Based on the interactions with $DPPH^{\bullet}$ and $ABTS^{+\bullet}$, the total *in vitro* antioxidant effect of GaAOA was higher than this of HAOA.

2. 5-aminoorotic acid was better hydrogen donor than its complex with Ga(III). The Ga(III) complex was more active in electron transfer than HAOA alone.

3. Both HAOA and GaAOA increased the antioxidant activity of rat blood serum, the effect being stronger in the presence of the complex than this in the presence of 5-aminoorotic acid alone.

4. It was proposed that because of its stability GaAOA might be promising anticancer agent with antioxidant effect in the blood stream.

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