STABILITY OF GADOLINIUM-BASED CONTRAST AGENTS IN THE PRESENCE OF ZINC AND CALCIUM IONS IN DIFFERENT MEDIA

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To improve the safety of Gd^{3+}-based contrast agents (GBCA) in clinical practice, it is recommended to use the most stable substances and to consider conditions determining their stability. The aim of this study was to compare the stability of GBCAs for magnetic resonance imaging in the presence of zinc and calcium ions and polyvinylpyrrolidone (PVP) in water, phosphate buffer solution and blood serum using proton NMR relaxometry. The study demonstrated that macrocyclic gadobutrol is more stable than all linear contrast agents. The addition of PVP (10 mg/ml) improved the stability of linear GBCAs in phosphate buffer solution and blood serum. Calcium ions have a much weaker destabilizing effect on GBCAs than zinc ions.

Keywords: gadolinium-based magnetic resonance contrast agents, polyvinylpyrrolidone, calcium ions, zinc ions, NMR relaxometry

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СТАБИЛЬНОСТЬ ГАДОЛИНИЙСОДЕРЖАЩИХ МАГНИТНО-РЕЗОНАНСНЫХ КОНТРАСТНЫХ СРЕДСТВ В ПРИСУТСТВИИ ИОНОВ ЦИНКА И КАЛЬЦИЯ В РАЗЛИЧНЫХ СРЕДАХ

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Для повышения безопасности клинического использования гадолинийсодержащих магнитно-резонансных контрастных средств (МРКС) рекомендовано применять наиболее стабильные препараты и учитывать условия, определяющие их стабильность. Целью исследования был сравнительный анализ стабильности Gd^{3+}-содержащих МРКС в присутствии ионов цинка, кальция и поливинилпирролидона в воде, фосфатном буфере и сыворотке крови с использованием метода протонной ЯМР-релаксометрии. Было показано, что макроциклический гадобутрол обладает большей стабильностью, чем все линейные МРКС. Поливинилпирролидон (10 мг/мл) способен улучшить стабильность линейных МРКС в фосфатном буфере и сыворотке крови. Ионы кальция обладают значительно менее выраженным дестабилизирующим действием на МРКС, чем ионы цинка.

Ключевые слова: гадолинийсодержащие магнитно-резонансные контрастные средства, поливинилпирролидон, ионы кальция, ионы цинка, ЯМР-релаксометрия

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It is known that gadolinium-based contrast agents have found their widest application in MRI studies [1]. Although gadolinium is present in them as a chelate, one should bear in mind that the toxicity of this rare earth element in its free form can be compared to that of mercury and lead [2] and that the stability of gadolinium-based magnetic resonance contrast agents (MRCAs) varies and is determined by two major factors: 1) a chemical structure of a chelator; 2) a presence of some organic and non-organic ligands in the medium that can compete for binding to Gd^{3+} ions or a chelating compound thus facilitating Gd^{3+} release.

Using an unstable contrast agent can be life threatening for patients with impaired renal function since free gadolinium retains in tissues and can cause nephrogenic systemic fibrosis [3–5].

Recent studies demonstrated an increased intensity signal in such brain structures as globus pallidus and dentate nucleus on unenhanced T_1-weighted MR images in patients [6] or laboratory animals [7] who had received low stability linear MRCAs before, which is possibly related to Gd^{3+} depositing. After administration of high stability macrocyclic MRCAs, no such "residual" increased signal was observed. It is also known that gadolinium release from MRCAs depends on the presence of various ions in the surrounding medium [8]. Therefore, a complex study on how the above mentioned factors interact can shed some light on the dynamics of Gd^{3+} release from a chelate complex in various media, as well as estimate the risk of administering certain contrast agents to patients with renal insufficiency or conditions accompanied by increased zinc or calcium ions concentration in blood. Improving the stability of these contrast agents, as by means of adding a substance with strong chelating properties, is also important. Polyvinylpyrrolidone (PVP) with its chelating and detoxifying properties can be regarded as such a substance [9].

The aim of this study is to conduct the comparative analysis of the stability of Gd^{3+}-based MRCAs in the presence of zinc ions, calcium ions and PVP in water, phosphate buffer solution and human serum.

METHODS

The following linear Gd^{3+}-based MRCAs were studied: gadopentetate dimeglumine (Magnevist 0.5 M, Bayer, Germany); gadobenate dimeglumine (MultiHance 0.5 M, Bracco, Italy); sodium gadopentetate + PVP (Dipentast 0.125 M, Epidbiomed Group of Companies, OOO, Russia); gadopentetate-β-cyclodextrin (Cyclogadopentetate 0.125 M, Epidbiomed Group of Companies, OOO, Russia), and gadobutrol, a macrocyclic MRCA (Gadovist 1 M, Bayer, Germany)

Contrast agents stability was assessed by proton NMR relaxometry (Minispec mq 20, Bruker, Germany). Gadolinuim release from a chelate affects proton relaxation times in the medium [10]. T_1 relaxation time was measured since MR signal intensity depends on this parameter. Stability assays of the substances listed above were performed in distilled water (pH 6.0), phosphate buffer and blood serum (pH 7.4). In the experiments with zinc, stability of five MRCAs was assessed, i.e. gadopentetate dimeglumine, sodium gadopenetate with PVP, gadopentetate-β-cyclodextrin, gadobutrol and gadobenic acid, whereas in the experiments with calcium only gadopentate dimeglumine was involved.

To obtain 0.2 M phosphate buffer (pH 7.4), aqueous solutions of NaH_2PO_4 and Na_2HPO_4 were prepared [11]. Blood serum was obtained from the patients of A.N.Ryzhikh State Scientific Centre for Coloproctology. All donors signed the informed consent to their biological material being used in the scientific research under the conditions of respecting their privacy and confidentiality. Blood was collected in sterile tubes with a clot activator and a barrier gel. Serum was obtained by centrifuging blood at 1200 g for 10 minutes and stored frozen at –20 °C for no more than 10 days. Prior to freezing, serum samples were tested for albumin concentration on Spotchem EZ SP-4430 clinical chemistry analyzer (Arkray Inc., Japan). Then the samples were diluted in phosphate buffer until albumin concentration of 10^{-4} M (close to physiological) was obtained.

A 200 mM $ZnCl_2$ aqueous solution (Komponent-reaktiv, Russia) was prepared by dissolving the weighted amount of 2.7 g in 100 ml distilled water. The final concentration of $ZnCl_2$ in the sample was 2 mM. While adjusting $ZnCl_2$ final concentration, we drew on the study by M. Taupitz et al. [12] that demonstrated the most illustrative results at this particular $ZnCl_2$ concentration. The concentration of the initial $CaCl_2$ aqueous solution (Komponent-reaktiv, Russia) was also 200 mM (2.2 g $CaCl_2$ in 100 ml distilled water), the final concentration in the sample was 2 mM. The initial aqueous solution of PVP (Kollidon® 17 PF, BASF) was prepared by dissolving 500 mg PVP powder in 1 ml distilled water.

To assess the stability of the studied MRCAs, two samples were prepared simultaneously. The first sample was a 0.2 mM MRCA solution. T_1 relaxation time of the 0.2 mM MRCA solution was measured at 40 °C (temperature value in the sample chamber of the MR relaxometer). Then a zinc chloride or calcium chloride solution was added to the sample until the final concentration of 2 mM was reached; then relaxation time was measured again. After that the sample was incubated in the thermostat at 40 °C; T_1 measurements were repeated in 1, 2 and 24 hours. The second sample was similar to the first one, the difference being a PVP solution with a final concentration of 10mg/ml added to it after adding zinc chloride or calcium chloride. In the second sample relaxation time was measured at the same time points.

Within the framework of this study all experiments were repeated sixfold to improve the reliability of the results. Using Statistica 10 software, mean values and standard deviations were computed. Because of the normal distribution of the obtained data (in all cases of sample checks using the Kolmogorov-Smirnov test, the p-value was substantially higher than 0.05), a statistical significance of differences between the means was determined by Student's t-test, the difference being significant with $p < 0,05$.

RESULTS

Effect of zinc ions on MRCAs stability

In distilled water T_1 longitudinal relaxation time of all linear MRCAs shortened by an average of 23-28% (Fig. 1) in the absence of PVP 24 hours after the addition of zinc chloride. In the gadopentetate dimeglumine sample T_1 value lowered by 25.7 ± 0.6 %, in the sodium gadopentetate sample — by 28.1 ± 0.7 %, in the Cyclogadopentetate sample (CGP)— by 22.0 ± 0.5 %, in the gadobenate dimeglumine sample— by 24.8 ± 0.4 %, respectively. For macrocyclic gadobutrol T_1 did not change significantly.

In phosphate buffer without PVP, T_1 of all linear MRCAs lowered by an average of 13–19 % 24 hours after the addition of zinc chloride. We observed a reduction in T_1 by 18.1 ± 0.7 % in the gadopentetate dimeglumine sample, a reduction by 19.3 ± 0.8 % in the sodium gadopentetate and PVP sample, a reduction by 12.8 ± 0.6 % in the CGP sample, a reduction by 15.9 ± 0.5 % in the gadobenic acid sample. In the gadobutrol

sample T_1 did not undergo any significant alterations (Fig. 2). The lowest T_1 value was observed 1 and 24 hours after the addition of zinc chloride as opposed to its immediate reduction in the previous series of experiments with distilled water being a medium.

After adding PVP to gadopentetate dimeglumine, T_1 decreased by 7.9 ± 0.7 %, in the sodium gadopentetate sample it decreased by 12.3 ± 0.7 % (Fig. 3). Thus these MRCAs showed a statistically significant improvement in stability in the presence of PVP by an average of 10% and 7 %, respectively. PVP improved the stability of CGP by 13 %, with T1 displaying no significant changes 24 hours after its addition. In the gadobenic acid sample T_1 final values in the presence and in the absence of PVP did not show a significant difference. No effect of zinc ions on gadobutrol relaxation time was observed in phosphate buffer in the absence or presence of PVP.

In blood serum in the absence of PVP T_1 of MRCAs decreased by an average of 31–61 %, the most significant reduction was observed 1 and 24 hours after the addition of zinc chloride to the solution (Fig. 4). Of all linear MRCAs the best stability figures were observed in CGP— T_1 decreased by an average of 31.2 ± 0.3 %; the worst results were observed in gadopentetic acid salts: in the dimeglumine salt sample T_1 decreased by 61.2 ± 0.6 %, in the sodium salt sample — by 56.1 ± 0.1 %. In the gadobenic acid samples T_1 lowered by 50.2 ± 0.1 %, in the gadobutrol samples no significant decrease in T_1 was observed. Stability improvement of gadobenic acid by PVP was slight, but statistically significant (by 5%). PVP did not have any effect on the stability of other MRCAs (Fig. 5).

Effect of calcium ions on MRCAs

Exposed to calcium and zinc ions, gadopentate dimeglumine showed no significant variation in T_1 in the absence of PVP in water, and a T_1 reduction by 7.8 ± 0.7 % and 9.1 ± 1.1 % in phosphate buffer and blood serum respectively. The addition of PVP resulted in a statistically significant improvement in gadopentetate dimeglumine stability in phosphate buffer and blood serum. In the tests with calcium ions gadopentetate dimeglumine stability did not change in the presence of PVP in water, which a constant T_1 value is indicative of.

DISCUSSION

According to the obtained results T_1 longitudinal relaxation time shortens in all linear MRCAs samples after the addition of zinc regardless of PVP presence. A shortened T_1 relaxation time can be explained by a transmetalation reaction between zinc and a MRCA molecule: zinc ions replace gadolinium ions in a chelate, while in its free form gadolinium can shorten the proton relaxation time in the medium. Zinc ions did not have any effect on macrocyclic gadobutrol.

The results of our work demonstrate a higher stability of macrocyclic MRCAs and confirm literature data on gadolinium dissociation in vivo when linear MRCAs are used as opposed macrocyclic [13]. They also confirm that zinc facilitates gadolinium release from linear but not macrocyclic chelates as a result of transmetalation [12].

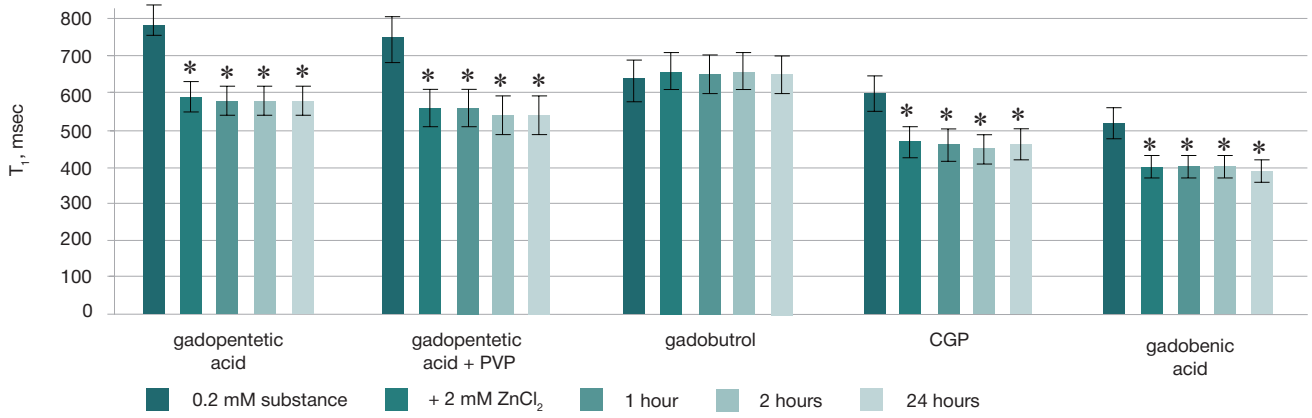

Fig. 1. Effect of zinc ions on T_1 water proton relaxation time in the studied MRCAs solutions in water (pH 6.0) Here and in fig. 2-5 below: CGP — gadopentetate-β-cyclodextrin .* — statistically significant difference from the control. Procedures and conditions of the experiment are described in "Materials and Methods"* — statistically significant difference from the control (p <0.05).

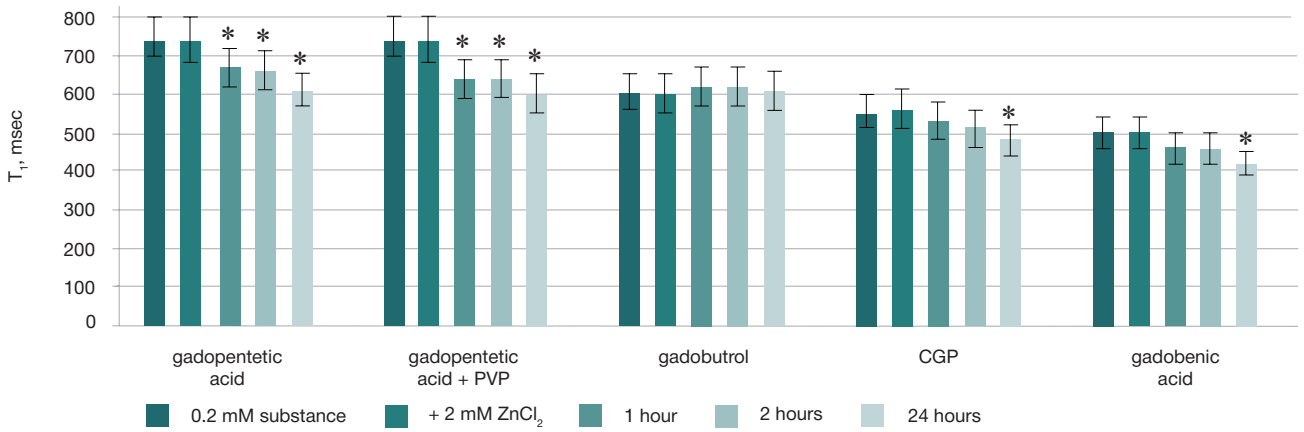

Fig. 2. Effect of zinc ions on T_1 water proton relaxation time in MRCAs solutions in phosphate buffer (pH 7.4)

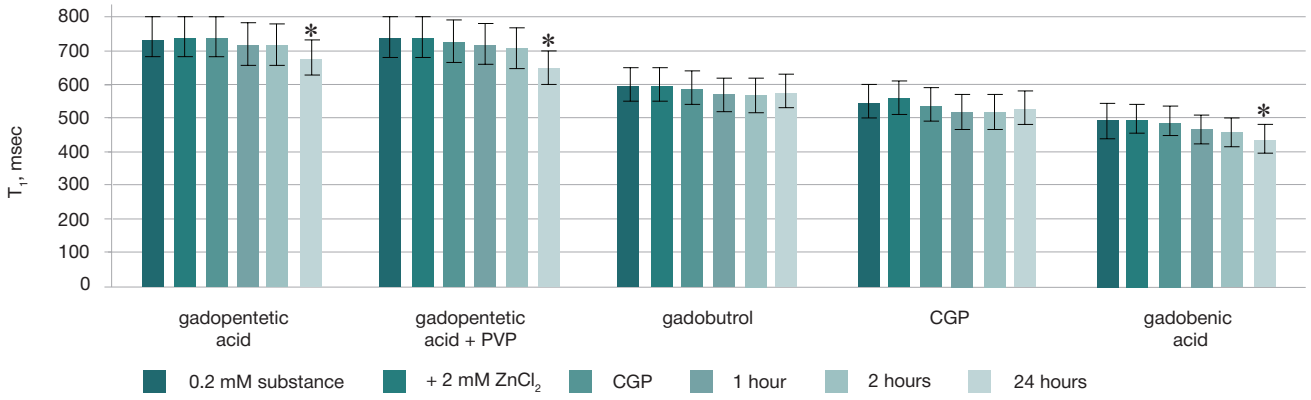

Fig. 3. Effect of zinc ions and PVP on T_1 water proton relaxation time in MRCAs solutions in phosphate buffer (pH 7.4)

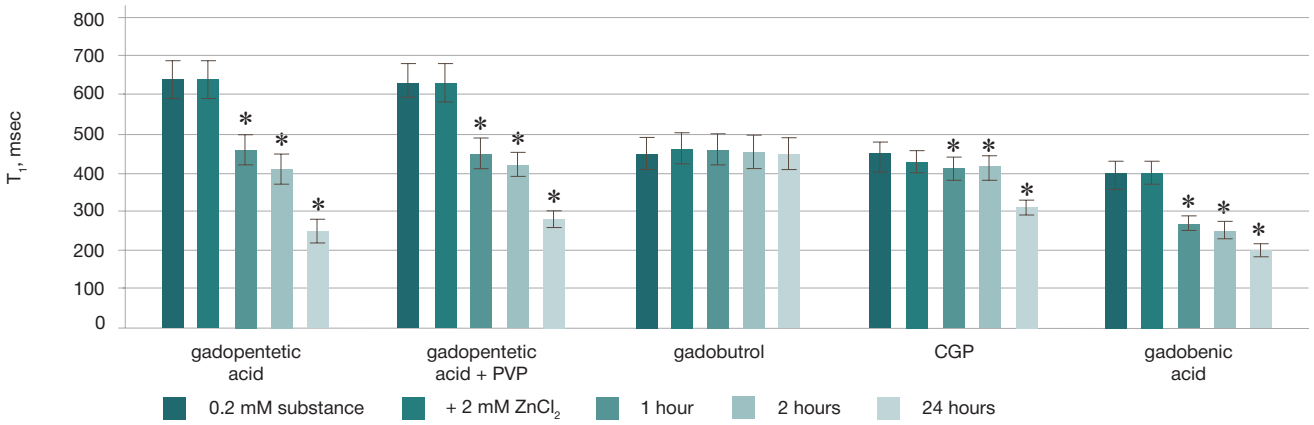

Fig. 4. Effect of zinc ions on T_1 water proton relaxation time in MRCAs solutions in blood serum diluted in phosphate buffer (pH 7.4)

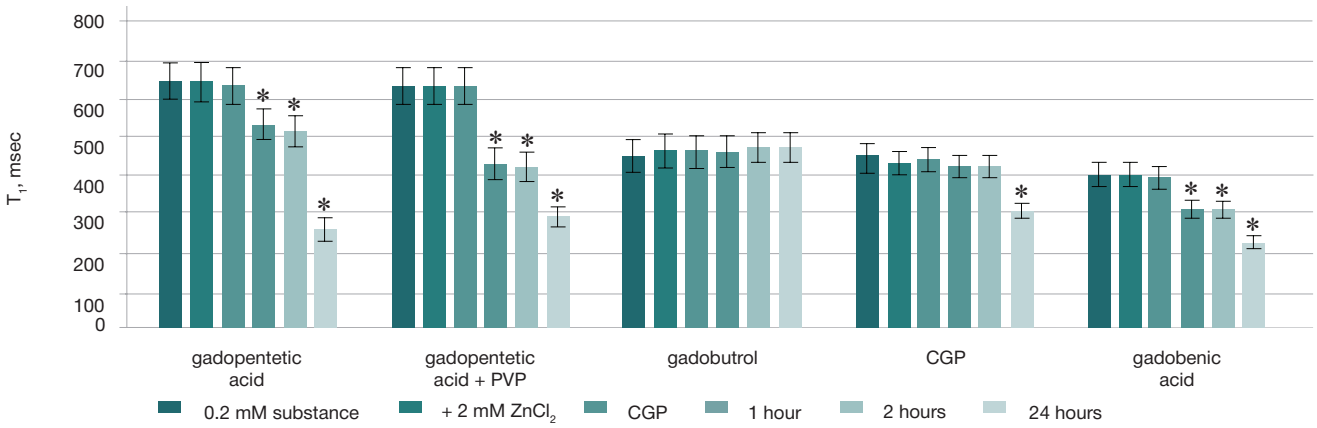

Fig. 5. Effect of zinc ions and PVP on T_1 water proton relaxation time in MRCAs solutions in blood serum diluted in phosphate buffer (pH 7.4)

In serum diluted down in phosphate buffer to albumin concentration of 10^{-4} M, T_1 decreased more than in two other media within 24 hours. It is probably the result of a larger number of compounds in the serum that can interact with both positively charged Gd^{3+} ions (phosphate, citrate, carbonate, heparin and others) and negatively charged chelates (metal cations), which leads to the destabilization of a large number of MRCAs molecules and creates a higher concentration of free gadolinium compared to other media. As a result, T_1 reduction in blood serum tests is the most considerable. The results of tests with zinc and calcium ions showed that calcium ions effect on gadopentetate dimeglumine stability is weaker.

PVP significantly improved stability of three studied linear MRCAs in phosphate buffer, as opposed to water solution. Thereby a question of adding PVP as an auxiliary component to the pharmaceutical forms of linear MRCAs should be raised.

In patients with renal insufficiency MRCAs half-life is prolonged. Administering linear MRCAs, specifically non-ionic that are less stable than macrocyclic, to such patients should be avoided [14]. This recommendation is also relevant for patients with conditions accompanied by increased zinc and phosphate levels in blood.

CONCLUSIONS

Macrocyclic gadobutrol is more stable than other studied linear magnetic resonance contrast agents. Zinc ions do not have any effect on its relaxation properties. Linear MRCAs show the highest stability in the presence of zinc ions in phosphate buffer, and the lowest stability in blood serum. Polyvinylpyrrolidone demonstrates a statistically significant although slight improvement of linear MRCAs stability in phosphate buffer (by 10% in gadopentetate dimeglumine, by 7% in sodium gadopentetate, by 9 % in CGP) and in blood serum (by 5% in gadobenic acid) and not in water. Calcium ions have a much less destabilizing effect on gadopentetate dimeglumine than zinc ions.

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