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ORIGINAL ARTICLE

In Vitro Cytogenotoxic Evaluation of Gadobutrol on MCF-7 Cell Line and Computational Molecular Docking Analysis

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Abstract

Background: Gadobutrol (Gd) is a highly water-soluble, hydrophilic gadolinium-based (Gd-based) contrast agent with thermodynamic stability, bound to a non-ionic, macrocyclic hard chelate complex. The aim of this study was to evaluate the possible genocytotoxic effects of gadobutrol, which is widely used worldwide, in breast cancer cells (MCF-7). Furthermore, the interaction energy level of gadobutrol with B-DNA was evaluated in silico using molecular docking.

Methods: Gadobutrol (0.1 mM, 1 mM, 10 mM and 100 mM) was applied to MCF-7 cells and the decrease in cell viability and IC 50 dose were evaluated by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) method. After determining the IC 50 values of gadobutrol (3.31 mM, 6.63 mM, 13.27 mM and 26.54 mM), the genotoxic effects of gadobutrol on MCF-7 cells were examined using Comet assay. Furthermore, a molecular docking experiment was performed using Schrödinger Maestro 13.9 to predict the possible interaction of gadobutrol in the crystal structures of the B-DNA molecule.

Results: All concentrations of gadobutrol did not cause a statistically significant change in terms of genotoxic effect in MCF-7 cells (ns $p > 0.05$). However, all concentrations applied for MTS statistically significantly decreased cell viability in MCF-7 cells (**$p < 0.01$ and ****$p < 0.0001$, respectively). According to the results of in silico analysis; Gadobutrol is located in the minor groove of DNA. Hydrogen bonds were formed between the hydroxyl groups of the molecule and DNA bases and the free binding energy was determined as -6.14 kcal/mol.

Conclusions: According to the results of the study carried out under in vitro conditions, it was determined that gadobutrol used in MRI imaging did not show genotoxic effects but statistically decreased cell viability. In addition, the interaction of gadobutrol with B-DNA suggested that it may induce apoptosis in MCF-7 cells. The cytogenotoxic effect of gadobutrol in MCF-7 cells may indicate a promising new strategy for breast cancer treatment.

Keywords: Cancer; MCF-7 cell line; Gadobutrol; Cytotoxicity; Genotoxicity; in silico molecular docking.

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INTRODUCTION

There are many imaging techniques used in the field of health. Magnetic resonance imaging (MRI) is one of the most preferred imaging techniques because it provides the highest anatomical resolution and highest contrast for soft tissues (1-3). Despite the various benefits of MRI, such as its high resolution and ability to detect soft tissues, contrast agents are administered to patients during MRI in 40-50% of examinations (4, 5). Contrast agents are used to solve imaging problems when contrast is insufficient for imaging. Due to their paramagnetic properties, Gd-based contrast agents (Gd-CAs) have been successfully used in MRI since the 1980s (6). Paramagnetic contrast agents are metal ions with unpaired electrons, unlike superparamagnetic contrast agents, which are colloid materials with thousands of magnetic ions. These agents are composed of unpaired electrons from dysprosium, Gd or manganese, providing the magnetic moment and forming chelates to reduce their toxicity (7). Gd (III), the most commonly used metal atom in contrast agents during MRI, has a larger magnetic moment and a stable structure compared to other metals (8).

Gadobutrol, a Gd-based contrast agent, reduces the toxicity of this substance by chelating the Gd ion in its structure to other molecules (9). However, Gd-CAs contain both linear and macrocyclic chelates; linear chelates are more likely to release free Gd ions into the body (10). Cells can accumulate free Gd ions released from the chelated molecule by transmetallation mechanisms, allowing the Gd ion to detach from the chelated molecule and bind to metals in the blood or extracellular fluids. During this process, Gd has been reported to accumulate in skin, kidney, brain and bone tissues (8). Due to its increasing popularity worldwide, a large number of people are exposed to contrast agents used in MRI scans (11). The literature contains a number of studies evaluating the genotoxicity of gadobutrol using different methodologies. Some of these studies reported that gadobutrol may be genotoxic (12), while others reported no genotoxic effect (9).

Due to the increased use of contrast agents, it is now necessary to assess the harmful effects of chemicals on human health. The MTS assay is generally known as a simplified and one-step MTT assay as it does not involve intermittent procedures (13). This method uses the mitochondrial dehydrogenase enzyme to convert the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide to a water-insoluble purple colour in living cells (14). Since formazan crystals are formed in living cells, the intensity of the colour is closely related to mitochondrial activity (15).

Genotoxicity tests are widely used to determine whether chemical agents cause chromosomal abnormalities, mutations and DNA damage. Among these methods, the comet assay is one of the short-term genotoxicity tests commonly used to detect DNA damage caused by various chemical agents, including cancer (16). The comet assay measures the degree of DNA damage using three parameters: tail density, length and tail moment. Comet tail density is considered to be the most useful measurement as it is not affected by experimental conditions and can measure the widest range of DNA damage. Tail density (the percentage of DNA in the tail) and tail moment (the integration of the percentage of DNA in the tail and the tail length) are frequently used (17).

Molecular docking techniques are crucial for the development of pharmaceuticals and to shed light on inhibitory mechanisms and interaction strategies. Molecular docking is used in in silico drug design to predict the bond conformations and free binding energies between ligands and macromolecular targets (18). There are two types of molecular docking: flexible body docking, where the ligand is flexible and the macromolecule is rigid, and rigid body docking, where the macromolecule and ligand are both rigid. These studies help to determine the interaction between ligand and macromolecule (19). The gadobutrol we used in our study is used as a contrast agent in MRI. In silico molecular docking study was performed to investigate the binding properties of this substance to DNA. This represents the first reported docking study of gadobutrol's interaction with DNA. MTS and Comet test systems were used to determine its genotoxic and cytotoxic potential.

MATERIALS AND METHODS

This study was conducted using commercially available cell lines and did not involve human participants or animal subjects. Therefore, ethical approval was not required. All experimental procedures were performed in accordance with the principles of the Declaration of Helsinki.

The test substance we used for our study was gadobutrol, which is used as a contrast agent in magnetic resonance imaging (Figure 1). This substance is chelated with other

molecules. If the chelation is weak, it causes Gd ion accumulation in living tissue and this accumulation may pose a genocytotoxic risk to the organism (8). MTS test was performed using MCF-7 to examine the effect of the substance on cell viability. The concentration that reduced the cell viability rate by 50% compared to the control group was found to be 26.54 mM and this value was determined as the IC50 value. The other three concentrations (13.27 mM, 6.63 mM and 3.31 mM) were prepared as 50% decreasing multiples of this value.

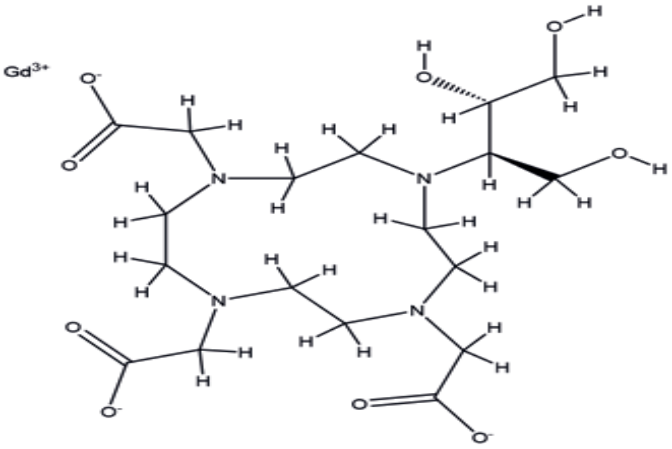


Figure 1: Chemical structure of Gadobutrol drawn with ChemDraw 22.2

MTS Assay

MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium] (Promega; Fitchburg, WI, USA) assay was performed according to the manufacturer's protocol. Briefly, 5000 breast cancer cells were plated in the wells of a 96-well plate and cells were treated with 4 different gadobutrol concentrations of 0.1, 1, 10 and 100 mM for 24 hours. After 4 h incubation with MTS reagent, the absorbance of the wells was measured at 490 nm with a spectrophotometer (Spectramax; BMG Labtech., Offenburg, Germany). The percentage of cell viability was used to evaluate the cytotoxicity-inducing effects of gadobutrol.

Comet Assay and Cell Culture

Human breast cancer cell line was purchased from American Type Culture Collection (ATCC), USA and plated in DMEM (Dubelco's Modification on Eagle's Medium, Gibco, USA) containing 10% FBS (Fetal Bovine Serum), 1% (v/v) Penicillin Streptomycin. Cells were incubated at 37 °C and 5% CO2 pressure. All experiments involving the handling of live cells were performed in a Class II Biosafety Cabinet. The cells were provided with a living environment by the incubator under 5% CO2 pressure. Cell viability was determined by trypan blue. After viability test, MCF-7 cells were treated with predetermined concentrations of gadobutrol (3.31 mM, 6.63 mM, 13.27 mM and 26.54 mM) for 1 h and incubated at 37 °C. 35% hydrogen peroxide (H2O2) (100 µl) was used as a positive control. Comet assay was performed by modifying the method developed by Singh et al. (20). The evaluation of the results was made as follows;

The evaluation of the results was performed as follows;

For each group, i.e. slide, 100 cell images were scored and recorded. Cells were scored according to increasing degree of nuclear damage.

Grade 0 = No damage, Grade 1 = Slightly damaged, Grade 2 = Moderately damaged, Grade 3 = Very damaged, Grade 4 = Extremely damaged.

Total Damage Score (Genetic Damage Index = GDI)

Genetic Damage Index (GDI) = (0 × G0 + 1 × G1 + 2 × G2 + 3 × G3 + 4 × G4) /N

The sum of the average number of damaged cells (grade 2, grade 3 and grade 4) was evaluated as the damaged cell index (DCI) (21).

Molecular Docking

A simulated screening technique called molecular docking studies was performed using the Schrodinger Maestro Version 13.9 program to predict the possible interactions and behaviors of the target molecule and ligand (22, 23). To study the binding mechanism between gadobutrol (PubChem CID: 6102852) and B-DNA (PDB ID: 1BNA), B-DNA was downloaded using the protein data bank. Cocrystals, metal ions, water molecules and cofactors were removed using Maestro Schrodinger's protein preparation wizard (24). A grid encompassing the entire DNA was created. Using the LigPrep module, the lowest energy conformers of Gadobutrole were generated. Once the ligand and protein were prepared, the docking wizard was used to complete the docking process. Interpretation of the molecular docking data was done by considering that the binding affinity with the target protein region increases with negative binding energy value.

Statistical Analysis

GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com) was selected for statistical analysis. The normal Gaussian distribution of the data was confirmed by the Shapiro-Wilk normality test. Comparisons of cell viability and genotoxicity values were made using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data are expressed as mean ± SD. Differences were accepted as significant when *$p < 0.05$. Significance levels are shown in the figures as follows: **or = $P < 0.01$, and **** or = $P < 0.0001$.

RESULTS

The cytotoxic and genotoxic effects of the test substance and MCF-7 cancer cell line were investigated. For this purpose, MTS and Comet test systems were applied and the findings are given in Figure. 2, Figure 3 and Figure 4. In addition, the findings of in silico molecular docking study to determine the affinity of the substance to DNA are given in Table 1, Figure 5 and Figure

Table 1. Types of binding and docking score between Gadobutrol and B-DNA

Chemical	DNA	Docking Score	Binding Type
Gadobutrol	B-DNA	-6.14 kcal/m	A:T8-H Bond
			A:T8-H Bond
			A:A6-H Bond
			B:C21-H Bond
			B:C21-H Bond
			B:G22-H Bond
			B:G22-H Bond

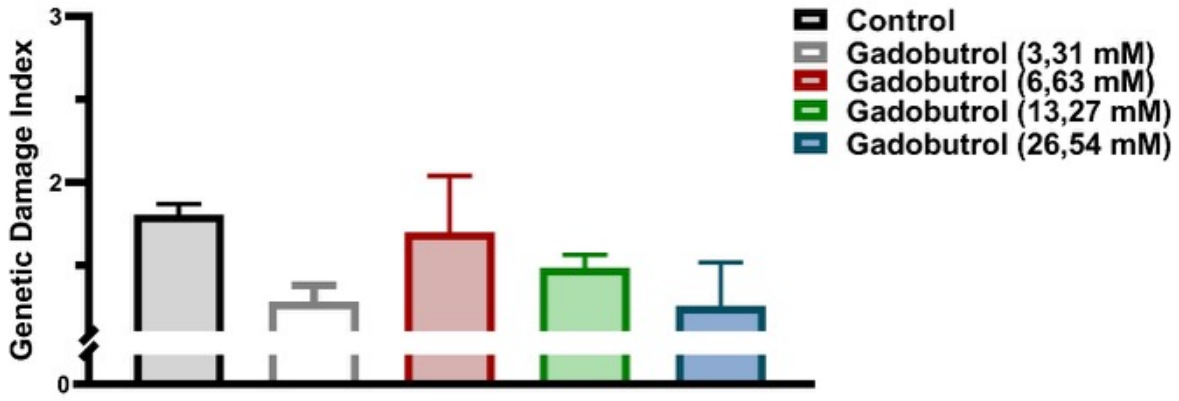


Figure 2: Mean ± SD values of Genetic Damage Index of gadobutrol (3,31, 6,63, 13,27 and 26,54 mM) in MCF-7 breast cancer cells. ns $p > 0.05$ vs control (one-way ANOVA, post-test Tukey)

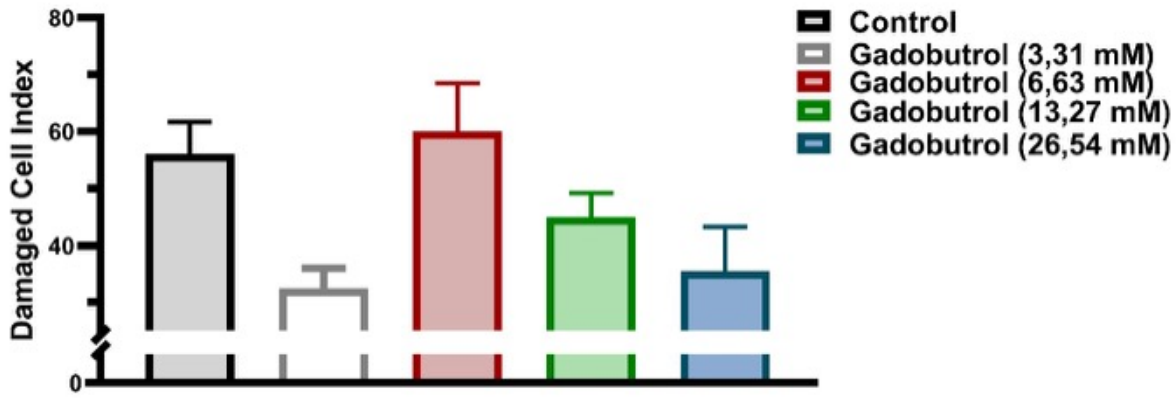


Figure 3: Mean ± SD values of Damage Cell Index of gadobutrol (3,31, 6,63, 13,27 and 26,54 mM) in MCF-7 breast cancer cells. ns $p > 0.05$ vs control (one-way ANOVA, post-test Tukey)

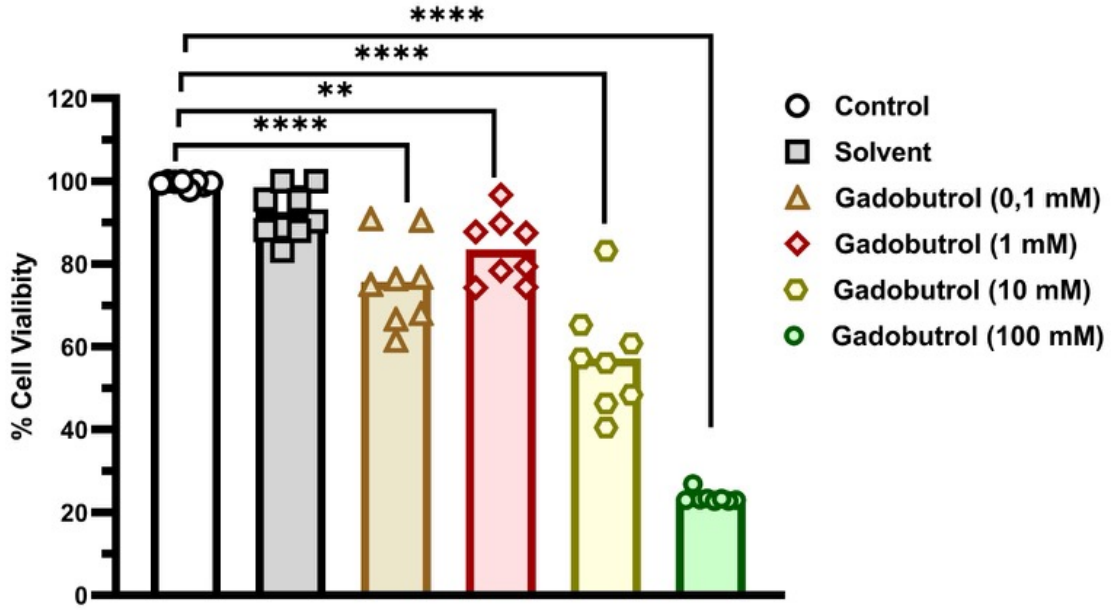


Figure 4: Effect of gadobutrol (0,1, 1, 10 and 100 mM) on cell viability in MCF-7 breast cancer cells. **$P < 0.01$ and ****$P < 0.0001$ vs control (one-way ANOVA, post-test Tukey)

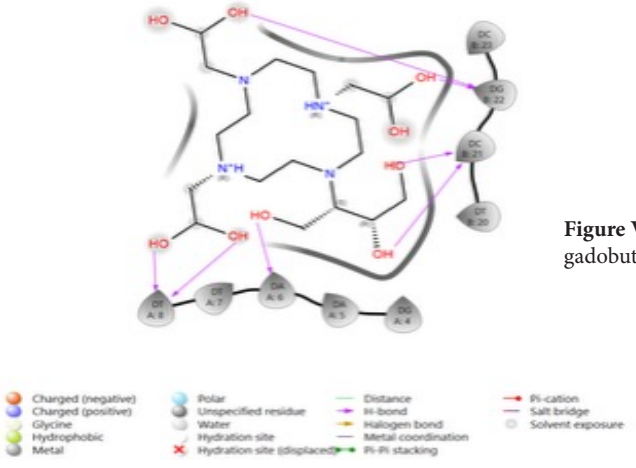
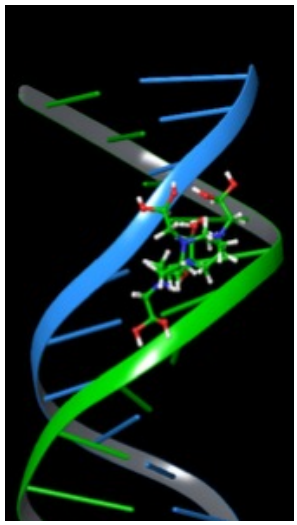
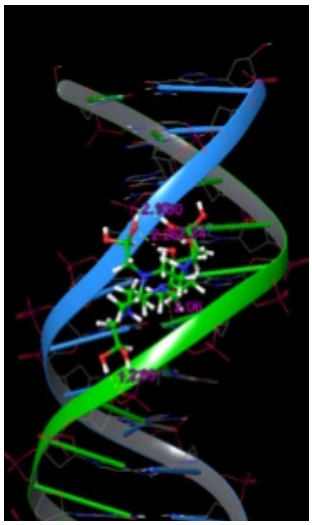


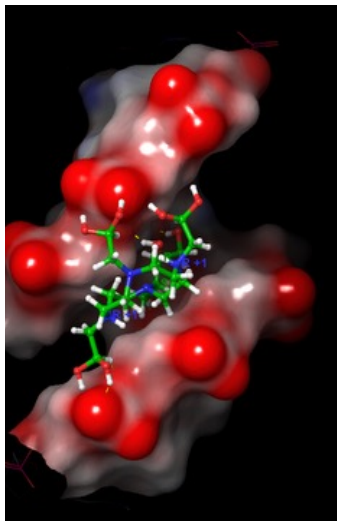
Figure V: B-DNA interaction diagram of gadobutrol in MCF7 cancer cells



Best docking pose



Receptor-ligand interaction measure



Receptor-ligand intraction surface

Figure VI: Best docking pose of gadobutrol between B-DNA, receptor-ligand interaction size and receptor-ligand interaction surface in MCF-7 cancer

Genotoxic effect of gadobutrol

Gadobutrol concentrations of 3.31 mM, 6.63 mM, 13.27 mM and 26.54 mM were applied to MC-7 under in vitro conditions. At all concentrations, no statistically significant difference was observed in both the genetic damage index and the percentage of damaged cells compared to the control. (ns $p > 0.05$).

Cell viability

All concentrations of gadobutrol showed an effective inhibition on cell viability by MTS assay. These values were statistically compared with the control groups. A statistically significant decrease in cell viability was observed at all concentrations of gadobutrol (0,1 1, 10 and 100 mm) compared to the control (**$p < 0.01$ and ****$p < 0.0001$)

Molecular docking

As a result of the molecular docking study, the interaction diagram between gadobutrol and DNA is shown below with the bond types and bond lengths. In order to predict the interaction between the ligand and the macromolecule, the docking score indicating the negative binding energy is considered (Table 1). The lowest negative score is indicative of high affinity and has been reported as a threshold value of -6.00 (25). In this study, the binding energy was calculated as -6.14 when looking at the docking score. Gadobutrol is localized in the minor groove of DNA. Hydrogen bonds were formed between the hydroxyl groups of the molecule and DNA bases. Chemical bond types and docking score in kcal/mol are shown in Table 1.

DISCUSSION

Gd-based contrast agents have been part of magnetic resonance imaging since the 1980s. The rapid and widespread use of magnetic resonance imaging (MRI) has led to increased use of contrast agents. Considering that Gd-based contrast agents are used in 35% of all MRI scans, it is very important to determine the potential risks in patients exposed to the substance (4-6). In the present study, MTS and comet assay tests were performed to determine the genocytotoxic effects of gadobutrol used as a Gd-based contrast agent. In addition, in silico molecular docking studies were performed to show the possible interactions between gadobutrol and DNA.

The MTS assay is one of the common methods used to measure cell viability (26, 27). In our study, all concen-

trations of gadobutrol (0.1, 1, 10, 100 mM) caused a statistically significant decrease compared to the control. When cells die, they lose the ability to convert MTS to formazan, so that color formation functions only as a useful and convenient marker of living cells. (28). Although the precise cellular mechanism for the reduction of MTS to formazan is not well understood, it may involve a reaction with NADH or similar reducing molecules that transfer electrons to MTS (15). In this case, it can be hypothesized that MTS measures mitochondrial activity (29). Under physiological conditions, reactive oxygen species (ROS) are produced in mitochondria and maintained at minimal levels (30). Gd causes an increase in cellular ROS levels and a decrease in ATP synthesis and mitochondrial metabolic activity. Furthermore, Gd can alter neuronal transmission by altering Ca2+ influx through voltage- or ligand-gated channels and can induce apoptosis, increased LDH release and oxidative stress in rat cortical neurons (31-35). It may cause ROS accumulation due to the inhibitory effect of Gd on mitochondrial metabolism (36). Akbas et al. (37) examined the cytotoxic effects of gadobutrol and reported that the substance showed cytotoxic effect. Similarly, Erdogan et al. (36) reported that gadobutrol significantly affected cell viability at increasing concentrations. These data support our findings. Çobanoğlu (38) reported that gadobutrol had no cytotoxic effect on human peripheral lymphocyte cultures. The results of this study differ from our findings. This may be due to the difference in the cells and concentrations used.

Comet assay is a widely used method to assess the stability of genetic material and the damage caused by environmental toxins or drugs to the genetic structure (39-41). According to the results of comet assay of gadobutrol using MCF-7 cell line, no statistically significant change was observed in the genetic damage index and damaged cell ratio parameters. This indicates that the substance does not cause statistically significant damage to the genetic material. When the studies conducted using different test systems to evaluate the genotoxic potential of the substance were examined, Wack et al. (9) reported that the substance did not show genotoxic effect in their study with gadobutrol. B These data support our study. Akbas et al. (37) reported that gadobutrol has genotoxic potential. In another study, using the cytokinesis-blocked micronucleus method, which is frequently used in genotoxicity studies, it was reported that the substance has genotoxic potential at increasing doses (38). Similarly, Fiechter et al. (12) conducted a study with 20 volunteers to examine the genotoxicity of gadobutrol in cardiac MRI. As a result of this study, they reported that it has genotoxic potential. Our findings differ from the results of their study. It is thought that the reason why the results differ from each other may be due to different concentrations, sensitivity of test systems, treatment times and cell differences.

In the docking analysis of gadabutrol and B-DNA, gadabutrol was located in the small groove of DNA. The H bonds formed between them provided more stable binding to the structure. The binding energy of gadabutrol to DNA was determined as -6.14 kcal/mol. This binding energy was close to the threshold value (-6.0 kcal/mol) (25). When the data we obtained as a result of docking are evaluated together, the binding tendency of gadobutrol to DNA supports its cytotoxic effect. Accumulated Gd binds to DNA, suggesting that it drives the cell to apaptosis. Although our in silico docking analysis contrasts with our comet assay results, the comet assay can detect double strand breaks in DNA. Gd may have caused single chain breaks in DNA and we may not have been able to detect these damages by comet assay technique. In this case, Gd may have caused statistically insignificant damage to DNA by localizing in small grooves of DNA and binding stably with hydrogen bonds. On the other hand, gadobutrol may have prevented the progression of the replication mechanism during DNA synthesis by disrupting intracellular mechanisms.(42, 43) It is known that reactive oxygen species accumulating in the cell as a result of weak chelation of gadobutrol cause cytotoxic effect by damaging DNA (44). Our in silico analysis supports both our genotoxicity and cytotoxicity findings. No docking study between gadobutrol and DNA was found in the literature. In this context, the study will provide original information to the literature.

A limited number of studies investigating the genotoxic and cytotoxic effects of gadabutrol using various cell types and assay methods were not found in the literature. When the data are evaluated comprehensively, in silico molecular docking results show that gadobutrol binds DNA with a free binding energy of -6.14. On the other hand, gadobutrol did not show genotoxic effect in MCF-7 cell line but showed cytotoxic effect. Although it partially explains the genocytotoxic effect of gadobutrol under in vitro conditions, it needs to be confirmed by in vivo models and clinical studies.

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Abbreviations list

Gd: Gadobutrol
MCF-7: Breast cancer cells
MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
Gd-CAs: Gd-based contrast agents
ATCC: American Type Culture Collection
FBS: Fetal Bovine Serum
H2O2: Hydrogen peroxide
GDI: Genetic Damage Index
DCI: Damaged cell index
ANOVA: One-way analysis of variance
ROS: Reactive oxygen species

Ethics approval and consent to participate

This study was conducted using commercially available cell lines and did not involve human participants or animal subjects. Therefore, ethical approval was not required. All experimental procedures were performed in accordance with the principles of the Declaration of Helsinki.

Consent for publication

The authors approve publication in this form

Availability of data and materials

Data will be provided upon request from the authors

Competing interests

The authors declare no conflicts of interest

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