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Cytogenetic Toxicity of Magnevist in Stems Cells for Bone Marrow Mice and Finds a Less Toxic Dose for Medical Use

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Abstract: ***This study was established to identify the cytogenetic effect of magnevist of the bone marrow ofmice and calculate the dose less toxic and safe for medical use. It was treated with a negative control group 0.2 ml of distilled water. Magnevist was given alone in mice with intraperetonially 0.6 mg / kg for 24 hours and 48 hours. This was considered positive controls. For the treatment groups have given Magnevist alone to mice intraperetonially with 0.4 mg / kg for 24 hours and 48 hours and given Magnevist to mice intraperetonially with 0.2 mg / kg for 24 hours and 48 hours. . The results showed high induction (P <0.05) of chromosomal aberrations (CAS) and micronuclei (MN) and a decrease in mitotic index (MI) when compared to cationic groups with negative group and positive groups. Interestingly it was observed that the treatment groups were not significant value that low doses with minimal impact on the security of the bone marrow cells.***

Keywords: Chromosomal aberrations, Micronuclei, Magnevist Mitotic index

1. Introduction

Magnetic Resonance imaging (MRI) and magnetic contrast agents play important roles for the diagnosis of diseases, so the demand for agents MRI contrast to the new, with high sensitivity and important functions, is necessary. Inorganic nanoparticles have unique characteristics, such as a large surface area, easy functionalization the surface, and the effect of the contradictory excellent and other characteristics that depend on the size, so they are usually used MRI contrast agents[1].Magnetic resonance imaging has good characteristics allows us to imagine the internal structure of different organs in the human body and its functions. The distinction between the soft tissues in the human body can be done using magnetic resonance imaging, which gives better visibility of computed tomography (CT). Nor is the use of ionizing radiation in the MRI, which means that there are no side effects that may arise from this radiation as in CT. Magnetic moment can be developed using magnevist, which is paramagnetic agent [2].Magnevist (gadopentetatedimeglumine) is one of the brand names for a gadolinium-based magnetic resonance imaging (MRI) contrast agent. Magnevist classify as extracellular gadolinium-based contrast agents (Gd-CAs) most broadly used contrast agents for MR imaging [3].Although there are restrictions on the use of a drug application of chemotherapy, according to high damage to non-cancerous tissue, but to prove the therapeutic efficiency of high-dose chemotherapy injection growing in importance every day [4].From our observation of former studies show that the dose agreed by magnevist have a significant effect on bone marrow cells, and earlier studies shown that the magnevist is toxic contrast agent, which shows the toxic impact significant genes on stem bones mouse cell in the marrow and there is a significant decline in MI and the increasing importance in California and MN.This study aims to assess the effects of stem cells magnevist toxic to bone marrow in addition to the expense of toxic doses lowest for magnevist in bone marrow cells.

2. Materials and Methods

Magnevist dose and concentration
Magnevist was the manufacture of (Payer, Germany); single dose of the magnevist was used (0.6 mg/kg). These dose adoptions from leaflet come back to Bayer HealthCare Pharmaceuticals Company. It was purchased from Al-Karma Teaching Hospital as vial. For mouse injection (intraperetonially), a dose of 0.6 mg/kg, 0.4 mg/kg and0.2 mg/kg were prepared by diluted the drug in distilled water to prepare the required dose and concentration [5].

Laboratory animals
Seventy Albino Swiss male mice were gained from National Center for Drug Control and Research / Ministry of Health / Baghdad. Their ages ranged between (8-12) weeks and weighting (25 ±2) gm. They were divided into 7 groups; each group was put in a separated plastic cage under optimal conditions in the animal house of Collage of Science-University of Waist.

Administration of laboratory animals
All animals' studies groups divided according to concentration of doses and injected time as fallow:

Control group.
Group I: Negative control, (10 mice) treated with 0.2 ml of D. W. The mouse bone marrow samples were taken for cytogenetic analysis (MI, CA, and MN).

Magnevist study groups
Group I: Positive control 1, the animals were treated with 0.2 ml Magnevist 0.6 mg/kg and sacrificed after 24 hrs.(10 mice).
Group II: Positive control 2, the animals were treated with 0.2 ml of Magnevist 0.6 mg/kg and sacrificed after 48 hrs.(10 mice).

The treatment studies

Group I: Treatment group 1, the animals (10 mice) were treated with Magnevist (0.4mg/kg) and sacrificed after 24 hrs.

Group II: Treatment group 2, the animals (10 mice) were treated with Magnevist (0.4 mg/kg) andsacrificed after 48 hrs.

Group III: Treatment group 3, the animals (10 mice) were treated with Magnevist (0.2 mg/kg) and sacrificedafter 24 hrs.

Group IV: Treatment group 4, the animals (10 mice) were treated with Magnevist (0.2 mg/kg) and sacrificedafter 48 hrs.

The mice bone marrow samples were taken for cytogenetic analysis (MI, CA, and MN).

3. Cytogenetic Experiments

Chromosome preparation from somatic cells of the mouse bone marrow

The experiment was done according to Allen *et al*[6]. Colchicine was injected 2 hr. before sacrifice. Mice were sacrificed by cervical dislocation. It was dissected and both of femur bones were excised. Bone marrow was aspirated by flushing with phosphate buffer saline (PBS) in the centrifuge tube. The suspension was flushed in the tube properly to get good cell suspension and centrifuged for 10 min at 2000 rpm. Supernatant was discarded and the pellet was treated with pre-warmed (37°C) KCl (0.56%) and shaken well. Suspension incubated in a water bath at 37°C for 20 min. Pellet was treated with freshly prepared fixative solution (Methanol: Glacial Acetic Acid, 3:1) and shaken well then centrifuged for 10 min at 2000 rpm. Fixative was repeated 3 times to get debris free white pellet. Few drops from the tube were dropped vertically on the slide. Slides were kept overnight to dry then stained with (Giemsa's stain) and observed under microscope in 40 x and then in 100x magnifications. A total of 100 well spread metaphase plates were scored for chromosomal aberrations) gap, chromatid break, polyploidy, acentric fragment, ring and fragmentation (were counted and data of scoring was expressed as percentage chromosomal aberrations.

4. Cytogenetic Analysis

1) Mitotic index (MI) assay

The slides were examined under high power (40x) of light microscope, and (1000) of divided and non-divided cells were counted and the percentage rate was calculated for only the divided ones (metaphase cells) according to the following equation:-

$$\text{Metaphase Index (\%)} = \left(\frac{\text{Number of Metaphase Cells}}{\text{Total number of the cell(1000)}}\right) \text{x } 100$$

2) Chromosomal aberration (CA) assay

The prepared slides were examined under the oil immersion lens (100x) of light microscope for 100 divided cells per each animal, and the cells should be at the metaphase stage of the mitotic division where the chromosomal aberrations were clear and the percentage of these aberrations could be estimated.

3) Micronucleus MN assay

This experiment was done according to method of Schmid [7] as follow:-

The femur bone cleaned from tissues and muscles, then gapped from the middle with a forceps in a vertical position over the edge of a test tube by a sterile syringe, (1 ml) of human plasma (heat inactivated) was injected so as to wash and drop the bone marrow in the test tube. Then the test tubes were centrifuged at speed of 1000 rpm (5 min).The supernatant was removed, and one drop from the pellet was taken to make a smear on a clean slide. The slides were kept at room temperature for (24 hr.). The slides were fixed with absolute methanol for (5 min.), then stained with Giemsa stain for (15 min), then washed with D.W and left to dry. Two slides for each animal were prepared for micronucleus test. The slides were examined under the oil immersion lens, and at least 1000 polychromatic erythrocytes (PCE) were examined for the presence of micronucleus. The micronucleus index was obtained using the following equation:

$$\text{Micronucleus Index} = \left(\frac{\text{Number of Micronuclei}}{\text{Total Count of PCE}}\right) \text{x } 100$$

5. Statistical Analysis

The values of the investigated parameters were given in terms of mean ± standard error, and differences between means were assessed by analysis of variance (Two-sampleT-test) using the computer program Minitab release (14.12) discovery Copyright 2004. The difference was considered significant when the probability value was less than $p<0.05$.

6. Results and discussion

The results of metaphase test are presented in Table (1). There is a significant different when we compare between negative control and positive control (I and II) and this differences was back to the toxic effect of MTX alone and magnevist alone too by reducing the mitotic index (MI). And there is no significant different when we compare the treatment groups (I, II and III, IV) with negative control (0.2 ml of D. W.).

Table 1: Percentages of mitotic index in bone marrow of mice for negative control, positive control groups and treatment groups (Mean ± SE)

Group's	Mitotic index% M+SE
Negative control (0.2 ml of D.W.)	6.600 + 0.30
Positive control I (Magnevist 0.6 for 24 hr.)	[a]*4.02 + 0.291
Positive control II (Magnevist 0.6 for 48 hr.)	[a]*3.52 + 0.159
Treatment group I (Magnevist 0.4 for 24 hr.)	[b]5.4 + 0.210
Treatment group II (Magnevist 0.4 for 48 hr.)	[b]6.28 ±0.299
Treatment group III (Magnevist 0.2 for 24 hr.)	[b]6.4+0.400
Treatment group IV (Magnevist 0.2 for 48 hr.	[b]6.5 + 0.2

[a]Positive control groups vs. Negative control, *Significant at ($p<0.05$)

[b]Treatment groups vs. Negativecontrol,no Significant at ($p<0.05$)

Table 2: Percentages of different types of chromosomal aberrations (CA) in bone marrow of mice for negative control, positive control groups and treatment groups (Mean ± SE)

Experimental Groups	Chromosomal aberrations %						Total %
	Acentric Fragment%	Ring %	Polyploidy %	Break %	Fragment %	Gap %	
Negative control (0.2 ml of D.W.)	4.40 ± 1.36	0.000 ± 0.000	0.000 ± 0.000	0.200 ± 0.200	5.000 ± 0.707	1.000 ± 0.447	11.60 ± 1.05
Positive control I (Magnevist for 24 hr.)	15.80 ± 2.24	1.600 ± 0.510	2.200 ± 0.970	3.200 ± 0.917	20.40 ± 3.36	10.20 ± 3.01	[a*]53.40 ± 3.23
Positive control II (Magnevist for 48 hr.)	16.00 ± 3.86	0.400 ± 0.400	1.000 ± 0.548	0.600 ± 0.400	39.40 ± 2.09	24.40 ± 6.10	[a*]81.80 ± 6.56
Treatment group I (Magnevist 0.4 for 24 hr.)	9.200 ± 0.735	0.000 ± 0.000	0.000 ± 0.000	0.300 ± 0.300	6.60± 1.57	8.20± 1.91	[b]24.3± 0.26
Treatment group II (Magnevist0.4 for 48 hr.)	7.800± 0.490	0.000 ± 0.000	0.000 ± 0.000	0.200 ± 0.200	7.20± 1.66	9.00 ± 1.12	[b]24.0± 0.258
Treatment group III (Magnevist0.2 for 24hr.)	5.000 ±0.949	0.0±0.0	0.0± 0.0	0.25 ±0.2	6.400 ±0.927	5.000 + 0.447	[b]16.67 ± 1.52
Treatment group IV (Magnevist 0.2 for 48 hr.)	8.00 ± 0.90	0.000 ± 0.000	0.000 ± 0.000	0.500 ± 0.500	5.20 ± 1.66	7.00± 1.67	[b]20.33± 1.76

[a]Positive control groups vs. Negative control, *Significant at ($p<0.05$)
[b]Treatment groups vs. Negative control, no Significant at ($p<0.05$)

Chromosomal aberrations findings present in Table 2. Animals treated with magnevist positive control (I and II) with dose (0.6 mg/kg) showed a high frequency of total chromosomal aberrations (53.4%, 81.8%) respectively in mice bone marrow cells, these findings were significant ($p<0.05$) when compared with negative controls (11.6%).

And the animals that treated with lower dose of magnevist treatment groups (I, II, III and IV) gave no significant different (24.3, 24.0, 16.67, 20.3) respectively in mice bone marrow cells, when compare with negative control.

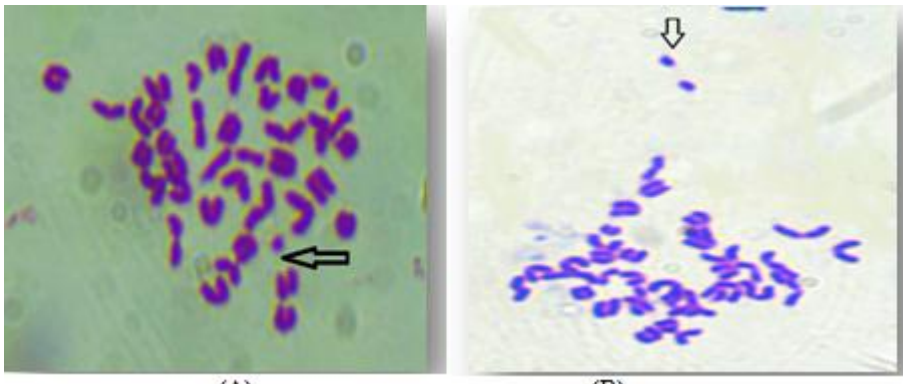
Table 3: Percentages of micronuclei (MN) in bone marrow of mice for negative control, positive control groups and treatment groups (Mean ± SE)

Groups	Micronucleus% M+SE
Negative control (0.2 ml of D.W.)	[a]*2.320±0.17
Positive control I (Magnevist for 24 hr.)	[a]*6.620 ± 0.17
Positive control II (Magnevist for 48 hr.)	[a]*7.440 ± 0.21
Treatment group I (Magnevist 0.4 for 24 hr.)	[b]3.32 ± 0.12
Treatment group II (Magnevist 0.4 for 48 hr.)	[b]3.52 ± 0.159
Treatment group III (Magnevist0.2 for 24 hr.)	[b]2.620 ± 0.12
Treatment group IV (Magnevist 0.2 for 48 hr.)	[b]2.820± 0.146

[a]Positive control groups vs. Negative control*Significant at ($p<0.05$)

[b]Treatmentgroups showed on significant differences when compared with all controlat ($p<0.05$).

The positive control (I and II) has shown a significant reduction in MI and a high increase in CAs and MN. The reason for this results was due to the toxic effect of magnevist that cause DNA damage by produce hydroxyl radicals (•OH), this suggested by Yamazaki *et al* [8] and Kim [9]. The cytotoxic and mutagenic impact for •OH can react with the deoxyribose DNA backbone and bases. Thus, it is probably cause a lot of lesions. The react which happen between •OH and DNA bases by add pi bonds to the electron-rich. The pi bond is situated between C5-C6 of pyrimidines and N7-C8 in purines [10]. The first event that appeared after the reaction between •OH with the deoxyribose sugar backbone represented by the removal of hydrogen from a deoxyribose carbon [11]. Thus creates a 1'-deoxyribosyl radical. After that, the radical may react with molecular oxygen and resulting a peroxyl radical, which can be reduced and dehydrated to form a 2'-deoxyribonolactone and free base. A deoxyribonolactone is mutagenic and resistant to repair enzymes. Thus, a basic site is created [12]. The figure below showed different chromosome aberrations from mice bone marrow for positive control (I and II).



(A) (B)

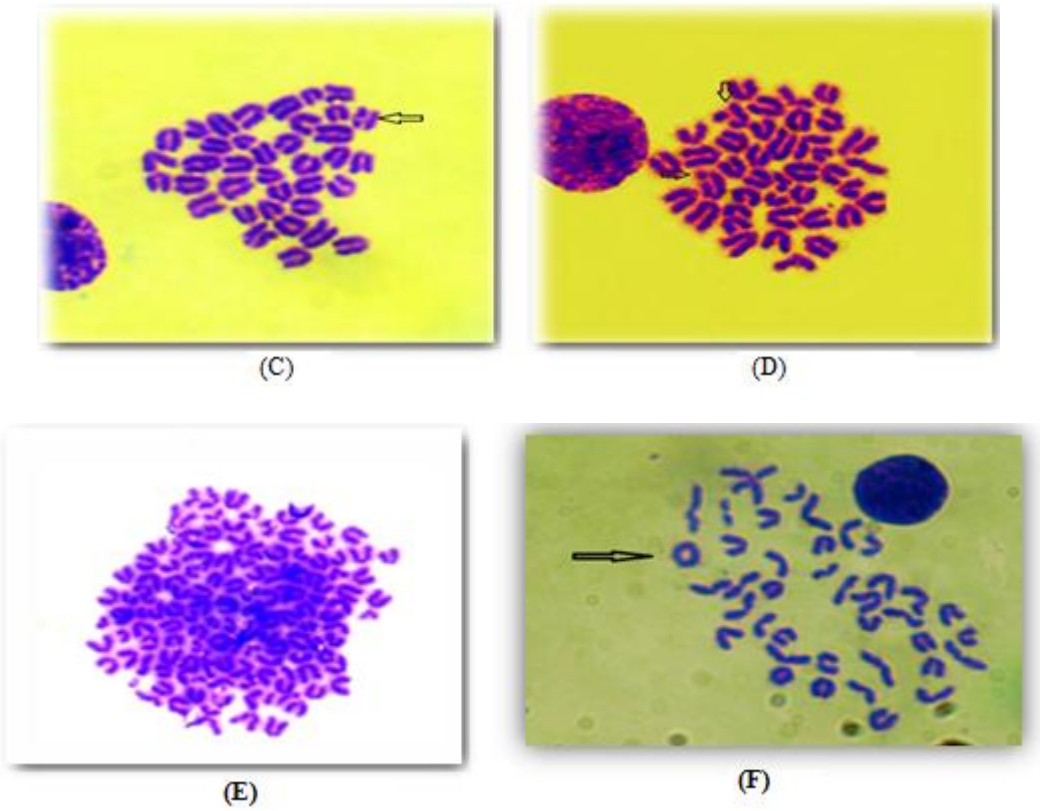


Figure1: Showed different chromosomal aberrations in mice bone marrow (100x) injected only with Magnevist. A: fragment. B: acentric fragment C: gap D: break E: polyploidy F: ring

All treatment groups for CAs and MN have no significant difference when compared with negative control and a significant different when compared with positive controls corresponding to it at ($p<0.05$).

The significant differences in positive control groups , may to the nested effect Gd-DTPA, which Gd-DTPA induce micronuclei and cause an increase in MN because it's effect on the synthesis of DNA and cause chromosome damage (CAs), thus induce MN formation. For the treatment groups were observed significant decrease in the proportion of chromosomal errors and in MN

Figure 2: Magnevist formation MN at (100x)

7. Conclusions

The study concluded that the magnevist is toxic contrast agent, which shows the toxic effects of important genes on bone stem cell mouse in the marrow and there is a significant decrease in MI and increase of MN, so concludes this study that the potions that have been adopted in this the study was less toxic to the bone marrow and with minimal impact and thus doses are considered safe to use.

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