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Evaluation of the mutagenic effect of the iodinated contrast medium Urografina® 292 using the micronucleus test in mouse bone marrow cells

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ABSTRACT

Contrast media (CM) are frequently used in diagnostic radiology and in radiotherapy as a diagnostic tool and in treatment planning. Previous studies have demonstrated that these compounds induce chromosomal aberrations. This study evaluates the mutagenic effects induced by the contrast medium Urografina® 292 (meglumine amidotrizoate and sodium-ionic dimmer) in bone marrow cells (BMC) of mice *in vivo*. Micronuclei assay was performed in BMC of CF-1 mice injected with CM 1.5 and 3.0 mL/kg intravenous doses and 1.0, 2.0, 3.0 mL/kg intraperitoneal doses. The animals were beheaded 24 h after treatment by cervical dislocation, and femur BMC from each animal were used in the micronucleus test. The group treated with the highest intravenous injection of Urografina® 292 (3.0 mL/kg) presented an increase in the frequency of micronucleated polychromatic erythrocytes (MNPCEs) in relation at the control group ($P<0.05$). The results obtained after intraperitoneal administration of CM showed that all doses (1.0 mL/kg, 2.0 mL/kg and 3.0 mL/kg) increased the frequency of MNPCEs, being significantly different from the negative control ($P< 0.01$). The present results suggest that iodinated contrast media Urografina® 292 may cause a significant increase of cytogenetic damage in bone marrow cells of mice.

Key words: chromosomes, contrast medium, micronuclei, Urografina® 292.

INTRODUCTION

Contrast media (CM) are widely used to enhance the contrast of body structures or fluids in medical imaging such as angiography, computed tomography, among others, especially the visibility of blood vessels and of gastrointestinal system structures (Araújo 2007). Therefore, in recent times CM has increasingly become the object of research, in an attempt to assist the evaluation of organs and soft tissues when testing for diagnostic imaging.

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Structurally, CM may have a benzene ring, forming monomers, or two benzene rings, forming dimers. CM can also be classified as negative and positive (Chuang and Morris 2000). Urografina® 292 (meglumine amidotrizoate and sodium-ionic dimmer), the focus of this study, is a dimeric ionic contrast with high osmolality.

Ionic iodinated CM is dissociated in ions when dissolved, and its osmolality is higher than that of so-called non-ionic compounds, which do not dissociate into electrically-loaded particles (Juchem and Dall'agnol 2007). Some

chemical and physical properties of CM, like density, number of atoms of iodine per milliliter of solution, viscosity and osmolality, are related to their efficacy and safety.

The most general adverse reactions to CM are thought to be idiosyncratic or pseudoallergic reactions. Unfortunately these reactions are unpredictable, though they are not dose-dependent and may involve the release of histamine as well as other biological mediators such as serotonin, prostaglandins, bradykinin, leukotrienes, adenosine, and endothelin. To date, no conclusive evidence that these adverse reactions to CM are allergic has been obtained, since antibodies to CM could not be consistently demonstrated. The chemotoxic effects of CM occur due to direct molecular toxicity and to their physiological properties. These effects are more common in debilitated and medically unstable patients (Namasivayam et al. 2006).

Anaphylactic or idiosyncratic reactions do not depend on the CM dose administered, and are usually similar to allergic reactions, like urticaria, nasal cold, hypotension accompanied by tachycardia, bronchial spasm and laryngeal edema, though more intense manifestations like shock and severe respiratory failure are also observed (Juchem and Dall'agnol 2007).

Higher osmolality CM (HOCM) are more likely to cause adverse reactions of different kinds. In turn, low-osmolality agents (LOCM) are associated with less discomfort and fewer cardiovascular and anaphylactic-type reactions. However, LOCM are significantly costlier than HOCM, which prevents them from being used exclusively (Thomas and Maddox 2002).

CM osmolality is yet another detrimental factor that may lead to toxicity in kidney. The use of HOCM has been shown to increase the risk of nephropathy, as compared to LOCM. Therefore, it is possible to say that different kinds of CM may have discrepant adverse effects on renal toxicity (Hsueh-Wei et al. 2007).

Apart from the systemic reactions, chromosomal damage in lymphocytes of patients undergoing radiology examinations based on CM has also been demonstrated. It is known that chromosomal damage in human somatic cells may be one of the events in a process that will eventually lead to the manifestation of diseases such as cancer (Mozdarani and Fadaei 1998). The clastogenic effect of both ionic and non-ionic X-ray CM was reported by various investigators in metaphase analysis or micronuclei scoring (Adams et al. 1977, Parvez et al. 1987, Cochran and Norman 1994, Norman et al. 1978). Although cytogenetic effects of CM were observed in *in vitro* lymphocyte cultures (Norman et al. 1978), most of the *in vivo* studies on this subject were conducted using relatively large doses of radiation such as in excretory urography (Cochran and Norman 1994), angiocardiography (Adams et al. 1977) and angiography (Parvez et al. 1987).

Urografina® 292, the focus of this study, was also studied in detail by Deimling et al. (2008). The authors reported the results of *in vivo* experiments conducted on bone marrow polychromatic erythrocytes (PCE) of mice treated with that CM. The results showed that Urografina® 292 significantly increased the frequencies of micronucleated polychromatic erythrocytes (MNPCEs) in both male and female mice treated with doses of 14.3 and 20.0 mL/kg body weight, respectively.

The possibility that the formation of nuclear anomalies such as micronuclei, chromosomal rearrangements and anaphase bridges induced by chemical and physical agents is associated with early carcinogenesis events increases the mid- to long-term risk in patients receiving iodine-containing X-ray CM (Deimling et al. 2008).

In this scenario, the aim of our study was to evaluate the mutagenic activity of the CM Urografina® 292 administered by two pathways, using the micronucleus test.

MATERIALS AND METHODS

Animals and Chemical Treatment

Male CF-1 mice (2 - 3 months, 45 - 55g) were obtained from our breeding colony (UNESC). The animals were housed five per cage with food and water available *ad libitum* and were maintained on a 12-h light/dark cycle (lights on at 7:00 am) at 22°C ± 1°C. All experimental procedures involving animals were performed in accordance with the National Institute of Heath's Guide for the Care recommendations for animal care. This study was approved by the local ethics committee (Comitê de Ética no Uso de Animais da Universidade do Extremo Sul Catarinense) under protocol number 19/2010.

Drugs

The CM used in the present study was Urografina®292 (Schering, Rio de Janeiro, Brazil), which contains a mixture of sodium amidotrizoate (0.08 g/mL) and meglumine amidotrizoate (0.52 g/mL) in aqueous solution, and iodine as 292 mg/mL. The doses chosen for *in vivo* experiments were clinically relevant concentrations also previously used in (Toulany et al. 2010).

Experimental Design

Two different experiments were conducted. In the first experiment, mice were divided in three groups, with five male mice per group. The control group received saline solution (NaCl 0.9%) intravenously (10 mL/kg), the second and third groups were treated with Urografina 292® as 1.5 and 3.0 mL/kg intravenous doses, respectively, after anesthesia with ketamine through of a surgical incision in the inguinal region of the femoral vein.

In the second experiment, mice were divided in four groups, with five male mice per group, as follows: control group, which received saline solution (NaCl 0.9%) via intraperitoneal injection (10 mL/kg); the second, third and forth groups received the doses of 1, 2 and 3 mL/kg Urografina® 292, respectively.

The animals were killed 24 h after treatment by cervical dislocation and femur bone marrow cells from each animal were used in the micronucleus test.

Micronucleus Test

The micronucleus assay was performed according to the US Environmental Protection Agency Gene-Tox Program (Mavournin et al. 1990). Bone marrow from both femurs was suspended in fetal calf serum and smears were prepared on clean glass slides. Next, slides were air-dried, fixed in methanol, stained in 10% Giemsa and coded for a blind analysis. Two thousand polychromatic erythrocytes were analyzed per mouse (1000 cells from each of two replicate slides). To detect possible cytotoxic effects, the proportion of PCE (polychromatic erythrocytes) and NCE (normochromatic erythrocytes) in 200 erythrocytes/animal was calculated. The slides were scored for blind analysis and inspected using a light microscope equipped with a 100X immersion objective.

Statistical Analysis

The normality of variables was assessed using the Kolmogorov-Smirnov test. Micronucleus test results were analyzed by an analysis of variance (ANOVA) followed by the Tukey post-hoc test, when ANOVA was significant. The critical level for rejection of the null hypothesis was considered to be a two-tailed P value of 5%, and the statistical package used was Bioestat 5.0.

RESULTS

To evaluate the cytotoxic effect of CM, the ratio of PCE/(PCE + NCE) was determined as shown in Fig. 1 and 3, representing the two different experiments, respectively. These figures show that Urografina® 292 administration pathway is cytotoxic.

The clastogenic effect of Urografina® 292 was analyzed by determining the frequency of micronuclei formation in bone marrow cells

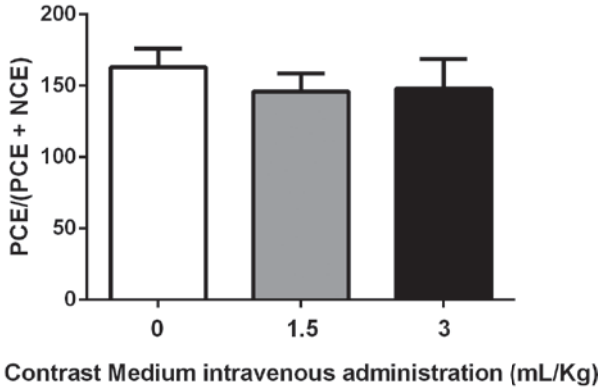

Fig. 1 - Cytotoxic effect of CM *in vivo*. Mice were injected by intravenous administration with the indicated concentration of CM. Data bars represent the mean PCE/(PCE + NCE) ± SD. The data obtained are not significantly different.

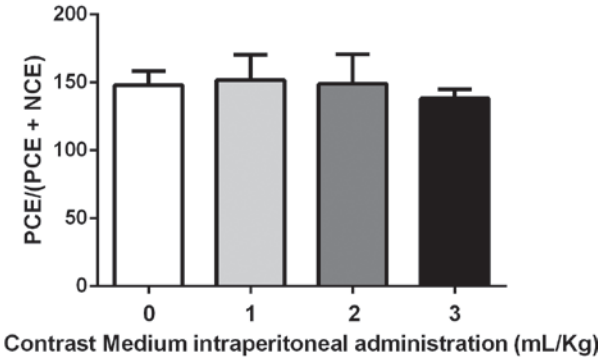

Fig. 3 - Cytotoxic effect of CM *in vivo*. Mice were injected by intraperitoneal administration injected with the indicated concentration of CM. Data bars shown represent the mean PCE/(PCE + NCE) ± SD. The data obtained are not significantly different.

(Fig. 2) 24 h after intravenous administration of CM in mice as 1.5 mL/kg and 3.0mL/kg doses. In this first experiment, the group treated with the highest dose of Urografina®292 (3.0 mL/kg body weight) present an increased MNPCEs frequency (ANOVA, post-hoc Tukey test, $P < 0.05$). Although micronuclei formation induced by Urografina® 292 as 1.5 mL/kg dose was apparently higher than in the negative group, this difference was not significant.

Results of the MN test 24 h after intraperitoneal administration of CM in bone marrow cells of mice are presented in figure 4, which shows that all doses of the CM tested produce increased frequencies

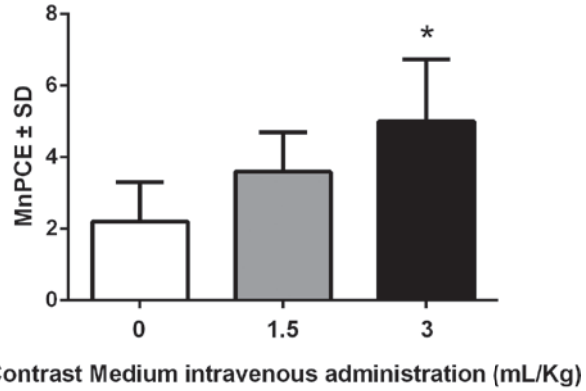

Fig. 2 - Clastogenic effect of ionic CM in bone marrow cells of mice injected by intravenous administration. By scoring 1000 polychromatic erythrocytes (PCEs) per 5 mice of each treatment condition the frequency of micronucleated polychromatic erythrocytes (MNPCEs) was determined and graphed. Asterisks indicate statistically significant differences between frequency of MNPCEs induced by Urografin and the control group (ANOVA, post-hoc Tukey *$p < 0.05$).

of MNPCEs. Comparison of the results obtained for the three doses (1.0 mL/kg, 2.0 mL/kg and 3.0 mL/kg) and negative control group using the ANOVA, post-hoc Tukey showed that all doses were significantly different from negative control ($P < 0.01$).

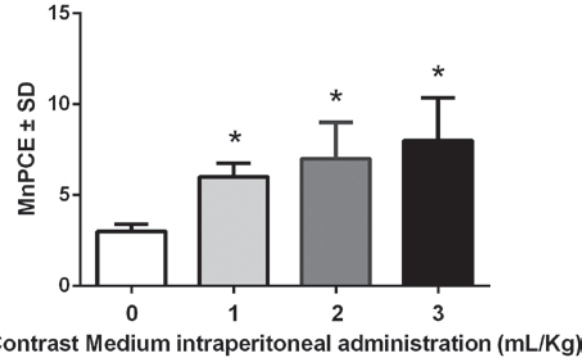

Fig. 4 - Clastogenic effect of ionic CM in bone marrow cells of mice injected by intraperitoneal administration. By scoring 1000 polychromatic erythrocytes (PCEs) per 5 mice of each treatment condition the frequency of micronucleated polychromatic erythrocytes (MNPCEs) was determined and graphed. Asterisks indicate statistically significant differences between frequency of MNPCEs induced by Urografin and the control group (ANOVA, post-hoc Tukey *$P < 0.01$).

DISCUSSION

Contrast media are frequently used in diagnostic radiology and in radiotherapy for treatment planning. These compounds have been demonstrated to induce chromosomal aberrations (Matsubara et al. 1982). Cytogenetic analysis results are greatly important, since cytogenetic events are involved in carcinogenesis mechanisms. It is generally accepted that chromosomal mutations are causal events in the development of neoplasia, and it has been postulated that an increased cytogenetic damage may be an indication of an enhanced cancer risk.

Adams et al. (1977) suggested that the disparity between the X-ray dose absorbed by the blood of patients undergoing angiocardiography and the dose estimated using chromosome count was due to two effects of CM: (a) an increased absorption of X rays as compared to blood, and (b) chromosomes breakage, even in the absence of X rays. Adams et al. (1977) also observed an increased micronuclei frequency in *in vitro* experiments in which human lymphocytes were placed in a suspension containing Renografin 76 (a 76% solution of methylglucamine diatrizoate and sodium diatrizoate) and Hypaque (a 50% solution of sodium diatrizoate).

Mutation frequency and chromosome aberration induction caused by several CM has been the object of a numerous studies. Weeler et al. (1980) reported that the diatrizoate group present in some CM produced chromosomal damage in lymphocytes of patients undergoing angiocardiography. Other reports indicate that CM caused chromosomal damage only when radiation was also used, resulting in enhanced yield of aberrations in lymphocytes (Toulany et al. 2010). It has also been shown that various CM induce micronuclei in peripheral blood lymphocytes (Parvez et al.1987). The results of these elegant studies indicate that irrespective of ionic and osmolarity differences, CM are indeed capable of leading to chromosomal damage in peripheral lymphocytes. Urografina® 292, used in this study, is a structurally ionic monomer and belongs to this group of CM.

These results were obtained in *in vitro* experiments (cultured human lymphocytes and Chinese hamster ovary cells), or were obtained by indirect deduction with regard to patients who had received iodine-containing X-ray contrast agents during radiologic procedures (Deimling et al. 2008).

Mozdarani and Fadaei (1998) evaluated the cytogenetic effects (chromosomal aberrations) *in vivo* of two ionic CM, Urografin 76% (a sodium-meglumine diatrizoate), and Telebrix 38 (a sodium-meglumine ioxythalamate) on lymphocytes of 15 patients undergoing brain computed tomography (CT), before and after examination. The results showed no difference in aberration frequency for patients who underwent brain CT without CM, when compared to controls. However, injection of CM resulted in a high frequency of chromosomal aberrations that significantly differed from controls ($P < 0.05$). The effect of Urografin 76% appeared to be similar to that of Telebrix 38. Therefore, both CM exhibited clastogenic effects on peripheral lymphocytes *in vivo*. In the present study, increases in chromosomal aberrations due to the CM used were similar to that reported for other ionic and non-ionic compounds. The mechanisms behind the chromosome damage caused by CM have yet to be more thoroughly understood, and it has been suggested that cytogenetic damage is not molecule-specific, but may be caused by iodine, ionicity or osmolality of CM (Mozdarani and Fadaei 1998).

In the present study, we observed that Urografina® 292 significantly increased MNPCEs frequency in mice bone marrow cells *in vivo* treated using two administration pathways (intravenous and intraperitoneal injections). The intravenous administration of CM as a 3-mL/kg dose body weight caused a more pronounced increase in the frequency of MNPCEs, in comparison to the control. However, the 1.5-mL/kg dose was not sufficient to

induce a significant increase in MNPCEs frequency, when compared with the negative control group (Fig 2). In addition, when CM was administered intraperitoneally, it was possible to observe that the effects of all doses were significantly different from those seen for negative control group (Fig 4). Micronuclei are formed due to clastogenic or aneugenic activity of chemical agents. Our results indicate that irrespective of the administration pathways, the CM tested here is able to cause chromosomal damage in bone marrow cells.

Toulany et al. (2010) investigated the cytogenetic effects of ionic and non-ionic CM using the Micronuclei assay in bone marrow cells (BMC) of Balb/C mice intraperitoneally injected with CM in the presence or absence of whole-body irradiation of 50 mGy. The authors observed that both compounds reduced proliferation of BMC significantly. In addition, concentrations of 0.5, 1 and 2 mL/kg meglumine or iohexol significantly enhanced the frequency of MNPCEs ($P < 0.01$) and 2 mL/kg of iohexol ($P < 0.05$). When used in combination with irradiation, meglumine as 0.5 and 1 mL/kg doses led to a higher frequency of MNPCEs, as compared to iohexol/IR ($P < 0.05$). The discrepancies in our results shown (figure 2) may be due to the different experimental conditions, administration pathway and differences in CM composition.

Deimling et al. (2008) reported the results of *in vivo* exposure of on bone marrow polychromatic erythrocytes (PCE) of mice to Urografina® 292 (the same compound used in our study) and to purified sodium amidotrizoate and meglumine amidotrizoate administered separately or in combination (at the same ratio and concentration as that of the highest dose of Urografina® 292 used in the experiment administered intraperitoneally). The results showed that Urografina® 292 significantly increased the frequencies of MNPCEs in both male and female mice treated with doses of 14.3 and 20.0 mL/kg body weight. However, when lower doses were used (5.7 and 8.6 mL/kg body weight), no significant increase in the frequencies of MNPCEs was observed, when compared with the negative control group. The same result was observed for both male and female animals treated with purified sodium amidotrizoate and meglumine amidotrizoate separately or in combination. In addition, there was a significantly positive correlation between the Urografina® 292 doses used and micronuclei frequency. These results are partly in agreement with the findings of the present study, since the lower doses administered through the same pathway led to a significant increase in MNPCEs, in comparison to the control group.

Our results and the conclusions arrived at by Deimling et al. (2008) lend strength to the hypothesis that small amounts of aryl amines present in all X-ray CM containing diatrizoate and closely related triiodobenzoates may be genotoxic. Using the Ames Salmonella assay (strains TA15348 and TA98) Weeler et al. (1980) observed that the diatrizoate molecule was not mutagenic (when used as doses up to 500 mg/plate), even in the presence of the S-9 liver fraction. However, 3-amino-5-acetamido-2,4,6-triiodobenzoate, the impurity present in commercial diatrizoates, proved to be mutagenic when used as doses of as little as 0.5 mg/plate, also in the presence of the S-9 liver fraction.

In the present study, the frequencies of PCEs observed were determined to estimate the toxicity of Urografina® 292 to bone marrow. The results indicated that the PCE frequencies did not vary statistically across the groups treated with the different Urografina® 292 doses stipulated, when compared with the negative control group. Identical result was observed by Deimling et al. (2008).

The fact that mutagenic agents are also generally carcinogenic raises concerns about the possible long-term risks these agents pose for patients who are exposed to iodine-containing X-ray CM during radiodiagnostic procedures (Mozdarani and Fadaei 1998). In a given population, increased frequencies of chromosomal aberrations may

indicate an increased risk of cancer. Structural chromosome changes can lead to the activation of proto-oncogenes and elimination of tumor-suppressor genes and therefore represent an important mechanism of tumorigenesis (Heim et al. 1989). The mechanism of action by which CM can cause chromosome damage has not been established. (Sinues et al. 1991), comparing the action of CM (diatrizoate and ioxaglate), concluded that the differences detected may be due to differences in the nature of the agents. In contrast, Cochran and Norman (1978) suggested that chromosomal damage may not be caused by a particular CM; on the contrary, these effects may be imputed to certain properties these CM share.

All in all, our results of the *in vivo* treatment with clinically relevant doses of Urografina® 292 strongly support the *in vitro* observations for Urografina® 292, although further investigations are necessary. Concerning the safe clinical use of these CM, the effects observed may stimulate further investigation into the capacity of CM to induce genetic instability.

ACKNOWLEDGMENTS

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RESUMO

Meios de contraste (MC) são freqüentemente utilizados em radiodiagnóstico e em radioterapia como uma ferramenta de diagnóstico e em planejamento do tratamento. Estudos prévios demonstraram que estes compostos induzem aberrações cromossômicas. Este estudo avaliou os efeitos mutagênicos induzidos pelo meio de contraste Urografina® 292 (Amidotrizoate de meglumina e sódio-dímero iônico) em células da medula óssea (BMC) de camundongos *in vivo*. Teste de micronúcleos foi realizado em BMC de camundongos CF-1, injetados com MC intravenoso nas doses 1,5 e 3,0 mL/kg e intraperitonealmente nas doses 1,0, 2,0 e 3,0 mL/kg. Os animais foram decapitados 24 horas após o tratamento por deslocamento cervical, e as BMC dos fêmures de cada animal foram utilizados no teste de micronúcleos. O grupo tratado com injeção intravenosa mais elevada de Urografina® 292 (3,0 mL/kg) apresentou um aumento na freqüência de eritrócitos policromáticos micronucleados (MNPCEs) em relação ao grupo controle ($P<0,05$). Os resultados obtidos após a administração intraperitoneal do MC demonstrou que todas as doses (1,0 mL/kg, 2,0 mL/kg e 3,0 mL/kg) aumentaram a freqüência de MNPCEs, sendo significativamente diferentes do controle negativo ($P<0,01$). Os presentes resultados sugerem que o meio de contraste iodado Urografina® 292 pode causar um aumento significativo de danos citogenéticos em células da medula óssea de camundongos.

Palavras-chave: cromossomos, meio de contraste, micronúcleos, Urografina® 292.

REFERENCES

ADAMS FH, NORMAN A, MELLOS RS AND BASS D. 1977. Effect of radiation and contrast media on chromosomes. Radiol 124: 823-826.

ARAÚJO A. 2007. Tomografia Computadorizada: Princípios Físicos e Aplicações, n. 1. São Paulo: Corpus, 160 p.

CHUANG S AND MORRIS TW. 2000. Assistência à vida em radiologia: guia teórico e prático. In: SILVA EA ET AL. (Eds), Meios de contraste iodado, São Paulo: Colégio Brasileiro de Radiologia, BRA, p. 116-114.

COCHRAN ST AND NORMAN A. 1994. Induction of micronuclei in lymphocytes of patients undergoing excretory urography with ioversol. Invest Radiol 29: 210-212.

DEIMLING LI, MACHADO FL, WELKER AG, PERES LM AND SANTOS-MELLO R. 2008. Micronucleus induction in mouse polychromatic erythrocytes by an X-ray contrast agent containing iodine. Mutat Res 672: 65-68.

HEIM S, JOHANSSON B AND MERTENS F. 1989. Constitutional chromosome instability and cancer risk. Mutat Res 221: 39-51.

HSUEH-WEI Y, HSIANG-CHUN L, WEN-TE L AND SHENG-HUSIUNG S. 2007. Effects of Acetylcysteine and Probucol on Contrast Medium-induced Depression of Intrinsic Renal Glutathione Peroxidase Activity in Diabetic Rats. Arch Med Res 38: 291-296.

JUCHEM BC AND DALL'AGNOL M. 2007. Immediate adverse reactions to intravenous iodinated contrast media in computed tomography. Rev Lat Am Enfermagem 15: 78-83.

MATSUBARA S, SUZUKI S, SUZUKI H, KUWABARA Y AND OKANO T. 1982. Effects of contrast medium on radiation induced chromosome aberrations. Radiology 144: 295-201.

MAVOURNIN KH, BLAKEY DH, CIMINO MC, SALAMONE MF AND HEDDLE JA. 1990. The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 232: 29-80.

MOZDARANI H AND FADAEI S. 1998. Similar cytogenetic effects of sodium-meglumine diatrizoate and sodium-meglumine ioxithalamate in lymphocytes of patients undergoing brain CT scan. Toxicol Lett 98: 25-30.

NAMASIVAYAM S, KALRA MK, TORRES WE AND SMALL WC. 2006. Adverse reactions to intravenous iodinated contrast media: a primer for radiologists. Emerg Radiol 12: 210-215.

NORMAN A, ADAMS FH AND RILEY RF. 1978. Cytogenetic effects of contrast media and triiodobenzoic acid derivatives in human lymphocytes. Radiol 129: 199-202.

PARVEZ Z, KORMANO M, SATOKARI K, MONCADA R AND EKLUND R. 1987. Induction of mitotic micronuclei by X-ray contrast media in human peripheral lymphocytes. Mutat Res 88: 233-239.

SINUES B, NUÑEZ E, BERNAL ML, ALCALA A, SAENZ MA AND CONDE B. 1991. Micronucleus assay in biomonitoring of patients undergoing excretory urography with diatrizoate and ioxaglate. Mutat Res 260: 337-342.

THOMAS G AND MADDOX M. 2002. Adverse reactions to contrast material: recognition, prevention, and treatment. Am Fam Physician 66: 1229-1234.

TOULANY M, KEHLBACH R, RODEMANN HP AND MOZDARANI H. 2010. Radiocontrast media affect radiation-induced DNA damage repair in vitro and *in vivo* by affecting Akt signaling. Radiother Oncol 94: 110-116.

WEELER L, NORMAN A AND RILEY R. 1980. Mutagenicity of diatrizoate and other triiodobenzoic acid derivatives in Ames Salmonella microsome test. Proc West Pharmacol Soc 23: 249-253.