VETERINARSKI ARHIV 93 (3), 341-350, 2023 DOI: 10.24099/vet.arhiv.1698

Chromosomal aberrations induced by Mitomycin C in canine lymphocytes

Martina Noel Caliri[1, 2], Daniela Marisol Ferré[1, 2], Valeria Ledda[3] and Nora Bibiana Maria Gorla[1, 2*]

[1]National Council of Scientific and Technical Research (CONICET), Argentina
[2]Laboratory of Genetics, Environment and Reproduction, Juan Augustin Maza University, Guaymallen, Mendoza, Argentina
[3]University of Anconcagua, Mendoza, Argentina

CALIRI, M. N., D. M. FERRÉ, V. LEDDA, N. B. M. GORLA: Chromosomal aberrations induced by Mitomycin C in canine lymphocytes. Vet. Arhiv 93, 341-350 2023.

ABSTRACT

Chromosomal aberrations comprise an abnormal number of chromosomes as well as changes in the structure of the chromosomes. Aberrations in the structure of chromosomes, such as gaps and breaks in chromatids and chromosomes, acentric fragments, telomeric associations, deletions, early chromatid-separation and large scale effects, such as pulverized metaphases, and sticky metaphases. In this study, the effect of Mitomycin C, a recognized clastogen on human lymphocytes, was assayed in cultures of canine lymphocytes, an animal species that is flourishing in comparative medicine. Blood cultures were performed on samples from a male and a female dog. Cytotoxicity using the mitotic index and genotoxicity testing were performed with 0.25 µg/ml Mitomycin C. The total chromosomal aberrations were significantly higher due to the effect of Mitomycin C (P=0.0247). The number of chromatid breaks nearly quintupled, while pulverized metaphases were found to be six times more frequent, and endoduplicated cells were three times higher than in negative control cultures. The quantitatively most relevant chromosomal aberration was the presence of sticky metaphases, related to adverse effects in chromatin proteins. The potential use of canine lymphocytes for chromosomal aberration assay is emphasized in the evaluation or re-evaluation of the genotoxic *in vitro* effect of xenobiotics, to evidence chromosomal damage.

Key words: canine cells; cytogenetics; clastogenic agents; chromosomal damage; lymphocyte cultures; mitomycin C

Introduction

Chromosomal abnormalities in the number and normal structure of chromosomes have been recognized as an indicator of genetic damage and as detrimental to human health (DHAWAN and BAJPAYEE, 2013). Any potential genetic damage may be evidenced by implementing genetic toxicology tests, such as those suggested by the FOOD AND DRUG ADMINISTRATION (2012). Numerous tests have been proposed composed of three main components which evaluate the following, at different levels of biological organization: *in vivo* studies with complete organisms in mammals; tools that reflect mutations in DNA; and *in vitro* tests with mammalian cells to

*Corresponding author:
Doctor in Biological Sciences Nora Bibiana Gorla, Laboratory of Genetics, Environment and Reproduction (GenAR), Juan Agustín Maza University, Lateral Sur Acceso Este 2245, Guaymallén, Mendoza, Argentina, C. P. 5519, +54 9 261 4056200 int 262, e-mail: noragorla@gmail.com

ISSN 0372-5480
Printed in Croatia

analyze chromosome aberrations (CA), which was the one carried out in the present study (UDROIU and SGURA, 2017).

Structural chromosomal damage can be produced in several ways, for example, by direct DNA breakage, replication from a damaged DNA strand, and inhibition of DNA synthesis. In addition, covalent binding of adducts can produce base pair substitutions and intercalation of molecules between base pairs, or induce insertions, intra- or inter-strand cross-links. Structural CA can involve a chromatid or both chromatids of a single chromosome, or a group of chromosomes. The structural damage is called clastogenic, and the genotoxic agents that cause this damage share the same name (MATEUCA et al., 2006; CHATTERJEE and WALKER, 2017).

Chromosome aberration testing is recommended to identify chromosome damage, and can be used with cultures of established cell lines, or primary cultures of human or rodent cells, according to the Organization for Economic Co-operation and Development, OECD (2016). Human peripheral lymphocyte cultures are a validated tool for evaluating the biological response to chemicals with genotoxic potential. The traditional method is to analyze metaphases with homogeneous Giemsa staining, although it is also possible to use cytomolecular techniques with resolution at the DNA kilobase level through fluorescence in situ hybridization (FISH) (HU et al., 2020). In any case, a positive control is necessary to demonstrate that it is possible to identify potential clastogens in the chosen biological material. Those which do not require *in vitro* metabolic activation are: methyl methanesulfonate, Mitomycin C (MMC), 4-nitroquinoline-N-oxide, and cytosine arabinoside (OECD, 2016).

MMC is an inhibitor of the "in vitro" proliferation of different cell types because it prevents the separation of double stranded DNA in replication. In addition, it forms covalent cross-links between the DNA of opposite strands (PONCHIO et al., 2000), and also impedes RNA synthesis through its alkylating power. It has also been reported that it hinders the activities of enzymes, such as: phagocyte NADPH-oxidase, NADPH-cytochrome P450, xanthine oxidase, and mitochondrial NADH dehydrogenase (ULUMAN and KILICLE, 2020), and can also promote the formation of free radicals. It has been widely used in human lymphocyte cultures as a genetic damage inducer, although there is no supporting literature with regard to its use on dog lymphocyte cultures. Canines are similar to humans in terms of environment and diseases, so they could also be used and validated for evaluation of chemicals with genotoxic potential.

The presence of genotoxic agents in the environment can cause damage through the different mechanisms of chromosomal mutation, which are associated with the induction of hereditary defects, as well as the development of cancers in the human population (DHAWAN and BAJPAYEE, 2013). Certain cancers that occur spontaneously in companion dogs resemble the histological alterations of human cancers, their progression and their genetic heterogeneity (GARDNER et al., 2019). Numerous known mutations, alterations in gene copy number, chromosomal translocations, and Single Nucleotide Polymorphism (SNPS) associated with increased risk of disease are similar in dogs and humans in numerous cancers (GARDNER et al., 2019). Also, canines are ideal for the study of chronic diseases, because they age five to eight times faster than humans, and generally live to a comparatively advanced number of years (SHEARIN and OSTRANDER, 2010; ALVAREZ, 2014). The possibility of using dog lymphocytes should also be considered beneficial for drugs that are being evaluated for use in humans, given the large areas of synteny and high genomic homology that have been detected in both species (LINDBLAD-TOH et al., 2005; GARDNER et al., 2019).

The aim of this study was to analyze the number and type of spontaneous CA in canine lymphocytes cultured "in vitro"; and to evaluate the potential of MMC to induce CA in this species.

Materials and methods

Animal selection. The study material included two mixed breed dogs (*Canis familiaris*), a male and a female, 3 years old. They were considered to be healthy on the basis of physical examination and

complete blood count to which they are routinely subject because they belong to the University Veterinary Practice Unit (VPU). The animals were kept at the VPU during the study (9 weeks). They were fed with high quality commercial pellets and had free access to drinking water. They were maintained and manipulated according to the statements of the Institutional Committee for the Care of Laboratory Animals, Experimentation and Teaching (CICUALID) of the University Juan Agustín Maza.

Blood sampling. Blood was extracted from the femoral vein under aseptic conditions with heparin. Extractions were performed early in the morning while the animals were fasting. The extracted blood was immediately taken to the laboratory to be processed.

Genotoxicity - mitomycin C testing. Six peripheral blood cultures from the male dog were performed, in different weeks but using the same batches of culture medium, solvents and dye. All cultures were performed in duplicate. Lymphocyte cultures were held in suspension for 72 h at 37° C according to MOORHEAD et al. (1960) modified by MARTIN et al. (2011). 0.5 ml of heparinized blood, 4 mL of RPMI 1640 culture medium, 1 mL of fetal bovine serum (Natocor®), 150 µl of phytohemagglutinin M (Gibco®) and 57 µl of streptomycin (10 mg/mL) - penicillin (10 000 IU/mL) (Sigma®) were placed in Falcon conical tubes. Each time, MMC (Fresenius Kabi®-Germany), dissolved in bi-distilled water to reach 0.25 µg/ml, was added to one of the cultures. The negative controls were cultured without MMC with an equal volume of solvent. After adding120 µL of Colcemid (Gibco) during the final 30 minutes of culture at 72 h, the tubes were centrifuged for 10 min at 3000 rpm; the supernatant was discarded and replaced with a hypotonic solution of cold 0.075 M KCl, and the contents were homogenized and allowed to incubate for 2- 3 min at 4 °C. They were centrifuged again and the supernatant was replaced with Carnoy methanol and acetic acid (3: 1) fixative solution. The samples were kept at between 4 and 8 °C for 24 h, then the fixative was renewed 3 times. They were then spread out on clean slides and stained with 10% Giemsa in distilled water for 10 min. We performed observations by optical microscope (Nikon Eclipse®).

In each culture, 1000 to 3000 cells were examined for their mitotic index (MI), calculated as the number of dividing cells/1000 cells.

Genotoxicity - mitomycin C testing. The In Vitro Mammalian Chromosomal Aberration Test was performed (OECD, 2016) with slight modifications. Three peripheral blood cultures from both animals were performed, in different weeks, using the same MMC concentration and negative control cultures used in the cytotoxicity testing. Cultures were also performed in duplicate.

One hundred metaphases/cultures were analyzed for number and type of CA. Those metaphases that presented no more than 4 overlapping chromosomes and differed in no more than 2 centromers (MOSESSO et al., 2013) from the diploid number of the species (2n=78) were analyzed. In each metaphase, the following observations were recorded: chromatid-chromosome gaps (ctg-csg) and breaks (ctb- csb), acentric fragment (ace), telomeric association by a chromatid-chromatid-association (at1), telomeric associations (at), deletions (del), pulverized metaphase (pvz), endoduplicated metaphase (end), early chromatid segregation (seg) (UDROIU and SGURA 2017) and sticky (sti) chromosomes (ADROVIĆ et al., 2016; ADESUYI et al., 2018). The results are expressed as the frequency of each variable analyzed/100 cells.

Statistical analyses. For the cytotoxicity testing, the mitotic indexes of the cultures with MMC were averaged to obtain a mean and standard error, and the same was done for the control group. Both groups were compared. For the genotoxicity evaluation variables, in each culture, the total number of CA was obtained as the direct sum of the individual frequencies of each aberration. Each type of alteration detected and the total average CA obtained in the cultures with MMC were compared with those of the negative control. We analyzed whether the mitotic indexes and the CA frequencies were distributed in a normal way according to Shapiro-Wilk analysis. Both groups were compared by the Student's unpaired t-test or Mann Whitney test, depending on whether their distributions were

parametric or non-parametric respectively. All tests were performed with a 95% confidence interval. Graph Pad Prism 8.4.3 software was used.

Results

MMC, at the concentration used, did not alter the mitotic index of the canine lymphocyte cultures (Table 1). No dividing cells were recorded in 33% of the cultures with MMC, which also occurred in 16% of the controls.

In the second round of tests, evaluating the genotoxicity of MMC, numerical and structural chromosomal alterations were observed. The former were represented by endoduplications. Of the 12 structural chromosomal aberrations searched for in the slides, 7 types were found in the analysis. At1, ace, csg, del and seg were not detected. Table 2 shows the type and number of chromosomal alterations observed. Some were structural in individual chromosomes (telomeric associations, gaps and breaks) and others affected the entire metaphase (pvz and sti). Some of the chromosomal alterations observed are shown in Fig. 1. The comparison between the total number of CA between the MMC cultures and the control was statistically significant (P=0.0247) since the presence of MMC increased the amount of spontaneous CA by seven times.

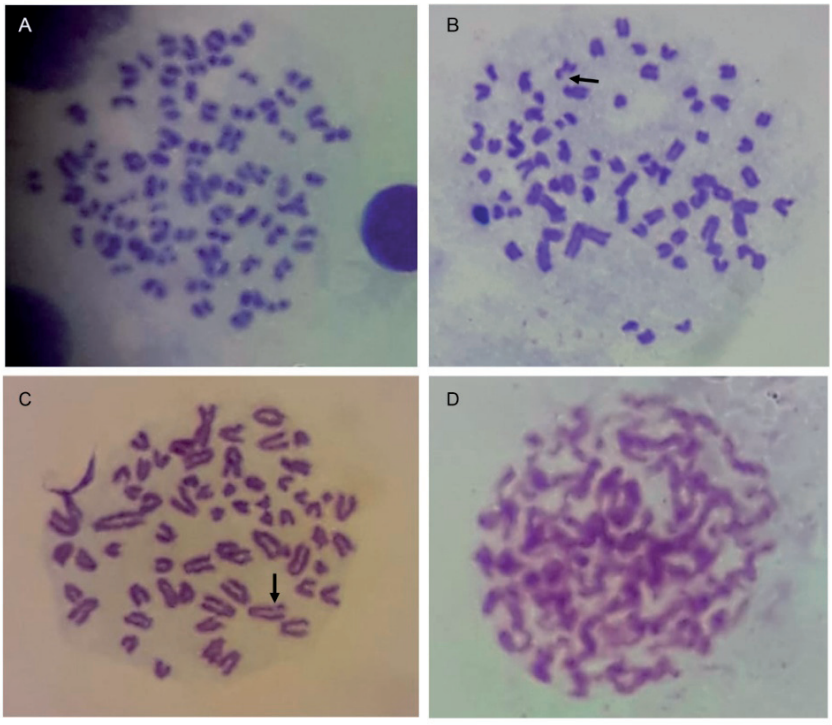

Fig. 1. A: endoduplication;
B: chromosome break;
C: chromatid gap,
D: sticky chromosomes.

Table 1. Mitotic index in peripheral blood lymphocyte cultures from a healthy adult male dog

Cultures	Sex	Mitotic index metaphases/1000 cells	
		Control	Mitomycin C 0.25 µg/ml
1	M	6	6
2	M	4	0
3	M	6.5	0
4	M	4	14
5	M	0	10
6	M	8	6
		4.75±1.14	6.00±2.25

M: Male Cultures were replicated on six different days. Each culture was done in duplicate. Around 1000 to 3000 cells per culture were analyzed and the mitotic index per 1000 cells was calculated. Results are presented as the mean ± standard error. There were no statistical differences between the control and mitomycin C cultures.

Table 2. Type and frequency of spontaneous and mitomycin C induced chromosomal aberrations in metaphases of peripheral blood lymphocyte cultures from healthy adult dogs

Cultures	Sex	Chromosomal aberrations							
		at1	ctb	csb	ctg	pvz	end	sti	T
Control									
1	F	0	2	0	0	0	0	2	4
2	M	2	1	0	0	0	2	4	9
3	M	0	1	0	0	1	0	0	2
		0.7±0.7	1.3±0.3	0	0	0.3±0.3	0.7±0.6	2.0±1.1	5.0±2.0
Mitomycin C 0.25 µg/ml									
1	F	2	10	3	1	0	2	14	32
2	M	0	0	0	0	5	2	43	50
3	M	1	9	0	2	1	2	7	22
		1.0±0.5	6.3±1.3	1.0±1.0	1.0±0.5	2.0±1.5	2.0±0.0	21.3±11.0	34.6±8.1*

M: Male F: Female. Cultures were performed on three different days. Each culture was done in duplicate. A hundred metaphases per culture were analyzed for chromosomal aberrations. At1: telomeric associations by only one chromatid, ctb: chromatid break, csb: chromosome break, ctg: chromatid gap, pvz: pulverized, end: endoduplication, sti: sticky, T: total. Results are presented as the mean ± standard error. * P= 0.0247 between control and mitomycin C cultures.

Discussion

The rate of mitotic activity in 72 h dog lymphocyte cultures was determined by metaphase count in total cells (Table 1). The MI found in negative control cultures in this study (4.75±1.14) was higher than that reported found in dog lymphocytes in the few cases in the literature, namely 1.98±0.11 (PALEJA, 2000) and 1.48±0.7 (KACHARE et al., 2009), which also used phytohemagglutinin as a mitotic inductor, and RPMI culture medium. Interpretation of the results for the MI induced by a genotoxic agent under study is done in contrast with the MI of the negative control cells (ADROVIĆ et al., 2016). In this comparison, the application of MMC from the beginning of the cultures did not decrease the MI, an indirect measure of cell proliferation and cytotoxicity. Having the appropriate concentration of MMC for each biological system is essential when it is to be used as a positive control of clastogenic effect for evaluation of *in vitro* tests of new substances based on the quantification of mitotic figures. Excessive toxicity can generate false negative results in later genotoxicity tests. The MI showed no differences due to the effect of MMC, indicating that the concentration used was not cytotoxic to canine lymphocytes and therefore suitable for evaluating genotoxicity in this system (OECD, 2016).The MI value is also a very important benchmark for analyzing tissue growth and multiplication.

In the CA testing, with and without exposure to MMC, the CA presented in UDROIU and SGURA (2017) were searched for, but only 7 of them were detected, plus others not mentioned in that article, such as endoduplications, pulverized metaphases and sticky metaphases. The total number of CA was significantly higher due to the effect of MMC (P=0.0247). Telomeric association by a single chromatid, and chromosome and chromatid gaps were found in small proportions. On the other hand, chromatid-breaks were almost five times higher in the MMC cultures than in the controls. It has previously been reported that antineoplastic antibiotics such as MMC, actinomycin, daunomycin and adriamycin induce chromatid-type chromosome aberrations (VIG, 1977). Pulverized metaphases were six times more frequent, and endoduplicated cells were tripled by the effect of MMC compared to the negative control cultures. Endoduplication is a process by which, after the S-period of the cell cycle, the nucleus does not enter mitosis but re-initiates another S-period. The most quantitatively relevant chromosomal alteration was the acquisition of sticky metaphases (Fig. 1D), which were 10 times more frequent in MMC cultures than in negative ones. The stickiness of the chromosomes can occur as a result of the physical adhesion of the chromosomes (chromatin material) to the proteins (LIU et al., 2012). It has been reported that this may be due to the failure to separate the chromosomes, due to the loss of the Ki-67 protein (BRANGWYNNE and MARKO, 2016). It could also be interpreted as the result of DNA depolymerization, partial dissolution of nucleoproteins, breakage and exchanges of the folded chromatid fiber, and the removal of non-histone proteins from the chromosomes (ADESUYI et al., 2018). These are entities in which the chromosomes are not free, but are united by a thin filament. This type of CA may be evidence of cytotoxicity (MARCANO et al., 2004). In the present study we did not contemplate this possibility because the main indicator of cell death is the decrease in MI, which was not observed here. The sticky chromosomes indicate the presence of a highly toxic substance, in this case MMC, which induces an irreversible effect on the physical state of chromatin. In this study, primary cultures of dog lymphocytes in suspension were used, for which it was confirmed that the concentration of MMC of 0.25 µg/ml, added at the beginning of the culture, is adequate to evaluate genotoxicity (Table 2). MMC is an anti-tumor antibiotic that has been shown to indicate a clastogenic rather than an aneugenic effect in MN assay (PARRY et al., 2002). MMC is also a recommended reference substance for assessing the competence of a laboratory (OECD, 2016). MMC has been used as a positive control in human lymphocyte cultures at a final concentration of 0.1 µg/mL, producing 10 times more aberrant cells than the negative control culture (SANTOVITO et al., 2020). In the present study, in dog lymphocytes the breaks of one or two chromatids were quintupled by the effect of MMC.

In previous assays of CA in human lymphocytes, we obtained a spontaneous average of 3.73±0.49 CA/100 cells in the reference group (negative control), observing gaps and chromatid-chromosome breaks, endoduplicated metaphases and acentric fragments (AIASSA et al., 2019). In relation to the effect of MMC on CA, we previously evaluated the effect on human lymphocytes (MAÑAS et al., 2009), observing the CA we found in dogs, but not the pulverized, nor the sticky nucleus, which are the characteristics of the present study. Other authors, using MMC 0.2 µg/ml in human lymphocytes, obtained 35% aberrant cells, with chromatid- and chromosome aberrations including 4% of polyploid cells (AYABAKTI and YAVUZ KOCAMAN, 2020). In future trials of CA in dog lymphocytes we suggest using MMC as a chromosomal damage agent, which is a use that we did not find to have been previously reported.

A strong similarity between dog and human genomes has been shown (PAOLONI and KHANNA, 2008) in terms of the pathophysiology of diseases such as cancer, which shows that the studies we carry out on dogs can also help to develop early warning systems for our own health (BREEN, 2008). Dog lymphocyte cultures are an available tool that can be found as pre-clinical models of cancer, with applications and utility in the discovery and evaluation of adverse drug effects. At the chromosomal aberration level, there are similarities in human and dog cancers, such as the fusion of the Abl gene (Abelson tyrosine kinase) with part of the BCR gene (breakpoint cluster region), which results in a constitutively active BCR-Abl tyrosine kinase in leukemia, or the presence of c-KIT mutations in both human and dog gastrointestinal tumors (BREEN and MODIANO, 2008; GREGORY-BRYSON et al., 2010). The dog genome is complex, due to its high number of chromosomes, the predominantly acrocentric morphology of all autosomes, and the small size of the autosomes (BREEN, 2008). This can be a disadvantage when performing G bands and location of specific breakpoints; but the CA assay, using conventional Giemsa staining, is informative and simple to perform, although it is very time consuming in microscopic analysis.

Conclusions

In this study, the effect was evaluated of MMC, which is frequently used in human lymphocytes as a recognized clastogen, but now in 72 h cultures of canine lymphocytes. At the concentration of 0.25 µg/ml, the total number of CA was significantly higher due to the effect of MMC. Chromatid-breaks, pulverized metaphases, endoduplicated and sticky nucleus were overexpressed. The potential use of canine lymphocytes for CA assay is emphasized, in the evaluation of the genotoxic effect of xenobiotics, to evidence chromosomal damage.

Acknowledgements

This research was possible thanks to the financial support of Juan Agustín Maza University UMAZA (Mendoza) and the National Council of Scientific and Technical Research CONICET (Argentina).

References

ADESUYI, A. A., K. L. NJOKU, A. L. OGUNYEBI, E. O. DADA, A. H. ADEDOKUN, A. O. JOLAOSO, M. O. AKINOLA (2018): Evaluation of the Cytogenotoxic Effects of Emulsifiable Concentrate form of Amitraz Pesticide on *Allium cepa* L. J Appl. Sci. Environ. Manage. 22, 1841-1847.

DOI: 10.4314/jasem.v22i11.20

ADROVIĆ, J., I. EMINOVIĆ, A. ČERKIĆ (2016): Genotoxic effects of some frequencies of low-voltage electrical currents on *Allium cepa* L. meristematic cells. Int. J. Mod. Biol. Res. 4, 25-34.

AIASSA, D. E., F. J. MAÑAS, N. E. GENTILE, B. BOSCH, M. C. SALINERO, N. B. GORLA (2019): Evaluation of genetic damage in pesticides applicators from the province of Córdoba, Argentina. Environ. Sci. Pollut. Res. Int. 26, 20981-20988.

DOI: 10.1007/s11356-019-05344-2

ALVAREZ, C. E. (2014): Naturally occurring cancers in dogs: insights for translational genetics and medicine. ILAR 55, 16-45.

DOI: 10.1093/ilar/ilu010

AYABAKTI, S., A. YAVUZ KOCAMAN (2020): Cytogenotoxic effects of venlafaxine hydrochloride on cultured human peripheral blood lymphocytes. Drug Chem. Toxicol. 43, 192-199.

DOI: 10.1080/01480545.2018.1486410

BRANGWYNNE, C. P., J. F.MARKO (2016): Cell division: A sticky problem for chromosomes. Nature 535, 234-235.

DOI: 10.1038/nature18904

BREEN, M. (2008): Canine cytogenetics–from band to basepair. Cytogenet. Genome Res. 120, 50-60.
DOI: 10.1159/000118740

BREEN, M., J. F. MODIANO (2008): Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans–man and his best friend share more than companionship. Chromosome Res. 16, 145-154.
DOI: 10.1007/s10577-007-1212-4

CHATTERJEE, N., G. C. WALKER (2017): Mechanisms of DNA damage, repair, and mutagenesis. Environ. Mol. Mutagen. 58, 235-263.
DOI: 10.1002/em.22087

DHAWAN, A., M. BAJPAYEE (2013): Genotoxicity Assessment: Methods and Protocols. Humana Press, New York, NY.
DOI:10.1007/978-1-4939-9646-9

FDA (FOOD and DRUG ADMINISTRATION) (2012): Guidance for industry.Avaiable at: www.fda.gov/downloads/Drugs/Guidances/ucm074931.pdf

GARDNER, H. L., P. LONDHE, C. A. LONDON (2019): Leveraging dogs with spontaneous cancer to advance drug development. In Animal Models in Cancer Drug Discovery, Academic Press, pp. 343-372.

GREGORY-BRYSON, E., E. BARTLETT, M. KIUPEL, S. HAYES, V. YUZBASIYAN-GURKAN (2010): Canine and human gastrointestinal stromal tumors display similar mutations in c-KIT exon 11. BMC Cancer 10, 559.
DOI: 10.1186/1471-2407-10-559

HU, Q., E. G. MAURAIS, P. LY (2020): Cellular and genomic approaches for exploring structural chromosomal rearrangements. Chromosome Res. 1-12.
DOI: 10.1007/s10577-020-09626-1

KACHARE, R. W., H. L. KANADKHEDKAR, D. H. PAWAR, A. A. ADGALE, S. H. SHAIKH, B. S. KATKADE, U. D. UMRIKAR (2009): Chromosome Architecture, G banding Studies and Media Comparison for Lymphocyte Culture of Karwani Dog. J. Bombay Vet. College 17, 5-7.

LINDBLAD-TOH, K., C. M. WADE, T. S. MIKKELSEN, E. K. KARLSSON, D. B. JAFFE, M. KAMAL, E. MAUCELI (2005): Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 438, 803-819.
DOI: 10.1038/nature04338.

LIU, P., M. LIU, H. E. JIANG, Z. ZHAO, J. WANG (2012): 'Chenguang', a new tetraploid Chinese jujube cultivar. Fruits 67, 293-296.

MAÑAS, F., L. PERALTA, N. GORLA, B. BOSH, D. AIASSA (2009): Chromosomal aberrations in workers occupationally exposed to pesticides in Córdoba. J. Basic. Appl. Genet. 20, 9-13.

MARCANO, L., I. CARRUYO, A. DEL CAMPO, X. MONTIEL (2004): Cytotoxicity and mode of action of maleic hydrazide in root tips of *Allium cepa* L. Environ. Res. 94, 221-226.
DOI: 10.1016/s0013-9351(03)00121-x

MARTIN, L., A. A. M. QUERO, D. M. FERRÉ, L. ALBARRACIN, V. HYNES, I. B. LARRIPA, N. B. GORLA (2011): A case of true hermaphroditism 78, XX Weimaraner bitch. Arch. Med. Vet. 43, 299-302.

MATEUCA, R., N. LOMBAERT, P. V. AKA, I. DECORDIER, M. KIRSCH-VOLDERS (2006): Chromosomal changes: induction, detection methods and applicability in human biomonitoring. Biochimie 88, 1515-1531.
DOI: 10.1016/j.biochi.2006.07.004

MOORHEAD, P. S., P. C. NOWELL, W. J. MELLMAN, D. T. BATTIPS, D. A. HUNGERFORD (1960): Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell. Res. 20, 613-616.
DOI: 10.1016/0014-4827(60)90138-5

MOSESSO, P., S. CINELLI, A. T. NATARAJAN, F. PALITTI (2013): In vitro cytogenetic assays: chromosomal aberrations and micronucleus tests. Methods Mol. Biol. 1044, 123-146.
DOI: 10.1007/978-1-62703-529-3_6

OECD (2016): Test No. 473: In Vitro Mammalian Chromosomal Aberration Test. OECD Guidelines for the Testing of Chemicals. Section 4.Paris:OECD Publishing

PALEJA, H (2000): Comparison between cytogenetic profile of wild wolf *(Canis Lupus)* and domestic dog *(Canis familiaris)*. Doctoral dissertation, AAU, Anand [India].

PAOLONI, M., C. KHANNA (2008): Translation of new cancer treatments from pet dogs to humans. Nat. Rev. Cancer. 8, 147-156.
DOI: 10.1038/nrc2273

PARRY, E. M., J. M. PARRY, C. CORSO, A. DOHERTY, F. HADDAD, T. F. HERMINE, J. WILLIAMSON (2002): Detection and characterization of mechanisms of action of aneugenic chemicals. Mutagenesis 17, 509-521.
DOI: 10.1093/mutage/17.6.509

PONCHIO, L., L. DUMA, B. OLIVIERO, N. GIBELLI, P. PEDRAZZOLI, G. R. D. CUNA (2000): Mitomycin C as an alternative to irradiation to inhibit the feeder layer growth in long-term culture assays. Cytotherapy 2, 281-286.
DOI: 10.1080/146532400539215

SANTOVITO, A., C. GENDUSA, P. CERVELLA, D. TRAVERSI (2020): In vitro genomic damage induced by urban fine particulate matter on human lymphocytes. Sci. Rep. 10, 8853.
DOI: 10.1038/s41598-020-65785-5

SHEARIN, A. L., E. A. OSTRANDER (2010): Leading the way: canine models of genomics and disease. Dis. Model. Mech. 3, 27-34.
DOI: 10.1242/dmm.004358

UDROIU, I., A. SGURA (2017): Cytogenetic tests for animal production: state of the art and perspectives. Anim. Genet. 48, 505-515.
DOI: 10.1111/age.12581

ULUMAN, E., P. A. KILICLE (2020):The investigation of the possible antigenotoxic in vivo effects of pomegranate (*Punicagranatum L.*) peel extract on mitomycin-C genotoxicity. Turk. J. Vet. Anim. Sci. 44, 382-390.
DOI: 10.3906/vet-1911-79

VIG, B. K. (1977): Genetic toxicology of mitomycin C, actinomycins, daunomycin and driamycin. Mutat Res. 39, 189-238.
DOI: 10.1016/0165-1110(77)90022-7

Received: 12 August 2021
Accepted: 8 March 2022

CALIRI, M. N., D. M. FERRÉ, V. LEDDA, N. B. M. GORLA: Kromosomske aberacije uzrokovane mitomicinom C u psećim limfocitima. Vet. arhiv 93, 341-350 2023.

SAŽETAK

Kromosomske aberacije jesu poremećaji koje obilježava abnormalan broj kromosoma odnosno promjene u njihovoj strukturi. Aberacije u strukturi kromosoma jesu kromatidni i kromosmski prekidi (*gapovi*) i lomovi, acentrični fragmenti, spojevi telomera, delecije, rano odvajanje kromatida i složeni učinci povezani sa metafazom (engl. pulverized metaphases i sticky metaphases). U ovom je radu učinak mitomicina C, poznatog ljudskog klastogena, analiziran u kulturi psećih limfocita, životinjske vrste koja se sve češće koristi u komparativnoj medicini. Krvne su kulture načinjene od mužjaka i ženke psa. Analiza citotoksičnosti praćene mitotskim indeksom i genotoksičnosti provedena je s 0,25 µg/mL mitomicina C. Ukupne su kromosomske aberacije bile znakovito veće zbog učinka mitomicina C (P = 0,0247). Broj lomova kromatida gotovo se upeterostručio, pulverizirane su metafaze bile šest puta češće, a endoduplicirane stanice tri puta brojnije nego u kulturama koje su činile negativnu kontrolu. Kvantitativno najrelevantnija kromosomska aberacija bila je prisutnost "sticky" metafaza povezanih s nuspojavama u proteinima kromatina. Ističe se potencijalna korist upotrebe psećih limfocita u analizi kromosomkih aberacija pri procjeni ili ponovnoj procjeni genotoksičnog *in vitro* učinka ksenobiotika kako bi se dokazalo oštećenje kromosoma.

Ključne riječi: pseće stanice; citogenetika; klastogeni agensi; kromosmsko oštećenje; limfocitne kulture; mitomicin C