Acta Veterinaria (Beograd), Vol. 59, No. 5-6, 601-611, 2009.

DOI: 10.2298/AVB0906601A UDK 619:615.099.092

EVALUATION OF FLUNIXIN MEGLUMINE GENOTOXICITY USING *IN VITRO* AND *IN VIVO*/*IN VITRO* MICRONUCLEUS TEST

AYDIN SA and ÜSTÜNER KELES O

Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Istanbul University, Turkey

(Received 15th April 2009)

The aim of this study was to investigate the genotoxic effects of flunixin meglumine on mice peripheral lymphocytes by in vitro and in vivo/invitro cytokinesis block micronucleus tests (CBMN). Flunixin meglumine was used at concentrations of 25, 50 and 100 µg/mL for the in vitro assay and 50, 75 and 100 mg/kg for the in vivo/in vitro assay. Mice were treated intraperitonally twice with a 24 h interval and sacrificed 6 h after the last dose. Cardiac blood was taken and added to the cultures for the in vivo/in vitro test. 21 h after the addition of the test compund for the in vitro test, and after the initiation of incubation for in vivo/in vitro test cytokinesis was blocked with the addition of cytochalasin-B and 20 h later the cultures were harvested. In both test systems, a negative and a positive control mitomycin C (MMC) were also included.

The micronucleated binuclear cell (MNBN) frequencies increased after both treatments, however, the differences between the treated cells and the control groups were found to be statistically significant only for the in vitro treatment. The increase was in a dose-dependent manner, significant elevations of MNBN cell ($p<0.05$ and $p<0.001$) were observed at concentrations 50 and 100 µg/mL respectively. In addition reduction in cytokinesis-block proliferation index (CBPI) was observed in both treatments, indicating cytotoxicity of flunixin meglumine.

According to these results, flunixin meglumine is genotoxic in mice lymphocytes treated in vitro, but has not mutagenic activity in vivo under micronucleus (MN) test conditions.

Key words: cytochalasin-B, flunixin meglumine, micronucleus, mice, peripheral lymphocytes

INTRODUCTION

Flunixin meglumine is a non-steroid anti-inflammatory drug (NSAID) used in food-producing animals and indicated for the regulation of inflammation in endotoxemia and control of pyrexia (Buur *et al*., 2006). Flunixin meglumine is a

cyclooxygenase inhibitor, blocks the biosynthesis of prostaglandins, which are believed to play a role in the development and progression of some forms of cancer (Jackman *et al.,* 1994; Zha *et al.,* 2004). Recent studies on prophylaxy and therapy of cancer established that NSAIDs have a cancer-chemopreventive action (Sheng *et al.,* 1997; Shiff *et al.,* 2003; Yao *et al.,* 2003). However, there is no published data about the anticancerogenic potential of flunixin meglumine. On the other hand, based on carcinogenicity studies, The European Medicines Agency report (EMEA, 1999) demonstrated that flunixin meglumine is not carcinogenic. To our knowledge, the International Agency for Research on Cancer (IARC) has no available evaluation on this molecule (IARC). There is considerable evidence of a positive correlation between the carcinogenicity of substances *in vivo* and their mutagenicity in long-term studies with animals (Ashby and Tennant 1991; Bernauer *et al.,* 2005). However, the genotoxicity profile of flunixin meglumine in short-term assays is somewhat equivocal due to the positive and negative results of the *in vitro* and *in vivo* genotoxicity tests. Committee on Mutagenicity (COM) reported that most of the mutagenicity data of flunixin meglumine were relatively old and had limitations (COM, 2001). Flunixin meglumine was not mutagenic in a limited number of *Salmonella typhimurium* strains in Ames test, unscheduled DNA synthesis assay in rat primary hepatocyte cultures and *in vivo* in a bone marrow micronucleus assay. In contrast flunixin meglumine had mutagenic potential *in vitro* in mouse lymphoma forward mutation assay, *in vitro* chromosome aberration assay in Chinese hamster ovary cells both in the absence and presence of S-9 metabolic activation and in a mitotic gene conversion assay in *Saccharomyces cerevisiae* (EMEA, 1999).

Flunixin meglumine is an ionic compound and *in vivo* dissociates rapidly in aqueous media at physiological pH to flunixin and meglumine. The primary purpose of meglumine is to act as a counter ion to keep flunixin soluble. Genotoxicity studies showed that there is no evidence to suggest flunixin to be mutagenic *in vivo*, in contrast meglumine mutagenicity results were inconsistent with some positive and negative results. The mutagenic activity seen *in vitro* with flunixin meglumine was believed to be due to the the meglumine component (COM, 2003).

One of the test systems applied as a cytogenetic assay for biomonitoring and identification of genotoxic effects of physical and chemical agents was the cytokinesis block micronucleus (CBMN) technique. MN are chromosomal fragments or whole chromosomes that are not incorporated into daughter nuclei during mitosis because of chromosomal breakage or dysfunction of the mitotic apparatus, respectively. Cytochalasin-B, an inhibitor of actin polymerisation, prevents cytokinesis and produces binucleated (BN) cells which can be easily and accurately scored for MN following one cell cycle (MacLean-Fletcher and Pollard, 1980; Fenech and Morley, 1985; Fenech, 1993).

Due to the controversial findings of earlier studies on the genotoxic effects of flunixin meglumine, the present study was undertaken to obtain additional data on the cytogenetic activity of flunixin meglumine and to investigate whether flunixin meglumine is mutagenic in cultured mouse lymphocytes both *in vivo* and *in vitro* by using CBMN assay as the genetic endpoint.

MATERIALS AND METHODS

Animals

Experiments were performed using male CD-1 mice, aged 8-12 weeks and weighing 20-25 g, obtained from Pendik Veterinary Control and Research Institute (Turkey). the mice were housed in polypropylene cages and acclimatised for two weeks in the animal house, maintained at 23 ± 2 ºC and humidity 50 ± 5% with a 12 h light/dark cycle. Feed and water were provided *ad libitum*. The experimental protocol was approved by Istanbul University Veterinary Faculty Ethic Committee (Regd, No. 2004/88).

Chemicals

Flunixin meglumine (2-[2-Methyl-3-(trifluoromethyl)phenyl]amino]-3-pyridinecarboxylic acid meglumine salt, Alke, Turkey, 99.5% purity) is soluble in physiological saline and was freshly prepared at concentrations 25, 50, 100 μg/mL for *in vitro* and 50, 75 and 100 mg/kg for *in vivo*/*in vitro* CBMN test before each experiment. Mitomycin C (MMC, Sigma, St. Louis, MO, USA), used as a positive control agent because of its clear response in the MN test, was dissolved in ultra-pure water to the concentration used, just prior to treatment.

In vitro micronucleus test

The doses for *in vitro* CBMN test were choosen according to the previous genotoxicity studies of flunixin meglumine (EMEA, 1999). CBMN was carried out using the Standard technique described by Fenech (2000; 2006) with slight modifications and the current OECD guideline (OECD, 2007). Briefly, blood samples were obtained by cardiac puncture with heparinised syringes from ether anaesthetised healthy mice. Whole blood (0.5 mL) was cultured in RPMI 1640 medium (Biochrom, Berlin, Germany) supplemented with 20% fetal bovine serum (FBS, Sigma, St. Louis, MO, USA), antibiotics (penicillin 100 IU/mL and streptomycin 100 μg/mL) and 2 % phytohaemagglutinin (PHA Sigma, St. Louis, MO, USA). The cultures were incubated for 62 h in a humidified environment with 5% CO_2 at 37ºC. The test substance flunixin meglumine was added at three different concentrations 21 h after PHA stimulation. Cytochalasin B (6 μg/mL) (Cyt-B, Sigma, St. Louis, MO, USA) was added at 42 h post-culture initiation, to arrest cytokinesis of dividing cells. Negative (physiological saline) and positive controls (MMC 0.2 μg/mL) were run simultaneously and similarly with flunixin meglumine treated cultures. The treatment protocol is shown in Figure 1.

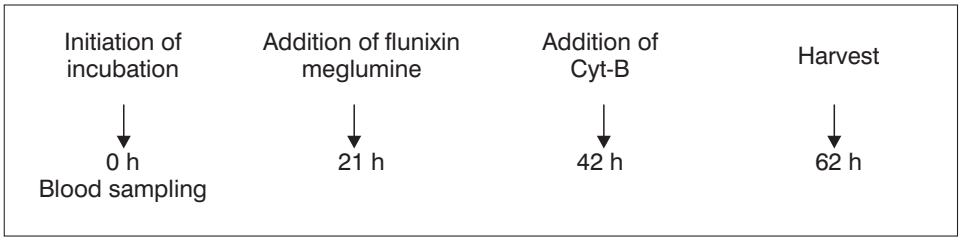

Figure 1. Treatment protocol of *in vitro* micronucleus test

In vivo/*in vitro micronucleus test*

Based on our preliminary experiment findings, doses of 50, 75 and 100 mg/kg b.w. of flunixin meglumine no observed mortality and toxicity signs were chosen for the *in vitro*/*in vivo* studies.

The experiments were performed as described by Moore *et al.,* (1995a,b), with application to lymphocytes. A total of 40 mice were divided into 5 groups. Flunixin meglumine (50, 75 and 100 mg/kg b.w.) was administered intraperitoneally twice with a 24-h interval at a volume of 10 mL/kg. In addition, a negative (physiological saline) and a positive (MMC, 2 mg/kg) control group were used to test the validity of the assay. Blood samples were obtained 6 h after the last treatment of flunixin meglumine and 48 h after physiological saline and MMC treatment. The test protocol was applied as described in *in vitro* assay. The treatment protocol is shown in Figure 2.

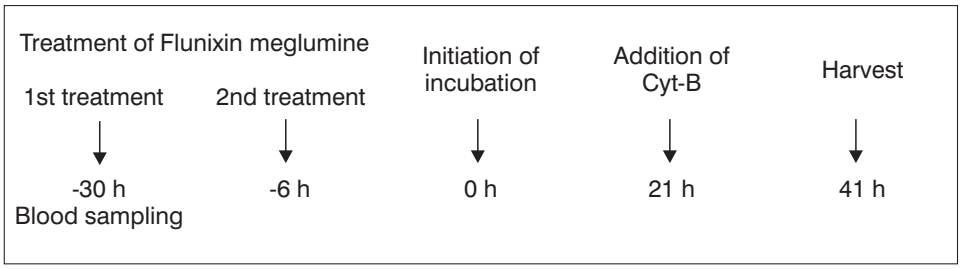

Figure 2. Treatment protocol of *in vivo*/*in vitro* micronucleus test

MN assay

The cells were harvested by centrifugation (1000 rpm, 8 min), and were suspended in a hypotonic solution of 0.075M KCl at room temperature. Next, cells were recentrifuged (2000 rpm, 3 min) and fixed three times in cold methanol: acetic acid (6:1). Slides were prepared by dropping and air-drying. Slides were stained with 5% Giemsa (pH 6.8) in phosphate buffer for 10 min, washed in distilled water and dried at room temperature (Lee *et al.,* 1994 a,b).

For MN identification, all slides were analysed in accordance with Fenech (1997; 2000) using a Olympus CX31 microscope. The induction of MN was evaluated by scoring a total of 1000 binucleated (BN) cells with well-preserved cytoplasm at 1000 × magnification.

From the data of MN analysis, cytokinesis-block proliferation index (CBPI), which can be considered as an index of cell kinetics or average cell division, was calculated by classifying 1000 cells according to the number of nuclei for *in vitro* and *in vivo*/*in vitro* treatments as CBPI = (M1 + 2M2 + 3(M3 + M4) / N where M1-M4 represents the number of cells with 1 to 4 nuclei, respectively, and N is the total number of scored cells (Suralles *et al.*, 1995). This value indicates the number of cycles per cell during the period of exposure to cyctochalasin.

Statistical Analysis

Statistical differences between the *in vitro* and *in vitro*/*in vivo* treatments and the controls groups were tested by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test using the "Instat" statistic computer program. A difference in the mean values of $p<0.05$ or less was considered to be statistically significant.

RESULTS

The frequency of binucleated cells with micronuclei (BNMN), cytotoxicity index (CBPI) obtained after *in vitro* and *in vivo*/*in vitro* treatment with flunixin meglumine is shown in Table 1 and Table 2, respectively. *In vitro* treatments in concentrations ranging from 25, 50 and 100 μg/mL were found to induce BNMN frequency with increasing concentrations of flunixin meglumine, reaching a statistical significance at 50 and 100 μg/mL concentrations ($p<0.05$ and $p<0.001$), respectively in the cytokinesis-blocked lymphocytes. Positive control MMC yielded a depression of cell proliferation and positive response in MN induction. The lowest concentration of flunixin meglumine (25 mg/kg) did not show any significant effect. The reduction in the frequency of CBPI and % BN cells with decreasing doses of the drug were observed in lymphocyte cultures, indicating cytotoxicity of flunixin meglumine in both treatments.

MN analysis in *in vivo*/*in vitro* micronucleus assay showed that the flunixin meglumine did not significantly increase the micronucleus frequency compared with the negative control. A reduction in cell proliferation was found, reaching statistical significance ($p<0.001$) at all test concentrations of flunixin meglumine compared to the control group.

DISCUSSION

The use of drugs in food-producing animals can lead to potentially harmful residues in edible products harvested from these animals. A risk assessment which offers a formal approach to the evaluation of the safety of veterinary drug residues is an essential component of the regulatory approval process for products containing these drugs. Genotoxic activity has an impact on the risk assessment of a veterinary drug (Gehring *et al.,* 2006).

Despite the expansive use of flunixin meglumine as a non-steroid anti-inflammatory drug, information on its toxicology is still incomplete. Since the results of earlier studies on evaluation of the genotoxicological profile of flunixin meglumine were contradictory and inconclusive (COM, 2005), this investigation was conducted to evaluate whether flunixin meglumine is capable of changing a normal cell cycle progression of mouse lymphocytes treated *in vitro* and *in vivo*/*in vitro* by analysing the cytogenetic endpoint MN. Our *in vitro* experimental results demonstrated a significant, partly dose-dependent increase of micronuclei at concentrations of 50 and 100 μg/mL flunixin meglumine. Previously, flunixin meglumine demonstrated clastogenic activity in Chinese hamster ovary cells

Table 1. Induction of micronuclei and CBPI values in mice lymphocytes treated with flunixin meglumine *in vitro*

Administrated compound and dose	No of mice	The total number of nucleated cells scored	The total number of binucleated cells scored	The total number of binucleated cells with micronuclei	Percentage of binucleated cells	Binucleated cells with micronuclei BNMN frequency (mean ‰±SD)	CBPI[2]
FS (NC)[1]	8	18 691	8000	22	42.8	2.75±1.28	1.44±0.013
Flunixin meg. (25 μg/mL)	8	21 680	8000	30	36.9	3.75±1.04	1.38±0.009***
Flunixin meg. (50 μg/mL)	8	31 008	8000	47	25.8	5.90±1.46*	1.27±0.008***
Flunixin meg. (100 μg/mL)	8	37 037	8000	64	21.6	8±1.31***	1.21±0.011***
MMC (PC)[1] (0.2 μg/ml)	8	35 635	8000	395	22.45	49±2.33***	1.36±0.014***

[1] (NC) Negative control. (PC) Positive control
[2] (CBPI) Cytokinesis-block proliferation index; by counting 1000 cells and calculated according to the formulation.
CBPI = (MoN cell count + 2 x BN cell count + 3 x PN cell count) / total cell count)
* $p<0.05$, *** $p<0.001$ (Compared with negative control group)

Table 2. Induction of micronuclei and CBPI values in mice lymphocytes treated with flunixin meglumine *in vivo*/*in vitro*

Administrated compound and dose	No of mice	The total number of nucleated cells scored	The total number of binucleated cells scored	The total number of binucleated cells with micronuclei	Percentage of binucleated cells	Binucleated cells with micronuclei BNMN frequency (mean ‰ ±SD)	CBPI[2]
FS (NC)[1]	8	14 774	8000	33	54.15	4.1±1.2	1.60±0.029
Flunixin meg. (25 μg/ml)	8	17 730	8000	39	45.12	5.1±0.1	1.49±0.023***
Flunixin meg. (50 μg/ml)	8	18 757	8000	45	42.65	5.6±1.4	1.47±0.023***
Flunixin meg. (100 μg/ml)	8	23 550	8000	41	33.97	5.1±1.2	1.39±0.018***
MMC (PC)[1] (0.2 μg/ml)	8	32 000	8000	205	25	25.6±1.4***	1.25±0.009***

[1] (NC) Negative control. (PC) Positive control
[2] (CBPI) Cytokinesis-block proliferation index; by counting 1000 cells and calculated according to the formulation.
CBPI= (MoN cell count + 2 x BN cell count + 3 x PN cell count) / total cell count) (Fenech, 2000)
* $p<0.05$ *** $p<0.001$ (Compared with negative control group)

without metabolic activation at a dose of 100 μg/mL, which is similar to the highest dose in our study, and with metabolic activation at a concentration of 200 and 400 μg/mL (EMEA, 1999). In addition, in the present study, a significant increase ($p<0.05$) in MN frequencies at an even lower concentration of 50 μg/mL of flunixin meglumine could be due to the fact that the (MN) frequency in peripheral blood lymphocytes in conjunction with the CBMN assay is among the most popular and effective biomarkers used for evaluating the effect of genotoxic agents. (Fenech *et al.,* 1999). Also, dose-dependent and reproducible positive results of flunixin meglumine were obtained in the mouse lymphoma forward mutation assay (EMEA, 1999).

In contrast to the occasional positive responses obtained by *in vitro* assays, *in vivo* data on the genotoxicity of flunixin meglumine are inconsistent with some positive and negative results (COM, 2003). Our negative results indicating a lack of chromosomal damage, measured as MN induction, agree with earlier studies performed in mammals *in vivo.* Flunixin meglumine was reported to give negative results in the mouse micronucleus test at dose levels of 40 and 80 mg/kg bw administrated intraperitoneally once a day for 2 days. Flunixin was negative in the same assay at dose levels of 100 and 150 mg/kg bw (EMEA, 1999). Meglumine, which is reported to be responsible for the genotoxicity of flunixin meglumine, was investigated in two separate micronucleus assays using BS1 and Alpk:ApfCD-1 mice. Positive results were obtained in BSI mice after intraperitoneal administration of 500 and 1000 mg/kg bw. However these results were not repeated in two bone marrow micronucleus assays in mice using an eqivalent treatment regime. In contrast, it was reported that negative results were obtained in a seperate *in vivo* micronucleus assay using intraperitoneal administration of two doses given 24 hours apart at up to 600 mg/kg bw of meglumine to CD1mice. It was mentioned that the observed effect could be complicated by toxicity and there could be a considerable individual variation (COM, 2005). In order to avoid false-positive responses generated by nonphysiological conditions because of extremely high concentrations and toxicity, we used 100 mg/kg as the highest concentration which does not cause toxicity signs and mortality in mice.

It is difficult to account for the discordance between positive results in *in vitro* and negative results in *in vivo*/ *in vitro* mouse lymphocytes micronuclei. The possible explanation of differences between the results of the *in vitro* and *in vivo*/ *in vitro* assays of flunixin meglumine may be due to the alterations in its metabolic pathway that may be metabolised *in vivo* to a less genotoxic derivate or *in vivo* may be formed an adaptive response to flunixin meglumine.

With respect to cytotoxic effects of flunixin meglumine on lymphocyte cultures, as measured by CBPI, in comparison with the control value, a dose-dependent significant decrease in cell proliferation indicates a delay in the cell cycle progression which is an overt sign of cellular toxicity. Although, the reduction of CBPI was observed in the present study, no effect on the *in vivo* genotoxicity was observed even at the highest dose. This could be due to interaction of the compound with different cellular components resulting in cytotoxic and genotoxic effects.

In conclusion, our results indicate that flunixin meglumine has mutagenic potential in the cytochalasin B block micronucleus assay treated *in vitro* and has not genotoxic activity after *in vivo* administration evaluated with *in vivo*/*in vitro* micronucleus assay under test conditions. In addition, from CBPI data it is concluded that flunixin meglumine showed cytotoxic effects in cultured mouse lymphocytes both *in vitro* and *in vivo*/*in vitro* tests.

ACKNOWLEDGEMENTS:
This study was supported by the Research Fund of Istanbul University. Project No. T-468/25062004, TUBÝTAK 1002-B and UDP-2028/23012008.

Address for correspondence
Oya Üstüner Keles
Department of Pharmacology and Toxicology
Faculty of Veterinary Medicine
Istanbul University
34320 Istanbul
Turkey
E-mail: oyakeles@istanbul.edu.tr

REFERENCES

1. *Ashby J, Tennant RW,* 1991, Definite relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP, *Mutat Res*, 257, 229-306.
2. *Bernauer U, Oberemm A, Madle S, Gundert-Remy U*, 2005, The use of *in vitro* data in risk assessment, *Basic Clin Pharmacol*, 96, 176-81.
3. *Buur JL, Baynes RE, Smith G, Riviere JE*, 2006, Pharmacokinetics of flunixin meglumine in swine after intravenous dosing, *J Vet Pharmacol Therap*, 29, 437-40.
4. COM – Committee on Mutagenicity, Flunixin meglumine (MUT/01/28), 2001.
5. COM – Committee on Mutagenicity, Flunixin, meglumine and flunixin meglumine (MUT/03/1), 2003.
6. COM – Committee on Mutagenicity, Statement on flunixin, meglumine and flunixin meglumine (COM/05/S1), 2005.
7. EMEA – The European Agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation Unit, EMEA/MRL/661/99-FINAL, Committee for Veterinary Medicinal Products, Flunixin Summary Report (1), August 1999.
8. *Fenech M, Morley AA*, 1985, Measurement of micronuclei in lymphocytes: *Mutat Res*, 147, 29-36.
9. *Fenech M*, 1993, The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxic studies in human population, *Mutat Res,* 285, 35-44.
10. *Fenech M*, 1997, The advantages and disadvantages of the cytokinesis-block micronucleus method, *Mutat Res*, 392, 11-8.
11. *Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S*. 1999, The human micronucleus Project. An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans, *Mutat Res,* 428, 271-83.
12. *Fenech M*, 2000, The in vitro micronucleus technique, *Mutat Res*, 455, 81-95.
13. *Fenech M*, 2006, Commentary on the SFTG international collaborative study on the *in vitro* micronucleus test: To Cyt-B or not to Cyt-B?, *Mutat Res,* 607, 9-12.
14. *Gehring R, Baynes RE, Rývýere JE*. 2006, Application of risk assessment and management principles to the extralabel use of drugs in food-producing animals, *J Vet Pharmacol Therap,* 29, 5-14.
15. IARC Website. http://www. iarc.fr [25 October 2008].

16. *Jackman BR, Moore JN, Barton MH, Morris DD*, 1994, Comparison of the effects of ketoprofen and flunixin meglumine on the *in vitro* response of equine peripheral blood monocytes to bacterial endotoxin, *Can J Vet Res*, 58, 138-43.
17. *Lee TK, Wiley AL, Esinhart JD, Blackburn LD*, 1994, Radiation dose-dependent variations of micronuclei production in cytochalasin B-blocked human lymphocytes, *Teratog Carcinog Mutagen*, 14, 1-12.
18. *Lee TK, Wiley AL, Means JA, Biggs L*, 1994, Preservation of cytoplasm in the human lymphocyte micronucleus assay, *Mutagenesis,* 9, 559-62.
19. *MacLean-Fletcher S, Pollard TD.* 1980, Mechanism of action of cytochalasin B on actin, *Cell*, 20, 329-41.
20. *Moore FR, Urda GA, Krishna G, Theiss JC*, 1995, An *in vivo*/in vitro method for assessing micronucleus and chromosome abberation induction in rat bone marrow and spleen 2. Studies with chlorambucil and mitomycin C, *Mutat Res,* 335, 201-6.
21. *Moore FR, Urda GA, Krishna G, Theiss JC*, 1995, An *in vivo*/in vitro method for assessing micronucleus and chromosome abberation induction in rat bone marrow and spleen 1. Studies with cyclophosphamide, *Mutat Res,* 335, 191-9.
22. OECD – Organisation for Economic Co-operation and Development, Guideline for the testing of chemicals draft proposal for a new guideline 487: In vitro Mammalian Cell Micronucleus Test (MNvit), 2007, 1-21.
23. *Sheng H, Shao J, Kirkland SC, Isakson P, Coffrey RJ, Morrow J et al*., 1997, Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2, *J Clin Invest*, 99, 2254-9.
24. *Shiff SJ, Shivaprasad P, Santini DL*, 2003, Cyclooxygenase inhibitors: drugs for cancer prevention, *Curr Opin Pharmacol*, 3, 352-61.
25. *Suralles J, Xamena N, Creus A, Marcos R,* 1995, The suitability of the micronucleus assay in human lymphocytes as a new biomarker of excision repair, *Mutat Res,* 341, 169-84.
26. *Yao M, Kargman S, Lam EC, Kelly CR, Zheng Y, Luk P et al.,* 2003, Inhibition of cyclooxygenase-2 by rofecoxib attenuates the growth and metastatic potential of colorectal carcinoma in mice, *Cancer Res*, 63, 586-92.
27. *Zha S, Yegnasubramanian V, Nelson WG, Isaac WB, De Marzo AM*, 2004, Cyclooxygenase in cancer: progress and perspective, *Cancer Lett*, 215, 1-20.

ISPITIVANJE GENOTOKSIČNOSTI FLUNIKSIN MEGLUMINA UPOTREBOM *IN VITRO* I *IN VIVO*/*IN VITRO* MIKRONUKLEUSNOG TESTA

AYDIN SA i ÜSTÜNER KELES O

SADRŽAJ

CIlj ovih ogleda je bio da se ispita genotoksični efekat fluniksin meglumina na periferne limfocite miša upotrebom *in vitro* i *in vivo*/*invitro* blok-citokinetičkog mikronuleusnog testa (CBMN). Fluniksin meglumin je korišćen u koncentracijama od 25, 50 i 100 μg/ml za *in vitro* esej i 50, 75 i 100 mg/kg za *in vivo*/*in vitro* test. Miševi su tretirani inraperitonealno, dva puta u roku od 24 h i žrtvovani 6 h posle druge aplikacije. Uzorci krvi su uzimani punkcijom srca i kultivisani za *in vivo*/*in vitro* test. Nakon isteka 21 sata, od dodavanja testirane supstance za *in vitro* test, i posle inicijacije inkubacije za *in vivo*/*in vitro* test, citokinezija je blokirana dodava-

njem citohalazina-B. Ćelijske kulture su analizirane dvadeset sati kasnije. U oba test sistema su korišćene negativne i pozitivne (mitomicin C - MMC) kontrole.

Frekvenca pojavljivanja mikronukleusnih binuklearnih ćelija (MNBN) je bila povećana nakon oba tretmana, ali su razlike između tretiranih i kontrolnih ćelija bile značajne samo pri tretmanu *in vitro.* Ovo povećanje je bilo dozno-zavisno i značajan porast broja MNBN ćelija ($p<0,05$ i $p<0,001$) je uočen pri koncentracijama od 50 i 100 μg/ml respektivno. Osim toga, oba tretmana su dovodila do smanjenja citokinetičkog blok proliferacijskog indeksa (CBPI) što ukazuje na citotoksičnost fluniksin meglumina.

Na osnovu ovih rezultata se može zaključiti, da fluniksin meglumin ispoljava genotoksične efekte prema limfocitima miša, tretiranim *in vitro,* ali nema mutagenu aktivnost *in vivo* koja se može dokazati mikronukleusnim testom.