Acta Veterinaria(Beograd), Vol. 48. No. 5–6, 351–356, 1998.

UDK 619:615.285.7

IN VITRO INDUCTION OF MICRONUCLEI IN SHEEP AND BOVINE LYMPHOCYTES AFTER EXPOSURE TO CHLORIDAZON

ELENA PIEŠOVA

University of Veterinary Medicine, Komenskeho 73, 041 81 Košice, Slovak Republik

(Received, 28. July 1998.)

The effect of the herbicide chloridazon on induction of micronuclei (MN) in sheep and bovine peripheral lymphocytes was evaluated in vitro.

Chloridazon affected MN at all the concentrations tested: $7x10^{-6}M$, $7x10^{-5}$ M and $7x10^{-4}$ M. A statistically significant increase of MN was seen in sheep and bovine lymphocytes at a chloridazon concentration of $7x10^{-4}M$ ($P<0.01$ and $P < 0.001$, resp.).

No significant differences in induction of MN were found at both lower concentrations tested.

Our results indicated that chloridazon exerted only a weak influence on induction of the chromosomal changes (MN) observed.

Key words: herbicide chloridazon, sheep and bovine peripheral lymphocytes, micronuclei

INTRODUCTION

Pesticides are a group of diverse chemical agents used to control insects, weeds or other pests (Hrelia et al., 1994). Genotoxic effects of these agricultural chemicals are among the most serious of all possible side effects. If a chemical reacts with nuclear DNA, it is usually mutagenic and carciogenic to the exposed organisms (Anwar, 1997). It is estimated globally that 5%-10% of all cancers may be caused by environmental exposures (Jones and Boffetta, 1997). Possible cancer hazards from pesticide residues in food have been much discussed in the scientific literature and the press (Chaisson et al., 1989). Farm animals are an important component of the human food chain and an accumulation of residues in meat poses an additional hazard to man (Pokorna et al., 1996). In particular, the diet has been estimated to play a role in about one-third of all cancers (Jones and Boffetta, 1997). Sheep and cattle on pasture may often be exposed to chemical agents. The effects of the chemicals discussed include hereditary genetic diseases, carcinogenesis, reproductive dysfunction, and birth defects (Anwar, 1997). Data from in vitro and in vivo tests indicate whether the compound is a potential mutagen and/or potential carcinogen (Hrelia et al., 1990). The

evidence for mutagenicity and carcinogenicity in different short-term tests is viewed as a useful diagnostic signal for potential genetic diseases and cancer. Generally, chromosome aberations (CA) are usually used to assess the risk from chemical agents (Tucker and Preston, 1996), while micronuclei (MN) and sister chromatid exchanges (SCE) serve as indicators of genetic damage (Norppa et al., 1993, Carrano et al., 1978).

This study is a part of some broader research aimed at evaluation of genotoxic risk from the herbicide chloridazon.

Chloridazon is the active ingredient of the preparation Burex Eko produced by Istrochem Bratislava (Slovak Republic). It is a herbicidal preparation intended for destruction of annual double germinating weeds in beet and sugar beet. It has been used in agriculture since 1962. Chloridazon is included in the group of triazols.

From the chemical point of view chloridazon is a complex of 5-amino-4-chloro-2-phenyl-pyridazin- 3-one. Its herbicidal effect results from strong inhibition of Hils photosynthetic reaction. The following clinical symptoms were observed under conditions of acute intoxication in rats: apathy, hyperventilation, dyspnoea, hypersalivation, paralysis, tonic-clonic convulsions as well as death (Mlynarčikova et al., 1998).

The present study evaluated the capability of this herbicide to cause genetic inpairment by the induction of MN in sheep and bovine lymphocytes in vitro.

MATERIAL AND METHODS

Chloridazon (5-amino-4-chloro-2-phenyl-pyridazin-3-one, purity 92.6%, Istrochem, Slovak Republic) was dissolved in dimethyl sulfoxide (DMSO) and prepared immediately before each experiment at concentrations of $7x10^{-4}$ M, $7x10^{-5}$ M and $7x10^{-6}$ M.

The positive control:mitomycin C (MMC, CAS no. 50-07-7, Sigma, St. Louis, MO, USA), was dissolved in redistilled water at the concentration of 0.4 μM and used in the MN test.

Lymphocyte cultures were prepared by adding 0.5 ml of heparinized whole blood from 2 healthy donors (2-year-old Merino sheep and Slovak spotted cattle, 3-years old) to 10 ml of chromosome medium RPMI 1640 supplemented with 15% foetal calf serum (BOFES, Workplace for special culture Sera, Brno, Czech Republic), antibiotics (penicillin 250 U/ml and streptoycin 250 μg/ml) and phytohaemagglutinin (PHA, 180 μg/ml, Welcome, Dartford, England).The lyphocyte cultures were incubated at 37^0C for 72 h. Cytochalasin B (Cyt. B, Sigma, St. Louis, MO, USA) was added 44 h after starting the culture, at a concentration of 3 μg/ml. The tested substance was added 24 h after culture initiation and was present until the end of cultivation (Surrales et al., 1995).

Slides for the MN test were stained with 5% Giemsa (Merck, Darmstadt, Germany) in Sörensen phosphate buffer, (pH 6.8) for 15 min.

The MNi were identified by means of criteria published by Countryman and Heddle (1976). The induction of MN was evaluated by scoring a total of 1000 binucleatd (BN) cells per donor and concentration.

The number of micronuclei was analysed statastacally using the chi-square test.

RESULTS AND DISCUSSION

Chloridazon was tested for its ability to induce micronuclei in sheep and bovine lymphocyte in vitro. Table 1 shows the results obtained in sheep and bovine lymphocyte cultures from two donors exposed to the herbicide. The treatments lasting 48 h provided cultures showing a dose-related increase which was statistically significant at the highest concentration tested ($7x10^{-4}$ M). The results obtained with sheep peripheral lymphocytes and bovine lymphocytes showed significant elevations in MN ($P< 0.01$ and $p < 0.001$, respectively). The exposure to lower doses of chloridazon ($7x10^{-5}$ M and $7x10^{-6}$ M) had no positive effect on the induction of MN.

Table 1. Induction of micronuclei in sheep and bovine lymphocytes from two donors after chloridazon treatment

Treatment	MN_{CB}	SD
Sheep		
DMSO (control)	20.0	0.14
Chloridazon 7×10^{-6} M	27.0[a]	0.17
" 7×10^{-5} M	28.5[a]	0.17
" 7×10^{-4} M	34.5**	0.18
MMC (0.4μ M)	67.0***	0.26
Cattle		
DMSO (control)	37.5	0.20
Chloridazon 7×10^{-6} M	39.5[a]	0.20
" 7×10^{-5} M	48.0[a]	0.21
" 7×10^{-4} M	66.0***	0.26
MMC (0.4μ M)	94.5***	0.30

MN_{CB}: mean number of MN in CB
SD: standard deviation
, *: Statistical significance ($P< 0.01$ and $P<0.001$, respectively, chi - square test)
*: nonsignificant differences

Figure 1 shows the percentage of micronuclei in CB cells. The data indicate that chloridazon increases the incidence of MN in lyphocytes in both the speices investigated.

The spontaneous induction of micronuclei amounted 10.0 MN (mean value) per 1000 binucleated cells in sheep lyphocytes and 18.7 MN (mean value) per 1000 binucleated cells in bovine lymphocytes. The number of micronuclei in 1000

binucleated cells of positive controls (Mitomycin) was 33.5 MN (mean value) in sheep lymphocytes and 45.9 MN (mean value) in bovine lymphocytes.

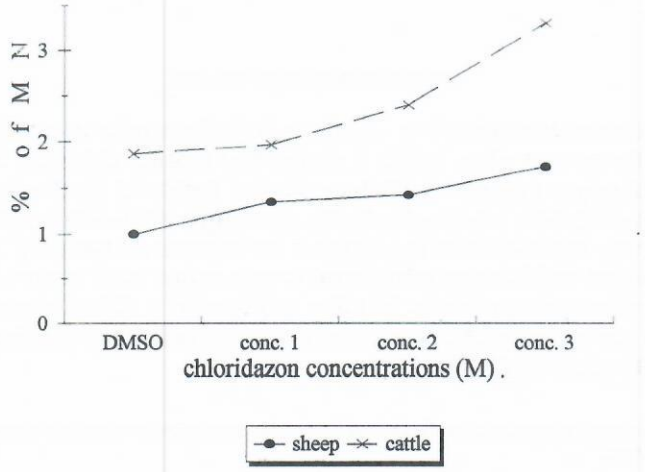

Figure 1. Percentage of MN in sheep and bovine lymphocytes in vitro after chloridazon treatment

Concentration 1: $7x10^{-6}$ M
Concentration 2: $7x10^{-5}$ M
Concentration 3: $7x10^{-4}$ M

The micronucleus test is a genotoxic endpoint widely used in vivo (mouse bone marrow test, Gudi et al., 1992), in vitro (short-tests in culture cells, Fritzenschaf et al., 1993) and for the biomonitoring of human populations (Fenech, 1993). Because of the dual origin of MN (fragments or whole chromosomes) MN induction can be a good in vitro tool in investigations of the effects of clastogens and aneuploidogens.

The genotoxic potential of herbicides has been investigated by several other researchers who used the MN test, too. Ribas et al. (1996 ab) evaluated the effects of herbicides such as alachlor, maleic hydrazide and trifluralin, for their genotoxicity to human peripheral blood lymphocyte cultures. Kevekordes et al. (1997) tested nitro musk compounds by the MN test in human lymphocytes in vitro and the human hepatoma cell line Hep G2. The nitro musk agents are used in some technical products such as herbicide formulations. Five pyrethroid insecticides were tested by Surralles et al. (1995)for their ability to induce micronuclei in both whole blood and isolated human lymphocytes.

Comparison our results with those of other short-term tests indicated similar responses. Šivikova and Buleca Jr. (1997) tested the affects of chloridazon on the induction of increased sister chromatid exchanges (SCE) in bovine lymphocytes in vitro. A statistically significant increase in SCE was seen at the doses

of $7x10^{-5}$ M and $7x10^{-4}$ M ($P < 0.05$ and $P < 0.01$, respectively). Piešova and Šivikova (1997) evaluated the effects of chloridazon on the induction of SCE in sheep peripheral lymphocytes in vitro. They found that the herbicide was capable of inducing SCE at the highest concentration of $7x10^{-4}$ M ($P<0.05$).

Our results demonstrate the sensitivity of sheep and bovine peripheral lymphocytes to chloridazon exposure in the MN test. The fact that the herbicide induced chromosome damage (MN) only at its highest concentration ($7x10^{-4}$ M) suggests only a weak genotoxic effect of chloridazon on sheep and bovine lymphocytes in vitro. According to our results, bovine lymphocytes exhibited higher susceptibility to chloridazon exposure than sheep lymphocytes, at least under our conditions of testing.

Acknowledgements

The present study was supported by grant No. 1/5150/98 of the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and Slovak Academy of Sciences.

REFERENCES

1. *Anwar A. W.* 1997. Biomarkers of human exposure to pesticides. *Environ. Health Perspect, Vol. 105, (Suppl. 4)*, 801-806.
2. *Carrano A. V., Thompson L. H., Linde P. A., Mikler J. L.* 1978. Sister chromatid exchanges as indicator of mutagenecity. *Nature*, 271-553.
3. *Chaisson C. F., Petersen B. J., Eickhoff J. C., Slesinski R. S.* 1980. Pesticides in Our Food *Facts, Issues, Debates, Perceptions. Technical Assessment Systems, Washington, DC.*
4. *Countryman P. I. and Heddle, J. A.* 1976. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. *Mutat. Res. 41, 321-322.*
5. Fenech M. 1993. The cytokinesis-block micronucleus technique: A detailed description of the method and its application to genotoxicity studies in human populations. *Mutat. Res. 285,* 35-44.
6. *Fritzenschaf H., Kohlopth M., Rusche B and Schiffman D.* 1993. Testing of known carcinogens and noncarcinogens in the Syrian hamster embryo (SHE) micronucleus test in vitro: micronucleus formation and cell transformation. *Mutat Res., 319,* 47-53.
7. *Gudi E., Xu Y. and Thilager A.* 1992. Assessment of the in vivo aneuplody/micronucleus assay in mouse bone marrow cells 16 chemicals. Environ. *Mol. Mutage. 20.* 106-116.
8. *Hrelia P., Vigagni F., Morotti M., Cantelli Forti G.* 1991. Genotoxic effects of pesticides ant their value in the prediction of risk to humans. *Pharmacol. toxicol. 69,* 18.
9. *Hrelia P., Vigagni F., Maffei F., Morotti M., Colacci A., Perocco P., Grilli S., Cantelli-Forti G.* 1994. Genetic safety evaluation of pesticides in different short-term tests *Mutat Res., 321,* 219-228.
10. *Jones S., Boffetta P.* 1997. AACR/IARC meeting on carcinogenesis from environmental. pollution: Assessment of human risk and strategies for prevention. *Annals of Oncology, 8,* 137-138.
11. *Kevekordes S., Zaulig A., Dunkelberg H.* 1997. Genotoxicity of nitro musks in the micronucleus test with human lymphocytes in vitro and the human hepatoma cell line *Hep G2 Toxicol.Lett. 91,* 13-17.

12. *Mlynarčikova H., Legath J., Guzy J., Elias V.* 1998. The toxic effects of chloridazon TP under conditions of acute intoxication in rats. *Slov. vet. čas 23,* 47-50.

13. *Norppa H., Luomahara S., Heikanen H., Roth S., Sorsa M., Renzi L., Lindholm C.* 1993. Micronucleus assay in lymphocytes as a tool to biomonitor human exposure to aneuploidogens and clastogens. Environ. *Health Perspect. Suppl. 101,* 139-143.

14. *Piešova E., Šivikova K.* 1997. Induction of micronuclei and sister chromatid exchanges in sheep lymphocyte culture by herbicide chloridazon. *Acta Vet. Brno, 66,* 229-234.

15. *Pokorna Z., Strnadova V., Rubeš J., Zudova Z.* 1996. Mutagenicity of feeds and bovine milk in districts with different levels of environmental pollution. *Vet. med.: Czech. 41,* 351-358.

16. *Ribas G., Surralles J., Carboneli E., Xamena N., Creus A., Marcos R.* 1996a. Genotoxicity of the herbicides alachlor and maleic hydrazide in cultured human lymphocytes. *Mutagenesis, 11,* 221-227.

17. *Ribas G., Surralles J., Carbonell E., Xamena N., Creus A., Marcos R.* 1996b. Genotoxic evaluation of the herbicide trifluralin on human lymphocytes exposed in vitro. *Mutat. Res., 371,* 15-21.

18. *Surralles J., Carbonell E., Marcos R., Degrassi F., Antoccia A., Tanzerella C.* 1992. A collaborative study on the improvement of the micronucleus test in cultured human lyphocytes. *Mutagenesis, 7, 407-410.*

19. Surralles J., Xamena N., Creus A., Catalan J., Norppa H., Marcos R. 1995. Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures. Mutat. Res., *341,* 169-184.

20. *Šivikova K., Buleca J., Jr.* 1997. In vitro induced sister chromatid exchanges after exposure to chloridazon in peripheral lymphocytes of cattle. *Slov. vet. čas. 22, 3,* 159-161.

21. *Tucker D. J., Preston R. J.* 1996. Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges, and cancer risk assessment. *Mutat. Res., 365,* 147-159.

INDUCIJA MIKROJEZGARA U OVČIJIM I GOVEĐIM LIMFOCITIMA POSLE EKSPOZICIJE HERBICIDOM CHLORIDAZONOM IN VITRO

ELENA PIEŠOVA

SADRŽAJ

U našem radu smo proučavali delovanje chloridazona na indukciju mikrojezgara u ovčijim i goveđim limfocitima. Chloridazon je delovao u svim testiranim koncentracijama $7x10^{-6}$ M, $7x10^{-5}$ M, $7x10^{-4}$ M povećanjem frekvencija microjezgara (MN). Statistički značajno povećanje broja N smo primetili kod koncentracije $7x10^{-4}$ M ($P < 0.01$) u ovaca, odnosno ($P < 0.001$) u goveda. Obe niže koncentracije nisu imale uticaj na indukciju MN.

Na osnovu naših rezultata, možemo zaključiti da je herbicid chloridazon imao samo slab učinak na indukciju posmatranih hromozomalnih promena (MN).