ELSEVIER

The Science of the Total Environment 180 (1996) 81–85

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

Assessment of radio-frequency electromagnetic radiation by the micronucleus test in Bovine peripheral erythrocytes

Zanda Balode

Department of Botany and Ecology, University of Latvia, Kronvalda blvd. 4, Riga, LV 1842, Latvia

Abstract

Previous bioindicative studies in the Skrunda Radio Location Station area have focused on the somatic influence of electromagnetic radiation on plants, but it is also important to study genetic effects. We have chosen cows as test animals for cytogenetical evaluation because they live in the same general exposure area as humans, are confined to specific locations and are chronically exposed to radiation. Blood samples were obtained from female Latvian Brown cows from a farm close to and in front of the Skrunda Radar and from cows in a control area. A simplified alternative to the Schiff method of DNA staining for identification of micronuclei in peripheral erythrocytes was applied. Microscopically, micronuclei in peripheral blood erythrocytes were round in shape and exhibited a strong red colour. They are easily detectable as the only coloured bodies in the uncoloured erythrocytes. From each individual animal 2000 erythrocytes were examined at a magnification of ×1000 for the presence of micronuclei. The counting of micronuclei in peripheral erythrocytes gave low average incidences, 0.6 per 1000 in the exposed group and 0.1 per 1000 in the control, but statistically significant ($P < 0.01$) differences were found in the frequency distribution between the control and exposed groups.

Keywords: Electromagnetic radiation; Cows; Peripheral erythrocytes; Micronuclei

1. Introduction

It has been suggested that in the direct zone of electromagnetic radiation exposure from the Skrunda Radio Location Station (RLS) pine trees have decreased their incremental growth (Balodis et al., 1995). This raised the question of possible exposure effects on humans and other species. In view of the somatic effects seen around the Skrunda RLS, it seemed important to investigate the possible genetic effects of the electromagnetic field.

Several authors have studied the genetic effects of electromagnetic radiation (McCann et al., 1993) on microbial systems (Juutilainen and Liimatainen, 1986), plants (Schmutz et al., 1994) and humans (Nordenson et al., 1988). However, controversial results were obtained, and usually 60-Hz fields were analyzed. The Skrunda RLS presents a unique case for the study of impulse

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SSDI 0048-9697(95)04923-O

radio-frequency electromagnetic radiation effects, due to its frequency (154–162 MHz) and pulse regime.

Combinations of different methods may be applied to assess the influence of one factor. Specific combinations of genotoxicity tests have been developed for the testing of chemicals, for instance, by the U.S. Environmental Protection Agency (Heddle et al., 1983). This has not been applied for the testing of EM radiation effects.

At present, the micronucleus test is the most popular short-term assay for evaluation of clastogenicity (Schmid, 1977; Chrisman and Baumgartner, 1979; Hayashi et al., 1984, 1990, 1992a, 1992b; Grawe et al., 1993; Watanabe et al., 1993). The micronucleus test described by Schmid (1982) in the early-1970s is often used as an indicator of chromosome damage. Micronuclei arise from chromosomal fragments or chromosomes that are not incorporated into daughter nuclei at the time of cell division. The peripheral blood micronucleus test is used to evaluate acute clastogenic effects and assess chronic damage of chromosomes (The Collaborative Study Group for the Micronucleus Test, 1992).

Schmid (1977) developed the May-Grünwald-Giemsa staining method in the micronucleus test. To improve the identification of micronuclei, several modifications have been developed. Gollapudi and Kamara (1979) described a simple Giemsa staining method and MacGregor et al. (1980) used a filtered Wright-Giemsa stain and Feulgen stain. The DNA stain Hoechst 33342 was used to identify and count micronuclei by flow cytometry (Grawe et al., 1993). Ultra-vital staining with acridine orange was recently introduced by Hayashi et al. (1990) for micronucleus assay with mouse peripheral blood cells.

In mutagenic testing protocols, usually the mouse has been used (Manna, 1991). Spontaneous frequencies of micronucleated erythrocytes in mice, rats and hamsters have been reported (Heddle et al., 1983; Zaickina and Ganassi, 1984). The test used for detection of micronuclei in human differs very little from the standard micronucleus test used in rodents and has been used to study individuals who have a high risk of chromosome damage (Jensen and Hüttel, 1976; Jensen and Nyfors, 1979; Mädki-Paakkanen, 1987; Nordic Study Group on the Health Risk of the Chromosome Damage, 1990).

Nordenson et al. (1988) reported chromosomal effects in lymphocytes of 400-kV substation workers. Compared to the control group, the exposed men displayed a statistically significant increase in chromosomal aberrations and cells with micronuclei. No increase was found in the frequency of sister chromatid exchange.

The current study is focused on the use of the micronucleus test in peripheral blood erythrocytes to indicate chromosome damage in cows living in the Skrunda RLS radiation zone. Cows were used for testing, since they live in uniform conditions and are exposed to the same type of radiation as humans in the area.

2. Materials and methods

2.1. Animals

Blood samples were obtained from 67 female Latvian Brown cows in a farm close to and in front of the Skrunda Radio Location Station and from 100 cows in a control area. The control area was selected based on the similarity to the exposed area, for all factors except electromagnetic radiation (Balodis et al., 1993). All animals were free from viral infections and had not been exposed to vaccinations and drugs during the previous 3 months. The animals had been living in the herd for at least 2 years.

2.2. Blood collection, slide preparation and staining

Blood samples were collected by puncture of v. jugularis in preservative-free heparin (10 IU heparin/ml blood). The blood was smeared on a clean glass slide, fixed with 96% ethanol for 5 min, air dried, hydrolysed in 1 N HCl at 60°C in a water bath for 8 min and stained with basic fuchsin in acid alcohol for 30 min by a simplified alternative to the Schiff method (Horobin and Kevill-Davies, 1971). Unreacted stain was removed by rinsing with tap water.

2.3. Microscopical analysis

Preparations were coded and observed blindly. The slides were screened at a magnification of

×100 for regions of suitable technical quality, where the cells were well spread and undamaged. Preliminary work indicated that counts of 1000 cells per animal were too low due to the rarity of micronucleated erythrocytes, but 2000 cells per animal provided suitable statistical power. A total of 2000 cells was observed at ×1000 magnification for each animal.

2.4. Statistical methods

For statistical evaluation, the Kolmogorov-Smirnov two sample test was used (Sokal and Rohlf, 1981).

3. Results

Microscopically, micronuclei in peripheral blood erythrocytes were round in shape and had a strong red colour. Since they were the only coloured bodies in the uncoloured erythrocytes, they were easily seen.

The frequency distribution of micronucleated erythrocytes was asymmetrical (Fig. 1). In the exposed group, 49% of cows had no micronucleated erythrocytes. In cows with micronucleated erythrocytes, 6% had outstanding high numbers of micronuclei. In contrast, 78% of control cows had no micronucleated erythrocytes, and no erythrocytes with high numbers of micronuclei were seen (Table 1).

The average incidences of micronuclei in peripheral erythrocytes were low, 0.6 per 1000 in the exposed group and 0.1 per 1000 in the control group. However, statistically significant ($P <$ 0.01) differences in the frequency distribution between the control and exposed group were found ($D = 0.29 > D_{0.01} = 0.26$).

Table 1
Frequencies of micronucleated erythrocytes in control and exposed cows

Micronuclei per 2000 cells	Exposed group	Control group
0	33	78
1	14	17
2	8	4
3	8	1
4	2	0
5	0	0
6	1	0
7	1	0

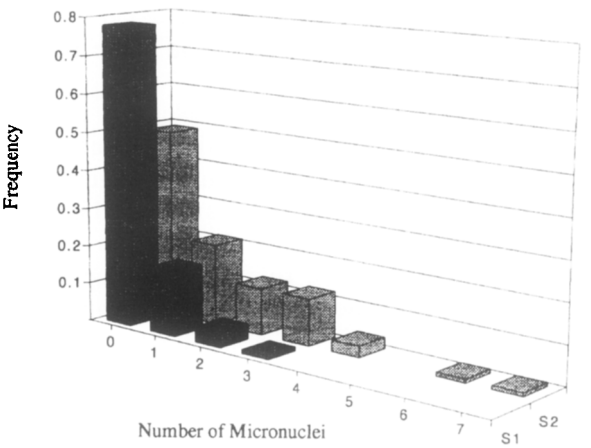


Fig. 1. Relative frequency distributions of micronucleated erythrocytes in control (S_1) and exposed (S_2) cows.

4. Discussion

Ecoepizoological methods are more suitable for assessment, since variation from factors other than pollution may be standardised. For example, livestock have enforced controlled conditions such as standard diet, enclosure and, for open grazing practice most common in Latvia, low social stress. The monitoring of chromosomal aberrations in farm animals is suitable for the assessment of the hygienic levels of herds with possible exposure to genotoxicants. For example, Rubes et al. (1992) carried out cytogenetic examinations of farm animals (cattle, horses and pigs) in areas with different levels of environmental contamination. Statistically significant differences of aberrant cells numbers were observed, despite the low animal sample size. Electromagnetic radiation effects are expected to be weak, and the study of chromosomal aberrations is expensive and time consuming. Therefore, the micronucleus test was more suitable for the present study.

A simplified alternative to the Schiff method was successfully used. It was introduced by Horobin and Kevill-Davies (1971) for staining DNA, but to our knowledge has not been used to

identify micronuclei in peripheral blood erythrocytes. This method is simpler and more precise counts can be made.

Cows with numerous micronuclei can indicate environmentally-caused genetical damage or abnormal haematopoiesis. In further studies, the chromosome aberration assay can be used in these cows to clarify the reasons for and the type of genetical damage. The role of Bovine spleen in the capture and destruction of micronucleated erythrocytes still requires clarification.

Acknowledgements

I am grateful to I. Muiznieks, M. Hayashi, A. Richters and an anonymous reviewer for helpful comments on the manuscript, and to Ilze, Valdis, Guntis, Didzis, Karlis, Niks, Kamils and my cows for good company and fun.

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