

The micronucleus frequency in cytokinesis-blocked lymphocytes of cattle in the vicinity of a nuclear power plant

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Cytogenetic and hematological analyses were performed on the peripheral blood lymphocytes (PBLs) obtained from Korean native cattle bred in the vicinity of three nuclear power plants (Wolsong, Uljin and Yeonggwang) and in a control area. The micronucleus (MN) rates for the cattle from the Wolsong, Uljin and Yeonggwang nuclear power plants and for the control area were 9.87 ± 2.64 , 8.90 ± 3.84 , 9.20 ± 3.68 and 9.60 ± 3.91 per 1,000 cytokinesis-blocked lymphocytes, respectively. The apparent difference is not statistically significant. The MN frequencies of PBLs from cattle bred in the four areas are within the background variation for this study. The MN frequencies and hematological values were similar regardless of whether the cattle were bred near a nuclear power plant or in the control area.

Key words: cattle lymphocyte, micronucleus, nuclear power plant

Introduction

It is often useful to match human epidemiology with animal studies. Animals monitored or evaluated *in situ* for an appropriate suite of endpoints can provide required information on both the exposure levels and any potential adverse health effects. Animals have served as sentinel indicators for various health effects associated with a number of environmental hazards, including radiation [25,27]. Domestic animals may be particularly valuable in this application because they also share the human environment.

One way of assessing the risk of genotoxins to humans is to develop nonhuman biological models where the dose, route of exposure, cell type, and end point examined are closely matched with those used for human screening. The peripheral blood lymphocyte (PBL) is the model cell type of choice for cytogenetic analyses. PBLs are relatively long-lived, initially nondividing cells that can be removed from human subjects with minimal discomfort, and have been used successfully as a biological dosimeter for examining the level of exposure to ionizing radiation [4,10,17,21].

The cytokinesis-blocked micronucleus (CBMN) assay has been used widely to assess *in vitro* radiation-induced chromosomal damage, and a satisfactory dose relationship has been reported [14,16,22]. The micronucleus (MN) frequency has also been shown to be a reliable biomarker in many biomonitoring studies involving human populations examining therapeutic [8,19], occupational [5,9,20,23], and accidental or environmental [6,7] exposure to ionizing radiation. Compared with classical cytogenetic methods for evaluating the level of chromosomal damage, the MN assay for PBLs is relatively simple to perform and allows the rapid scoring of a large number of cells by personnel who are not specifically trained in chromosomal analysis. The ease of MN assays, as well as the reliability of the cytokinesis-blocked (CB) approach is an obvious advantage in radiobiological monitoring [1,12,18].

The ultimate goal of any mutagen testing program is to test the potential mutagen directly on human genetic material or at least to confidently extrapolate the results from other test systems to humans [2,3,13,15]. From a purely genetic standpoint, it is inconceivable that controlled testing will ever be performed on humans. Therefore, an extrapolation method must be developed. In order to accomplish this, it is essential that parallel experiments be carried out on either identical, or closely related, biological systems.

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The aim of this investigation was to determine the MN frequencies in PBLs of cattle bred in the vicinity of three nuclear power plants in the Korea (Wolsong, Uljin and Yeonggwang) and in a group of cattle bred in a control area, using the CBMN assay and hematological analysis.

Materials and Methods

Subjects and samples

Blood samples were obtained from 35 Korean native cattle in farms located about 2 km away from each nuclear power station (Wolsong, Uljin and Yeonggwang) and 15 cattle in a control area (an area 100 km away from any of the nuclear power plants). All animals were free from viral infection and had not been exposed to vaccinations and drugs during the previous 3 months. The animals had been living in a herd for at least 1.5 years.

Measurement of hematograms and hematocrits

Whole blood was collected from the jugular vein of the cattle and the hematological parameters (hemoglobin, erythrocyte count, leukocyte count, platelet count and hematocrit) were determined by using an automated counter (Hemavet 850+; CDC Technologies, USA).

Cell culture

Lymphocytes were separated from the whole blood of cattle on Ficoll-Hypaque gradients, washed twice in Hank's balanced salt solution and were resuspended in RPMI 1640 medium containing Hepes buffer, 15% heat inactivated fetal calf serum, 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 100 U/ml penicillin and 100 µg/ml streptomycin. The lymphocytes were cultured in 12-well tissue culture plates (Corning, USA) at concentration of 5×10^5 cells/ml. The optimum concentration (2%) of phytohemagglutinin (PHA; Gibco BRL, USA) was used to stimulate the lymphocytes to transform and divide. The cells were cultured in a humidified atmosphere containing 5% CO₂ at 37°C.

Cytokinesis-block method

Cytochalasin B (Cyt-B; Aldrich Chemical, USA) was dissolved as a stock solution in dimethylsulfoxide at a concentration of 2 mg/ml, and was divided into small portions, and stored at -70°C. The Cyt-B stock solution was thawed, diluted in the medium and was added at a concentration of 4.0 µg/ml in medium 44 h after beginning the culture. A concentration of 4.0 µg/ml Cyt-B was chosen as this concentration was found to yield the largest number of binucleated cells. After a 72 h incubation period, the cells were resuspended and harvested onto glass slides using a cytocentrifuge (Cellspin; Hanil Science, Korea). The slides were air-dried, fixed in a mixture of methanol:glacial acetic acid (3 : 1) for 10 min and were stained using May-Gruenwald Giemsa for 10 min.

Scoring of MN

The MN in a total of 1,000 CB cells were scored using a microscope (Nikon Eclipse E600; Nikon, Japan) at a magnification of 1,000× with oil emulsion. Published criteria were used to identify the MN [1].

Statistic analysis

The data is presented as the mean and standard deviation. Statistical analysis was performed using the Student's *t*-test to express the difference between the two groups. All analyses were performed using computer software (Graph PAD In Plot; Graph PAD Software, USA) and a personal computer.

Results

Table 1 shows the hematograms and hematocrits determined for the cattle from farms adjacent to the nuclear power plants and from a control area. Hematological analysis was performed to evaluate the general biological state of the animals. No significant difference was found in the counts between animals located near a nuclear power plant and animals in the control area.

Table 1. Hematological values from cattle in the vicinity of the Wolsong, Uljin, Yeonggwang nuclear power plants and a control region

Test	Unit	Control	Wolsong	Uljin	Yeonggwang
Erythrocyte	10 ⁶ /µl	7.16 ± 0.50	7.48 ± 1.30	8.78 ± 1.10	8.57 ± 1.34
Hemoglobin	g/dl	9.83 ± 0.52	10.15 ± 1.21	13.52 ± 1.62	12.30 ± 1.89
Hematocrit	%	29.90 ± 1.96	33.13 ± 3.51	33.02 ± 3.26	34.31 ± 2.71
Leukocyte	10 ³ /µl	8.18 ± 1.89	8.38 ± 2.14	8.70 ± 1.51	9.18 ± 2.16
Neutrophil	10 ³ /µl	3.37 ± 1.13	3.66 ± 1.58	4.01 ± 1.33	2.97 ± 0.94
Lymphocyte	10 ³ /µl	4.49 ± 1.47	3.99 ± 1.12	4.14 ± 0.42	5.24 ± 1.66
Monocyte	10 ³ /µl	0.25 ± 0.10	0.37 ± 0.12	0.26 ± 0.14	0.80 ± 0.25
Eosinophil	10 ³ /µl	0.13 ± 0.10	0.28 ± 0.15	0.22 ± 0.10	0.12 ± 0.08
Basophil	10 ³ /µl	0.04 ± 0.03	0.10 ± 0.05	0.07 ± 0.04	0.05 ± 0.04
Thrombocyte	10 ³ /µl	240 ± 59	226 ± 60	279 ± 42	390 ± 84

*mean ± SD.

Table 2. Micronucleus frequency in binucleated lymphocytes of cattle in the vicinity of the Wolsong, Uljin, Yeonggwang nuclear power plants and a control region

Subject	Number of MN per 1,000 CB cells			
	Control	Wolsong	Uljin	Yeonggwang
1	10	13	10	7
2	4	9	15	11
3	7	8	7	8
4	7	6	8	11
5	21	11	3	10
6	10	7	14	6
7	10	12	9	13
8	13	7	4	4
9	7	10	11	6
10	7	6	8	16
11	9	13		
12	8	10		
13	11	10		
14	8	12		
15	12	14		
mean	9.60	9.87	8.90	9.20
± SD	± 3.91	± 2.64	± 3.84	± 3.68

The peak of binucleated cell formation (16%) was found at a concentration of 2% PHA and 4 µg/ml Cyt-B. Table 2 shows the incidence of MN observed in cattle from all of the four areas. All of the cells with micronuclei were seen with only one micronucleus per binucleated cell. The incidence in Wolsong, Uljin, Yeonggwang and the control area were 9.87 ± 2.64 , 8.90 ± 3.84 , 9.20 ± 3.68 and 9.60 ± 3.91 , respectively. The apparent difference is not statistically significant.

Discussion

Environmental release of low levels of radionuclides associated with mining, nuclear power and weapons production, as well as the medical use of radionuclides is a topic of continuing public concern [11,26]. Ecoepizootological methods are more suitable for assessment, because variations from factors other than pollution can be considered. For example, livestock have enforced controlled conditions such as a standard diet, enclosure, and in open grazing practice, low social stress, which make them an excellent sentinel animal for assessing the level of chromosomal damage that is most likely to be relevant to ecological risk from chronic, low-level exposure to radionuclides (and other clastogens) in the environment. The monitoring of chromosomal aberrations in agricultural animals is suitable for assessing the hygiene levels of herds that might have been exposed to genotoxicants. The radiation effects of ecodosimetric fields are expected to be weak. Moreover, a study of chromosomal

aberrations is expensive and time consuming. Thus, a micronucleus test is more suitable for this study. The use of the MN assay would make a good screening test after an accidental release at a known point in time, and it would be useful in elucidating mechanisms of biological response in this species.

Ecological dosimetry could revolutionize ecological risk assessment by providing a direct, accurate and precise method for estimating the dose that organisms have received in the field. Direct investigations of the effects of chronic, low-level environmental exposures in humans are impossible for obvious ethical reasons. Therefore, in addition to being ecologically relevant, ecodosimetric investigations could provide valuable insight into the effects of this type of exposure on humans [25].

The difference in the MN values between those of cattle bred near the nuclear power plants and those of cattle in the control area were not significantly different. The MN frequencies of PBLs from cattle bred in four areas show values that are within the background variation in this study. The MN frequencies and hematological values were similar regardless of whether the cattle were bred near a nuclear power plant or the control area.

The cytogenetic biomarkers of radiation exposure in the cattle lymphocytes for environmental biodosimetry represent a useful methodology to employ for determining the level of exposure of a radionuclide-contaminated environment and provide genetically relevant measurement endpoints for ecological risk assessments [24]. The technique can be more sensitive when the MN frequencies are determined only in B-lymphocytes [22]. Another method to consider is the determination of the number of MN with centromeres. For this purpose, hybridization with pancentromeric DNA probes and fluorescence labeling is advantageous to perform [22] under the same experimental conditions.

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