A Cytogenetic Study of Korean Native Goat Bred in the Nuclear Power Plant using the Micronucleus Assay

Chang Mo KANG1, Hae June LEE2, Young Hoon JI1, Tae Hwan KIM3, Si Yun RYU4, Se Ra KIM2, Sung Kee JO5, Jong Choon KIM2 and Sung Ho KIM2*

Micronuclei/Radiation/Goat/Nuclear power plant.

Cytogenetic and hematological analysis was performed on the peripheral blood lymphocytes (PBLs) obtained from Korean native goats bred in two nuclear power plants (Wolsong and Uljin) and a control area. The frequencies of gamma-ray-induced micronuclei (MN) in the cytokinesis-blocked (CB) lymphocytes at several doses were measured in three Korean native goats. The measurements performed after irradiation showed dose-related increases in the MN frequency in each of the donors. The results were analyzed using a linear-quadratic model with a line of best fit of $y = 0.1019D + 0.0045D^2 + 0.0093$ ($y =$ number of MN/CB cells and $D =$ irradiation dose in Gy). The MN rates in the goats from the Wolsong and Uljin nuclear power plant, and the control area were 9.60 ± 2.88, 6.83 ± 1.47 and 9.88 ± 4.32 per 1,000 CB lymphocytes, respectively. The apparent difference is not statistically significant. The MN frequencies of PBLs from goats bred in three areas means that the values are within the background variation in this experiment. The MN frequencies and hematological values were similar regardless of whether the goats were bred in the nuclear power plant or the control area.

INTRODUCTION

It is often useful to match human epidemiology with animal studies. Animals monitored or evaluated in situ for the appropriate suite of endpoints can provide the 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.1,2) Domestic animals may be particularly valuable 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 PBL is the model cell type of choice for cytogenetic analyses. PBLs are relatively long-lived, initially nondividing, 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.3–6) The micronuclei (MN) assay in the cytokinesis-blocked (CB) lymphocytes has been used widely to assess the in vitro radiation-induced chromosomal damage, and a satisfactory dose relationship has been reported.7–9) The MN frequency was also shown to be a reliable biomarker in many biomonitoring studies involving human populations examining therapeutic,10,11) occupational,12–15) and accidental or environmental16,17) exposed to ionizing radiation. Compared with the classical cytogenetic methods for evaluating the level of chromosomal damage, the MN assay for PBLs is relatively simple 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 CB approach is an obvious advantage in radiobiological monitoring.18–20) The ultimate goal of any mutagen testing program is to test the potential mutagen directly on the human genetic material or at least to confidently extrapolate the results from other test systems to humans.21–24) From a purely genetic standpoint, it is inconceivable that a controlled testing will ever be carried out 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.

The aim of this study was to examine the MN frequency in the CB cells taken from Korean native goats after various gamma-rays doses in order to determine if these species are a suitable target organism for an environmental study.

**MATERIALS AND METHODS**

**Subjects and samples**

Blood samples were obtained from 11 Korean native goats in the barn of a nuclear power plant (Wolsong and Uljin) and 8 goats in a control area (Hwasoon, an area 250 km away from the nuclear power plants). All the animals were free from viral infections and had not been exposed to vaccinations or 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**

The whole blood was collected from the jugular vein of the goat at 7 am and the hematological parameters (hemoglobin, erythrocyte count, leukocyte count, platelet count and hematocrit) were determined using an automated counter (HEMA VET 850+, CDC Technologies, Inc. U.S.A.).

**Cell culture**

The lymphocytes were separated from the whole blood of the goats on Ficoll-Hypaque gradients, washed twice in Hank’s balanced salt solution and resuspended in RPMI 1640 medium containing Hapes buffer, 15% heat inactivated foetal calf serum, L-glutamine, 2-mercaptoethanol and antibiotics. The lymphocytes were cultured in multi-well tissue culture plates (Corning) at concentration of $5 \times 10^5$ cells/ml.

The optimum concentration (2%) of phytohaemagglutinin (PHA, GIBCO BRL) was used to stimulate the lymphocytes to transform and divide. The cells were cultured in a humidified atmosphere containing 5% CO$_2$ at 37°C.

**Gamma-ray irradiation in vitro**

The lymphocytes were separated from 5 ml blood samples that were obtained from 3 healthy Korean native goats using Ficoll sedimentation. The isolated lymphocytes were irradiated at room temperature in a sterile polystyrene tube (Corning) before adding the PHA in order to avoid the effects of the drug on the radiosensitivity. The medium including PHA was added immediately after irradiation. One sample served as the control for determining the spontaneous MN frequency. The others were irradiated with 246, 492, 739, 985 and 1,969 mGy of $^{60}$Co gamma-rays (Theratron-780 teletherapy unit, AECL, Canada) at a rate of 2,110 mGy/min, respectively. The doses were measured using a Capintec PR-06C farmer type chamber and a Capintec 192 electrometer (Capintec, U.S.A)

**Cytochalasin B**

Cytokinetic-block method

Cytochalasin B (Cyt-B, Aldrich Chemical Co.) was made up as a stock solution in dimethylsulphoxide at a concentration of 2 mg/ml, divided in small portions and stored at -70°C. The Cyt-B stock solution was thawed, diluted in the medium and added at a concentration of 4.0 µg/ml of medium 44 h after beginning the culture. A concentration of 4 µg/ml Cyt-B was chosen because this concentration was found to yield the largest number of binucleated cells. After a 72 hr incubation period, the cells were resuspended and harvested onto glass slides using a cytocentrifuge (Cellspin, Hanil Science Industrial Co. Korea). The slides were air-dried, fixed in a mixture of methanol : glacial acetic acid (3 : 1) for 10 min and stained using May-Gruenwald Giemsa for 10 min.

**Scoring of MN**

The MN in a total of 500 or 1,000 CB cells were scored using a microscope with a magnification of 1,000 × with an oil emulsion. In the in vitro experiment for obtaining the dose-response curve, MN counted in 500 of the CB cells in each sample, and the MN were counted in 1,000 CB cells from the goat in the control and nuclear power plant areas. The published criteria were used to identify the MN.$^{18}$

**Statistics analysis**

The data is presented as a mean and standard deviations. Statistical analysis was performed using a Student’s t-test to express the difference between the two groups. All analyses were performed using a Graph PAD In Plot computer program (GPIP, Graph PAD Software Inc.) and a personal computer.

**RESULTS**

Table 1 shows the hematograms and hematocrits. Hematological analysis was performed to evaluate the general biological state of the animals. No significant difference was found in the counts between the Nuclear power plant and the control area.

The peak of the binucleated cell formation (16%) was found at a concentration of 2% PHA and 4 µg/ml Cyt-B. Table 2 summarizes the data obtained in the dose-response study for the MN from the goat. The MN frequencies in the unexposed lymphocytes were similar regardless of the donor. The baseline number of MN per CB cell in the non-irradiated lymphocytes was quite low, approximately 9.3 per 1,000 cells. The MN frequencies in the unexposed lymphocytes were similar regardless of the donor. The baseline number of MN per CB cell in the non-irradiated lymphocytes was quite low, approximately 9.3 per 1,000 cells. There was a significant correlation between the frequency of the induced MN and the gamma-ray dose ($\rho = 0.996$). Regression analyses using a linear-quadratic model ($y = aD + bD^2 + c$; where D is dose in GY and y is the number of MN per CB cell) gave an excellent fit. The line of best fit was: $y = 0.1019D + 0.0045D^2 + 0.0093$.  

Table 1. Hematological values in goat bred in Wolsong, Uljin nuclear power plant and control region (mean±S.D.).

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>Control</th>
<th>Wolsong</th>
<th>Uljin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte</td>
<td>10³/µl</td>
<td>16.89±0.95</td>
<td>14.65±3.26</td>
<td>14.81±2.62</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>g/dL</td>
<td>11.13±0.95</td>
<td>9.98±1.78</td>
<td>7.62±1.16</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>%</td>
<td>28.64±2.22</td>
<td>26.77±4.81</td>
<td>31.33±6.13</td>
</tr>
<tr>
<td>Thrombocyte</td>
<td>10³/µl</td>
<td>452±186</td>
<td>300±164</td>
<td>356±142</td>
</tr>
<tr>
<td>Leukocyte</td>
<td>10³/µl</td>
<td>15.69±3.69</td>
<td>13.31±2.21</td>
<td>15.33±3.75</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>10³/µl</td>
<td>3.75±2.00</td>
<td>3.95±0.98</td>
<td>4.72±1.39</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>10³/µl</td>
<td>9.55±2.04</td>
<td>7.42±1.48</td>
<td>8.60±2.21</td>
</tr>
<tr>
<td>Monocyte</td>
<td>10³/µl</td>
<td>2.12±0.67</td>
<td>1.58±0.62</td>
<td>1.73±0.55</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>10³/µl</td>
<td>0.21±0.17</td>
<td>0.26±0.23</td>
<td>0.19±0.10</td>
</tr>
<tr>
<td>Basophil</td>
<td>10³/µl</td>
<td>0.07±0.05</td>
<td>0.11±0.11</td>
<td>0.09±0.04</td>
</tr>
</tbody>
</table>

Table 2. Micronuclei (MN) per 500 cytokinesis-blocked lymphocytes following gamma-irradiation of goat peripheral blood.

<table>
<thead>
<tr>
<th>Donor Dose (mGy)</th>
<th>No. of cells without MN</th>
<th>Number of MN per cell</th>
<th>Frequency of MN/1,000cell (mean±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>496</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>495</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>495</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.33±1.155</td>
</tr>
<tr>
<td>1</td>
<td>246</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>246</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>246</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>45.33±4.163</td>
</tr>
<tr>
<td>1</td>
<td>492</td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>492</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>492</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60.66±5.033</td>
</tr>
<tr>
<td>1</td>
<td>739</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>739</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>739</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>83.33±8.083</td>
</tr>
<tr>
<td>1</td>
<td>985</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>985</td>
<td>47</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>985</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>112.66±5.033</td>
</tr>
<tr>
<td>1</td>
<td>1,969</td>
<td>76</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>1,969</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>1,969</td>
<td>65</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>228.00±5.292</td>
</tr>
</tbody>
</table>

Table 3 shows the incidence of MN observed in the goats from the 3 areas. The incidence is expressed as the number of micronuclei per 1,000 binucleated lymphocytes. None of the cells containing the MN had more than one MN. The incidence in Wolsong, Uljin and the control area were 9.60±2.88, 6.83±1.47 and 9.88±4.32, respectively. The apparent difference is not statistically significant. The estimated MN rates were determined using the linear-quadratic model based on the radiation-induced MN data over the range, 0 mGy to 1,969 mGy, from the goat lymphocytes with in vitro irradiation. In order to determine the relative dose, the equation, \( y = aD + bD^2 + c \), was transformed to \( D = \left[-a ± \sqrt{(a^2 - 4b(c-y))}\right] / 2b \). According to the dose response relationship, the mean incidences of MN in the goats bred in the nuclear power plant (Wolsong and Uljin) and control (Hwasoon) area might be equivalent to the results obtained from the experiments involving the in vitro radiation exposure of gamma-rays at doses of under 100 mGy exposure.

Table 3. Micronucleus frequency in binucleated cells of goat lymphocytes from Wolsong, Uljin nuclear power plant and control region.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Number of MN per 1000 CB cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

DISCUSSION

A comparative study of different species is of interest in determining the extent of cytogenetic damage induced by genotoxic agents. Several reports have shown that most MN formed in human lymphocytes after exposure to ionizing radiation are derived from acentric fragments, either formed directly as a result of the action of ionizations and free radicals or they are derived from asymmetrical exchanges and the loss of whole chromosome. A comparison of the MN data with other chromosome-aberration data indicates an apparent difference in radiosensitivity in the formation of MN. Some of the factors suggested to explain the different relationships observed between the cytogenetic...
effect and cell death are the nuclear volume and DNA content,28 the chromosome number and the number of chromosome arms,22,29 but nothing conclusive has yet been published.30 The frequency of MN in the lymphocytes observed in this in vitro study was approximately 7 per cent lower on average than those of dicentrics reported by Debuyst et al.31 However, a direct quantitative extrapolation from the MN frequency to the chromosome aberration frequency of goat appears unreliable because there is an apparent difference in efficiency in the formation of MN from the acentric fragments in goats. The direct application of dose-response curves from goats is important when extrapolating the data from goats to humans or even from goats to other animal species.

Environmental releases of low levels of radionuclides associated with mining, nuclear power and weapons production, as well as the medical use of radio-nuclides is a topic of continuing public concern.32,33 Ecoepizootiological 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 for 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 the 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 genotoxins. The radiation effects of ecodosimetric fields are expected to be weak. Moreover, a study of the chromosomal aberrations is expensive and time consuming. Therefore, a micronucleus test is more suitable for this study.

Ecological dosimetry can revolutionize the 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 can provide valuable insight into the effects of this type of exposure in humans. According to the dose response relationship, the mean incidences of MN in the goats bred in the nuclear power plant (Wolsong and Uljin) and control (Hwasoon) area might be equivalent to the results obtained from the experiments involving the in vitro radiation exposure of gammarays at doses of under 100 mGy exposure, if it is under the conditions of acute exposure and without taking into account the decay of MN-bearing cells in vivo. The micronucleus determination is not as sensitive as that of chromosomal aberrations. Generally, doses above 0.1 Gy are required.6,9 So, the MN frequencies of PBLs from goats bred in three areas means that the values are within the background variation in this experiment. The MN frequencies and hematological values were similar regardless of whether the goats were bred in the nuclear power plant or the control area. This study developed a biomarker of radiation exposure for goats, which appears to be the first application of a MN assay-based bios dosimetry in this species.

The cytogenetic biomarkers of radiation exposure in the goat lymphocytes for environmental biodosimetry represents a useful methodology for determining the level of exposure to radionuclide-contaminated environments, and would provide genetically relevant measurement endpoints for ecological risk assessments. The technique can be more sensitive when the MN are determined only in B-lymphocytes. Another possibility exists by determination of the number of MN with centromeres. For this purpose the hybridization with pancentromeric DNA probes and fluorescence labeling is of advantage,9 under the same experimental conditions.

ACKNOWLEDGEMENTS

This work has been carried out under the Nuclear R&D Program by MOST of Korea.

REFERENCES


Received on November 30, 2004
1st Revision received on April 26, 2005
Accepted on April 27, 2005