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Dose-Rate Effects for Apoptosis and Micronucleus Formation in Gamma-Irradiated Human Lymphocytes

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We have compared dose-rate effects for γ-radiation-induced apoptosis and micronucleus formation in human lymphocytes. Long-term assessment of individual radiation-induced apoptosis showed little intraindividual variation but significant interindividual variation. The effectiveness of radiation exposure to cause apoptosis or micronucleus formation was reduced by low-dose-rate exposures, but the reduction was apparent at different dose rates for these two end points. Micronucleus formation showed a dose-rate effect when the dose rate was lowered to 0.29 cGy/min, but there was no accompanying cell cycle delay. A further increase in the dose-rate effect was seen at 0.15 cGy/min, but was now accompanied by cell cycle delay. There was no dose-rate effect for the induction of apoptosis until the dose rate was reduced to 0.15 cGy/min, indicating that the mechanisms or signals for processing radiation-induced lesions for these two end points must be different at least in part. There appear to be two mechanisms that contribute to the dose-rate effect for micronucleus formation. One of these does not affect binucleate cell frequency and occurs at dose rates higher than that required to produce a dose-rate effect for apoptosis, and one affects binucleate cell frequency, induced only at the very low dose rate which coincidentally produces a dose-rate effect for apoptosis. Since the dose rate at which cells showed reduced apoptosis as well as a further reduction in micronucleus formation was very low, we conclude that the processing of the radiation-induced lesions that induce apoptosis, and some micronuclei, is very slow in quiescent and PHA-stimulated lymphocytes, respectively. © 2000 by Radiation Research Society

INTRODUCTION

Apoptosis is an evolutionarily conserved form of cell death that has distinctive morphological and biochemical characteristics (*1*). Many signals, both extracellular and intracellular, are known to elicit this genetically regulated response in a variety of cells (*2, 3*). Organisms use apoptosis to eliminate extraneous (*4*) or genetically damaged cells (*5*). Ionizing radiation damages DNA and is one agent that induces apoptosis in certain cells, including human lymphocytes.

Human lymphocytes undergo apoptosis in a time- and dose-dependent manner (*6–8*). A characteristic biochemical event that occurs in apoptotic cells, and distinguishes them from other cells and other modes of cell death, is the fragmentation of nuclear DNA (*9*). The time required for DNA fragmentation in a cell undergoing apoptosis varies depending on the organism, cell type, and the type of inducing signal (*10*). The appearance of characteristically fragmented DNA in human lymphocytes undergoing radiation-induced apoptosis *in vitro* ranges from 6 to 72 h (*11*). Because of the kinetics and the relative sensitivity of lymphocytes to low doses of radiation, lymphocytes may be a useful biological dosimeter for radiation exposure (*12*). Recently, it has been suggested that structural changes in membranes of isolated unstimulated human lymphocytes could be used as early biological indicators of radiation exposure (*13*).

There are two kinetically distinctive pathways for cell death by apoptosis in human lymphocytes. Slowly repaired or persistent DNA lesions, including those produced by ionizing radiation, induce a slow, protein synthesis-dependent process, compared to membrane-oxidizing agents which trigger a fast process of apoptosis independent of protein synthesis (*14*). It has also been shown that human lymphocytes can be sensitized to radiation-induced apoptosis by a prior exposure to low doses of ionizing radiation (*15*).

Micronuclei are also useful biological indicators of radiation exposure, and their production has been studied extensively to understand many biological responses to ionizing radiation.

The occurrence of micronuclei has been used as an indicator of DNA damage by both chemical and physical agents (*16, 17*) including ionizing radiation (*18–21*). Micronuclei are small round bodies found in the cytoplasm outside the main nucleus and contain fused or fragmented chromosomes, as well as whole chromosomes. They appear to be structurally similar to the main nucleus (*22, 23*).

Micronucleus formation and apoptosis have been compared previously in several studies attempting to understand the biological relationship between these two indicators of radiation damage (*24, 25*). Previous reports using human

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lymphocytes indicated that micronucleus formation clearly shows a dose-rate effect (*26*) whereas cell death by apoptosis does not (*27*). It was suggested that induction of apoptosis in human lymphocytes is signaled by initial DNA damage and not by DNA repair (*27*). Micronucleus formation, however, is dependent on cellular DNA repair capacity (*16, 26, 28*). Together, these results suggest that the two radiation-induced end points, micronucleus formation and apoptosis, are signaled by different events and may not be biologically correlated.

In the work reported here, we have compared micronucleus formation and death by apoptosis induced at dose rates lower than those tested previously. We confirmed that micronucleus formation shows a dose-rate effect, but present new evidence showing that γ-ray-induced apoptosis in resting human lymphocytes also has a dose-rate effect. The dose rate required to produce an effect for apoptosis, however, was lower than that where an effect was seen for micronucleus formation.

METHODS

Cell Cultures for Apoptosis

Blood samples from healthy male volunteers were collected in heparinized tubes. Histopaque-1077 (Sigma, St. Louis, MO) was used to separate the mononuclear lymphocytes. The lymphocytes were washed twice in Hanks' balanced salt solution (Sigma) containing 1% fetal bovine serum at room temperature. The washed cells were placed into T-25 culture flasks at a concentration of 4.0×10^5 cells/ml in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD) containing 20% fetal bovine serum, 2 m*M* L-glutamine, 10 U/ml penicillin, and 10 mg/ml streptomycin sulfate.

Gamma Irradiations for Apoptosis

Cobalt-60 γ irradiation of cells at high dose rate was performed using a Gamma-Cell 200 (AECL, Canada) with a dose rate of 70.2 cGy/min. Flasks were placed into the irradiator with the temperature of the medium at 37°C. Immediately after the 4-Gy exposure, all flasks containing cells were incubated (37°C at 95% relative humidity and 5% CO_2) for 48 h, or longer times for studies of kinetics, to allow lymphocytes undergoing apoptosis to fragment their DNA prior to being assayed (*11, 12*). Low-dose-rate radiation exposures were delivered using a ^{60}Co GammaBeam 150 (AECL, Canada). Cells were irradiated with a total dose of 4 Gy at different dose rates (1.3, 0.7, 0.29 and 0.15 cGy/min) by placing the flasks at various distances from the ^{60}Co source. Dose rate was verified using a Keithley Model 35040 Therapy Dosimeter with a New England ionization chamber (Model 2530/1) calibrated against a National Research Council of Canada standard source. Flasks were irradiated while inside a 37°C incubator at 95% relative humidity and 5% CO_2. Cells were incubated after the end of the low-dose-rate exposure for additional time that was equivalent to a total of 48 h from the start of the high-dose-rate exposure [time to deliver 4 Gy (h) + incubation time (h) = 48 h] and then were assayed. To test for possible alterations in the kinetics of apoptosis at low dose rate, cells were also incubated for longer periods totaling 72 and 96 h from the start of the high-dose-rate exposure.

Apoptosis Measured by TdT and FADU Assays

The two assays used to detect radiation-induced apoptosis in these experiments were the terminal deoxynucleotidyl transferase (TdT) assay (ApopTag®, Oncor) and fluorescence analysis of DNA unwinding (FADU) assay. The detection of apoptotic cells using these two assays is based on a DNA fragmentation event which is characteristic of apoptosis in human lymphocytes. We have previously compared these two techniques and have reported that radiation-induced apoptosis in unstimulated human lymphocytes can be quantified using either technique (*11, 12*). Our results using the comet assay to measure apoptosis also agree with results from the TdT and FADU assays, further supporting the validity of these detection systems to quantify levels of apoptosis in human lymphocytes (*14*).

The TdT assay is a biochemical detection system that fluorescently labels fragmented DNA within an apoptotic nucleus by attaching digoxigenin-tagged nucleotides to the cleaved DNA ends through a terminal deoxynucleotidyl transferase reaction. The incorporated digoxigenated nucleotides were detected with fluorescein-labeled anti-digoxigenin antibodies and were scored using an epifluorescence microscope. A minimum of 1000 nuclei were scored for each data point per replicate experiment. Values for the TdT assay are given as percentage apoptosis.

The FADU assay is also a biochemical assay that measures fragmented DNA of apoptotic cells, but the technique involves analysis of bulk DNA from a population of cells. Approximately 2×10^6 cells were assayed per data point, and results are expressed as the amount of double-stranded DNA remaining and exhibiting ethidium bromide fluorescence after alkali unwinding (*29*). The extent of DNA unwinding, which is proportional to the amount of fragmented DNA, in a population of apoptotic cells for each treatment (D), was calculated from the mean of fluorescence measurements obtained from independent assays of quadruplicate aliquots from the same sample. The relative amount of fragmented DNA in a population of apoptotic cells in each treatment sample was then expressed in Qd units, where $Qd = -100\log(Dt/Dc)$ and Dt and Dc are the percentages of double-stranded DNA remaining after unwinding (determined fluorometrically) in the treated and untreated control cells, respectively (*29*). Therefore, the FADU assay quantified apoptotic DNA fragmentation and was expressed as "Apoptosis (Qd)" (*11, 12, 14, 15*). In our previous reports, measuring apoptosis using this technique had less error and was more reproducible than the TdT (*11*) or the comet assay (*14*).

Cell Culture for Micronucleus Assay

Peripheral blood lymphocytes were isolated from blood samples donated by nonsmoking male volunteers. Blood samples were collected by venipuncture and lymphocytes were isolated using a standard Histopaque gradient (Sigma, Oakville, Ontario). After separation, cells were counted on a Z2 Coulter Counter (Coulter, Burlington, Ontario) and resuspended in a T-25 flask containing RPMI medium (Canadian Life Technologies, Burlington, Ontario) supplemented with 10% fetal bovine serum, 2 m*M* glutamine, and 0.25% gentamycin. Cell cultures were set at 1.0×10^6 cells/ml. Phytohemagglutinin (Canadian Life Technologies) at 10 μg/ml was added immediately to the cell cultures to stimulate cell division. Cytochalasin B (Sigma) was also added immediately to the cell cultures at 5 μg/ml to block cellular cytokinesis, allowing identification and enumeration of micronuclei in binucleate cells that had completed one nuclear division but not cell division.

Gamma Irradiations for Micronucleus Assay

Cell suspensions were exposed to 4 Gy γ rays using a GammaCell 200 (AECL, Canada) irradiator for high-dose-rate exposures delivered at approximately 70 cGy/min or a GammaBeam 150 (AECL, Canada) irradiator for doses delivered at lower dose rates (0.29 and 0.15 cGy/min). Cells were then incubated at 37°C in a 95% humidified atmosphere of 5% CO_2 in air and harvested 72 h after the start of the irradiation. Cells were collected by centrifugation at 200*g* for 15 min. The cells were then preserved using 5% acetic acid fixative followed by addition of a mixture of 3:1 methanol:glacial acetic acid for 10 min. Cells in suspension were collected by centrifugation (200*g* for 15 min), and the fixative was removed. Then 0.5 ml of new fixative was used to resuspend the cell pellet and approximately 50 μl was spread onto a microscope slide. The slide

was air-dried and stored at room temperature prior to staining for microscopy.

To score micronucleus frequency, slides were stained for 6 s with 0.25 mg/ml acridine orange and then washed twice for 6 s each in distilled water. The stained cells were then covered with a No. 1 glass cover slip (Fisher, Ottawa, Ontario) using one drop of water as the mounting medium. Micronuclei were scored using a Zeiss Axiophot microscope (under FITC filter at 490 nm absorbance, 520 nm emission) according to criteria outlined by Fenech (*30*).

Data and Statistics

Mean values represent data from a minimum of three independent experiments. Standard error bars are shown for each data point except where the error is equal to or less than the symbol size. *P* values were calculated using Student's *t* test or analysis of variance (*31*).

RESULTS

Intra- and Interindividual Variation

We have compared apoptosis in lymphocyte samples taken from the same individuals over extended periods. Long-term assessment of individual donors showed very little intraindividual variation but significant interindividual variation. Blood lymphocytes from Donor 21, a 50-year-old male, randomly sampled eight times over a 22-month period, had minor variability in apoptosis 48 h after a high-dose-rate 4-Gy exposure, with a mean and standard error of 19.6 ± 1.2% apoptosis. Blood lymphocytes from Donor 6, a 47-year-old male, randomly sampled seven times over a 12-month period also showed minor variability but had a significantly higher level of induced apoptosis (43.9 ± 1.9% apoptosis) compared to Donor 21 ($P < 0.001$). We have previously reported only minor intraindividual variation for other male donors, although repeat samples did not span over long periods (*12*). Similar results have been observed using lymphocytes from female donors (*32*).

Dose-Rate Effects

The average response of lymphocytes from a single donor to 4 Gy of γ radiation delivered at five different dose rates and repeated four times is given in Fig. 1. There may have been a slight increase in the level of radiation-induced apoptosis when the dose rate of the exposure was reduced from 70.2 to 1.3 and 0.7 cGy/min, but the difference was not statistically significant. If real, it may reflect the same process of sensitization by a prior dose described previously (*15*). At 0.29 cGy/min, a slight decrease in the frequency of apoptotic cells was evident, but again the difference was not statistically different from cells exposed at a high dose rate (70.2 cGy/min). However, there was a significant reduction ($P < 0.01$) in the level of radiation-induced apoptosis after a 4-Gy exposure when the dose rate was reduced further to 0.15 cGy/min.

Intra- and Interindividual Levels of Apoptosis Related to Dose Rate

We have previously shown that there is significant interindividual variation between donors (*12, 33*). Therefore, to control for interindividual variability, we compared apoptosis in paired blood samples from the same donor (repeated in triplicate) for irradiation at different dose rates (Fig. 2). Figure 2A shows the responses of lymphocytes from three donors to a 4-Gy dose delivered at either 70.2 or 0.29 cGy/min, while Fig. 2B shows responses at 70.2 and 0.15 cGy/min. There was no significant difference in the level of any individual response induced by 4 Gy delivered at 70 cGy/min compared to the same dose delivered at 0.29 cGy/min (Fig. 2A). Mean responses calculated from the three paired samples also show no significant difference. However, the data in Fig. 2B demonstrate that that there is a significant difference ($P < 0.01$) in the level of apoptosis for each of the three individuals when the dose rate of the 4-Gy dose is reduced further to 0.15 cGy/min. The mean responses of all the donors were also significantly different ($P < 0.001$).

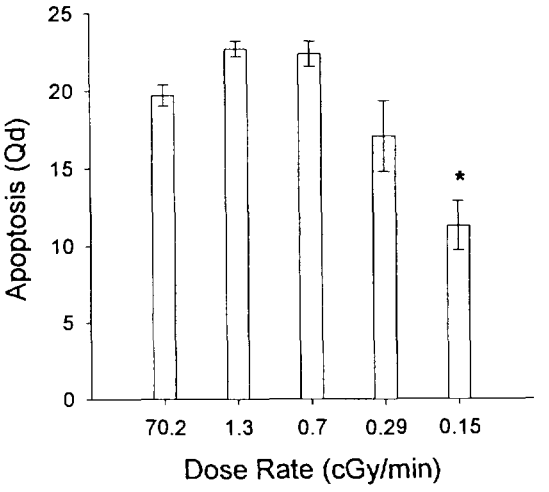

FIG. 1. Radiation-induced apoptosis and the effect of dose rate. The mean response of lymphocytes from a single donor after a 4-Gy dose delivered at five different dose rates and measured at 48 h (*significance at $P < 0.01$). Mean ± SE represents the combined results from replicates of four independent experiments.

Dose-Rate Effects and Kinetics of Apoptosis

We have previously reported that human lymphocytes undergo a slow radiation-induced apoptosis *in vitro* (*11, 12, 14*). Changing the dose rate of the exposure could alter the kinetics of apoptosis induced by radiation, and we therefore compared kinetics at different dose rates. Figure 3A compares the appearance of apoptotic nuclei in lymphocytes from three different donors irradiated with 4 Gy delivered at 70.2 and 0.29 cGy/min. There was no significant difference at any time in the level of apoptosis induced at either dose rate. At 48 h, the largest difference between means was observed, although it was not significantly different. The response agreed with the results shown in Fig. 1, indicating a trend that doses delivered at 0.29 cGy/min are marginally less effective at inducing apoptosis. The data in Fig. 3B show that when the dose rate was reduced further to 0.15 cGy/min, there was significantly less apoptosis (*P*

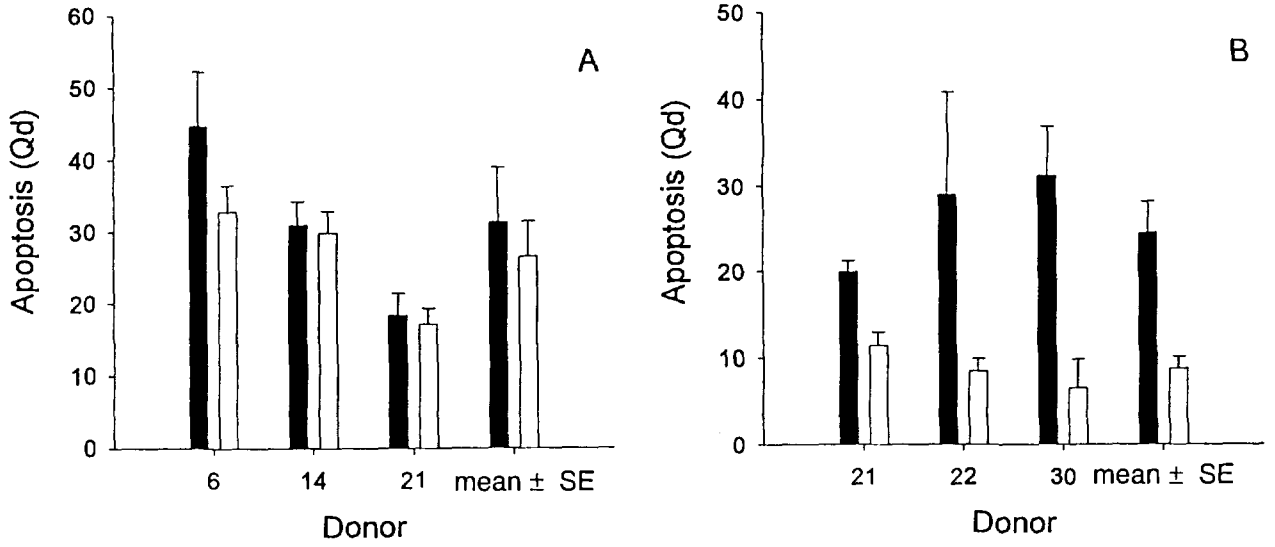

FIG. 2. Intraindividual levels of apoptosis in response to dose rate. The mean response of lymphocytes from individual donors (repeated in triplicate) to a high-dose-rate 4-Gy exposure delivered at 70.2 cGy/min (closed bars) compared to a 4-Gy low-dose-rate exposure (open bars) delivered at 0.29 cGy/min (panel A) or 0.15 cGy/min (panel B). Mean ± SE represents the combined results from all donors.

< 0.001) induced by a 4-Gy dose compared to the same dose delivered at high dose rate. Note that the mean individual donor responses to the 4-Gy high-dose-rate exposure delivered at 70.2 cGy/min shown in Fig. 3A and B are not the same because a different cohort of donors was used in the two sets of experiments.

Radiation-Induced Micronucleus Formation

Figure 4 compares micronucleus formation induced by a 4-Gy dose in samples from five different donors repeated in triplicate. In all donors, there was a significant decrease in micronucleus formation when the dose rate of the 4-Gy dose was decreased from 70 cGy/min to 0.29 cGy/min ($P < 0.001$) (Fig. 4A). The mean response at 4 Gy for all donors at 70.2 cGy was 48.0 ± 2.0%, significantly different ($P < 0.001$) from the 34.8 ± 2.1% observed at 0.29 cGy/min. At either dose rate, these five donors showed no significant interindividual variation with respect to micronucleus formation. Even though there was a dose-rate effect for micronucleus formation, there was no significant difference for binucleate cell frequency (19.5 ± 2.9% compared to 20.5 ± 3.0%) at the high and low dose rates, respectively (Fig. 4B). When the dose rate was reduced further to 0.15 cGy/min, not only was there a further reduction in micronucleus formation and a significant difference between the two dose rates for each individual donor

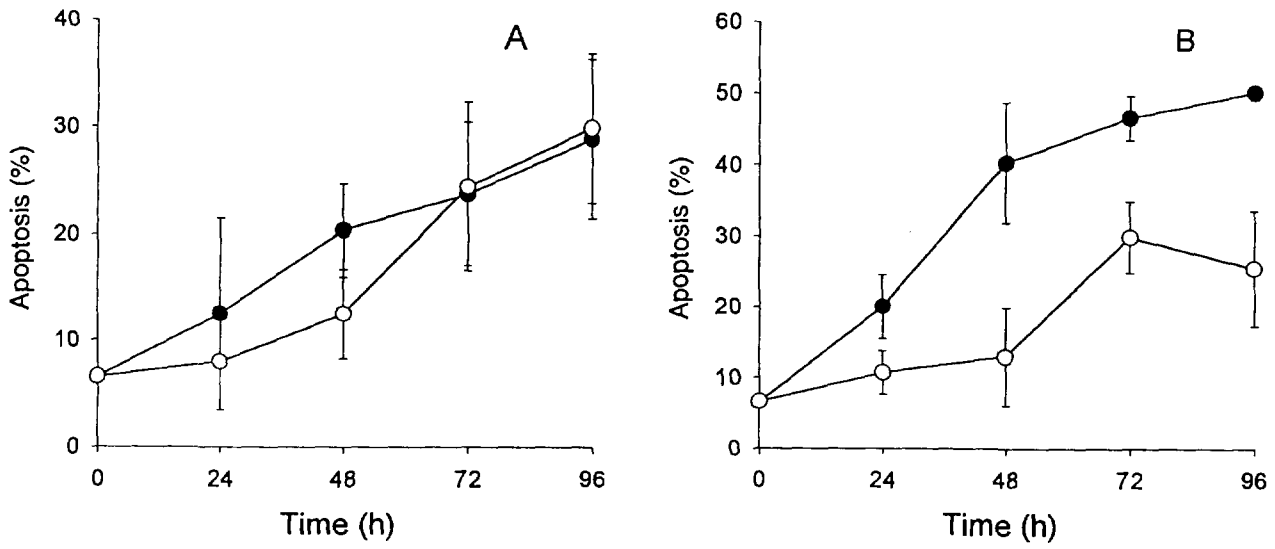

FIG. 3. Dose-rate effects and the development of apoptosis. The appearance of apoptotic nuclei with time in lymphocytes from three different donors irradiated with 4 Gy delivered at high dose rate (closed circles) at 70.2 cGy/min or low dose rate (open circles) at 0.29 cGy/min (panel A) or 0.15 cGy/min (panel B). Mean ± SE represents the combined results from all three donors.

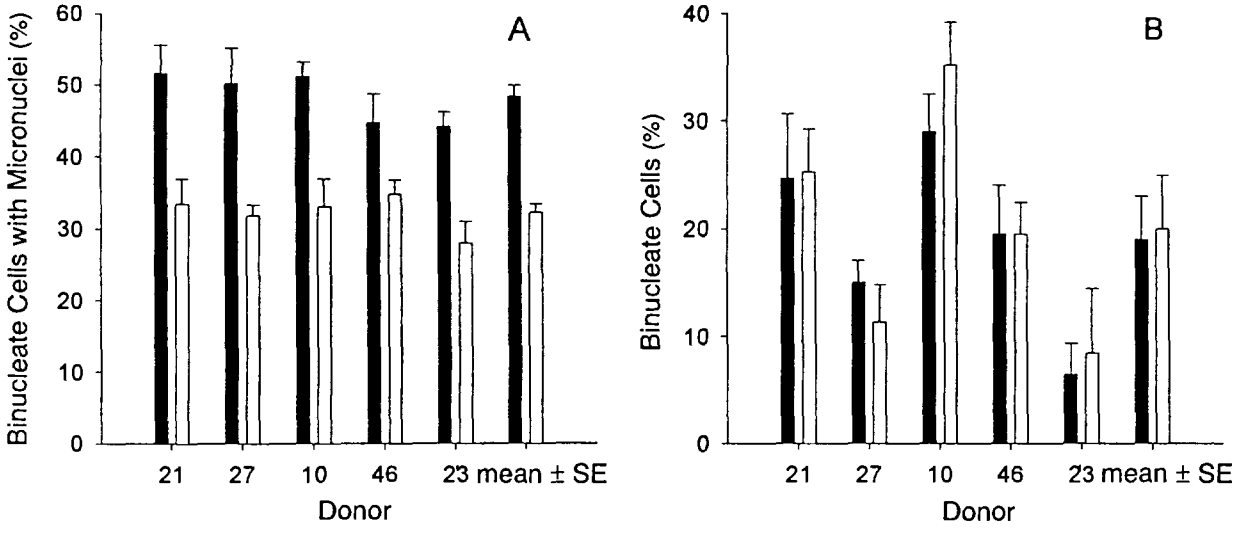

FIG. 4. Radiation-induced micronucleus formation. A comparison of micronucleus frequency (panel A) or binucleate cell frequency (panel B) induced by a 4-Gy dose delivered at 70.2 cGy/min (closed bars) or 0.29 cGy/min (open bars) in lymphocytes from five different donors repeated in triplicate. Mean ± SE represents the combined results from all donors.

($P < 0.001$) (Fig. 5A), there was also a concomitant significant reduction ($P < 0.001$) in the binucleate cell frequency of each donor (28.5 ± 1.5% to 8.0 ± 2.0%). The mean responses for micronucleus formation for all donors were also significantly reduced (48.0 ± 2.0% to 20.0 ± 3.0%) ($P < 0.001$) (Fig. 5A). At 4 Gy, micronucleus formation was reduced 1.4-fold when the dose rate was lowered to 0.29 cGy/min (Fig. 4A), and this effect increased to 2.4-fold when the dose rate was lowered further to 0.15 cGy/min (Fig. 5A).

Intra- and Interindividual Variation for Micronucleus Formation

There was a very small intra- and interindividual variation between donors for micronucleus formation. Although there were significant differences in the absolute level of response at the two different dose rates, at each dose rate there was little interindividual variation, and all donors showed similar levels of response (compare Figs. 4A and 5A). There was no correlation between the rate of cell division after a high-dose-rate exposure in any individual (Fig. 4B) and the extent of micronucleus formation in that individual (Fig. 4A).

Dose-Rate Effects and Kinetics for Micronucleus Formation

The results shown in Figs. 4 and 5 are from cell samples assayed 72 h after the beginning of the exposure. To confirm that low-dose-rate exposures did not slow the kinetics,

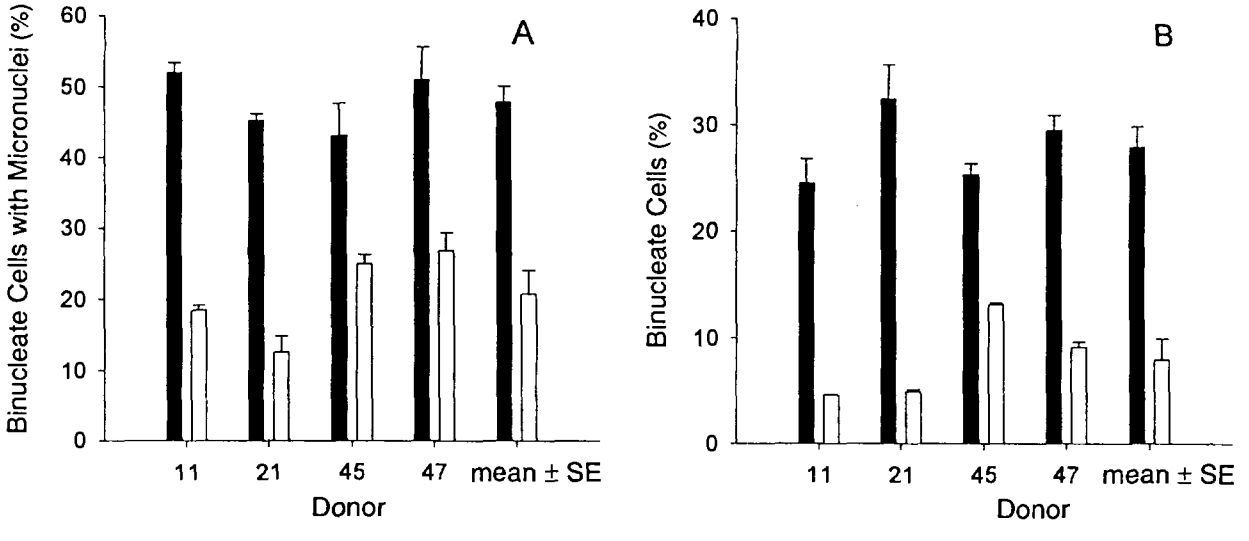

FIG. 5. Radiation-induced micronucleus formation. A comparison of micronucleus frequency (panel A) or binucleate cell frequency (panel B) induced by a 4-Gy dose delivered at 70.2 cGy/min (closed bars) or 0.15 cGy/min (open bars) in lymphocytes from four different donors repeated in triplicate. Mean ± SE represents the combined results from all donors.

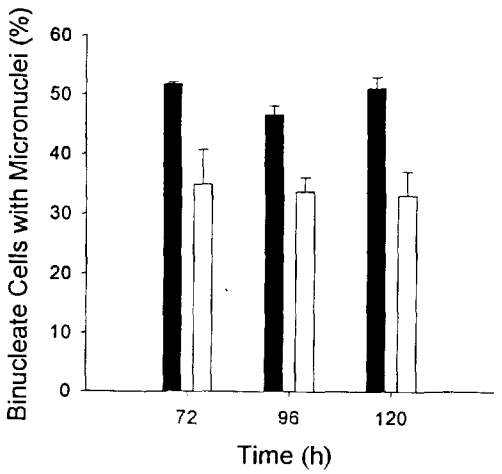

FIG. 6. Dose-rate effects and formation of micronuclei with time. The mean response in lymphocytes from three different donors for micronucleus formation after a 4-Gy dose delivered at 70.2 cGy/min (closed bars) or 0.29 cGy/min (open bars) at different times. Mean ± SE represents the combined results from three all donors.

and hence the apparent extent of micronucleus formation, samples from three donors were also assayed at 96 and 120 h. There was no significant difference with time between the absolute levels of micronucleus formation at any given dose rate (Fig. 6). The reduced effect of radiation delivered at 0.29 cGy/min was unchanged 96 and 120 h postirradiation. Unirradiated control values for micronucleus formation at 72, 96 and 120 h were 3.7 ± 1.0, 6.0 ± 0.3 and 6.0 ± 1.0, respectively.

DISCUSSION

The primary goal of this work was to investigate the effects of dose rate on radiation-induced apoptosis and micronucleus formation in human lymphocytes. The work has also confirmed our previous conclusion (*12*) that there is small intraindividual variation in radiation-induced apoptosis. Remarkable reproducibility from samples taken from the same individual over extended periods further supports the idea that this biological end point is a useful biological dosimeter and may be a good indicator of intrinsic individual radiosensitivity or cancer susceptibility. Others have drawn similar conclusions and also suggest that this end point could have applications in the clinic to predict individual responses to radio- or chemotherapy or identify individuals at altered risk from exposure to ionizing radiation (*35–38*). The results reported here also support our previous conclusion that variation in radiation-induced apoptosis can be large between individual donors (*12*). However, this work showed that interindividual variation for radiation-induced micronucleus formation was not significantly different between donors. Since a direct comparison of the same donors for radiation-induced apoptosis and micronucleus formation was not performed, we cannot speculate on the relationship between these two end points for determining individual responses to radiation. Our observed lack of interindividual variation for radiation-induced micronucleus formation was probably a coincidence of the donor selection since others have shown that there is significant interindividual variation for micronucleus formation which is positively correlated with age and influenced by gender (*39*).

The results of this work have shown that apoptosis in human lymphocytes exhibits a dose-rate effect. As reported previously (*27*) and confirmed here, there was no dose-rate effect for the induction of apoptosis by low-LET γ radiation when the dose rate was decreased to around 0.6 cGy/min. Even at 0.29 cGy/min, this work shows that there was no significant dose-rate effect for the induction of apoptosis by 4 Gy γ rays. However, when the dose rate was reduced further to 0.15 cGy/min, a dose-rate effect was apparent. This result indicates that it is not initial DNA damage that signals the response, as hypothesized by Vral *et al.* (*27*), but that DNA repair plays a role in this response. Since apoptosis shows a dose-rate effect only at very low dose rates, the types of lesions initiating the response must be persistent lesions which are only slowly repaired. This conclusion is consistent with the results of Cregan *et al.* (*14*), who demonstrated that slowing the repair of DNA lesions was sufficient to trigger apoptosis in human lymphocytes. For ^{60}Co γ rays, a dose rate of 0.29 cGy/min produces an average of about three electron tracks per nucleus per minute (*34*), and there was no measurable dose-rate effect. However, at 0.15 cGy/min, where on average each nucleus receives only one or two electron tracks per nucleus per minute, there was a dose-rate effect. This small difference in dose rate is presumably sufficient to allow the lymphocytes to repair some of the lesions involved in the triggering event.

It is known that at least two kinetically distinct pathways exist for apoptosis in human lymphocytes, one signaled by DNA-damaging agents (slow process) and the other by membrane-oxidizing agents (fast process) (*14*). Since different agents signal different pathways, the possibility exists that the dose-rate effect seen at the lowest dose rate may have simply been a consequence of altered kinetics. We considered the possibility that the extra 24 h of irradiation time required to deliver the 4-Gy dose at 0.15 cGy/min could have altered the kinetics of radiation-induced apoptosis and may have produced the observed dose-rate effect at the lowest dose rate. Therefore, we compared the kinetics of development of apoptosis after high-dose-rate exposures (70.2 cGy/min) to that after low-dose-rate exposures where there was not (0.29 cGy/min) or was (0.15 cGy/min) a dose-rate effect (see Fig. 3). Since the lack of a dose-rate effect at 0.29 cGy/min was persistent over time for up to 96 h, we conclude that there is not a reduced effect at this dose rate after a dose of 4 Gy (Fig. 3A). However, because there was a dose-rate effect at 0.15 cGy/min at all times, we conclude that there was a dose-rate effect at this dose rate after a dose of 4 Gy and that the

observed effect was not an artifact of altered kinetics. A similar test was performed for micronucleus formation, and we confirmed that the dose-rate effect observed at the higher dose rate of 0.29 cGy/min was also not a consequence of altered kinetics (Fig. 6).

It is known that micronucleus formation shows a dose-rate effect at dose rates higher than those tested here. Dose rates from 2.6 cGy/min to 0.7 cGy/min have been shown previously to be less effective than high-dose-rate exposures (40 Gy/h) at inducing micronuclei in human lymphocytes (*26*), demonstrating the importance of DNA repair mechanisms in micronucleus formation. Therefore, it was not surprising that the two low dose rates tested in these experiments (0.29 and 0.15 cGy/min) also showed a dose-rate effect (compared to 70.2 cGy/min) since they were even lower than rates previously shown to produce an effect. However, an interesting effect on binucleate cell frequency was observed only when the dose rate was reduced to 0.15 cGy/min, which was coincident with the same dose rate required to produce an effect for apoptosis. Binucleate cell frequency is an indicator of the number of cells progressing through one cell division after irradiation. Therefore, a change in the frequency of binucleate cells is indicative of changes in cell cycle kinetics. Even though there was a reduced micronucleus frequency when cells were irradiated at 0.29 cGy/min compared to high-dose-rate exposures, there was no significant difference in the binucleate cell frequency (Fig. 4A and B). This was not the case when cells were irradiated at 0.15 cGy/min, where cells from all donors showed a large decrease in binucleate cell frequency (Fig. 5B) as well as a further reduction in micronucleus frequency (Fig. 5A). Therefore, a reduction in dose rate from 0.29 to 0.15 cGy/min may have induced a cell cycle delay not seen at the higher dose rates. Since this possible cell cycle delay induced at 0.15 cGy/min was not required for the dose-rate effect on micronucleus formation observed at 0.29 cGy/min (Fig. 4A), this may indicate that cell cycle delay is not essential for part of the increased ability of cells to repair chromosome breaks at reduced dose rates. However, when there was a change in binucleate cell frequency (at 0.15 cGy/min) (Fig. 5B), there was also an additional dose-rate effect for micronucleus formation (Fig. 5A). Therefore, cell cycle delay may be an additional component of dose-rate effects at very low dose rates.

In conclusion, we have shown that there is a dose-rate effect for the induction of apoptosis in human lymphocytes. The effectiveness of a protracted radiation exposure to cause either apoptosis or micronucleus formation was reduced by low-dose-rate exposures, but the reduction was apparent at different dose rates for these two radiobiological end points. Since apoptosis showed a dose-rate effect at a dose rate lower than that for micronucleus formation, we suggest that the mechanisms or signals for processing radiation-induced lesions for these two end points must be different in part. There appear to be two mechanisms which contribute to the dose-rate effect for micronucleus formation, one independent of cell cycle delay and one dependent on a cell cycle delay induced only at very low dose rates, the same low dose rates which allowed a reduction in apoptosis. Since the dose rate at which cells showed reduced apoptosis as well as a further reduction in micronucleus formation was very low, we conclude that the processing of the radiation lesions that induce apoptosis, and some micronuclei, is very slow.

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