The Dependence of the Magnitude of Induced Adaptive Response on the Dose of Pre-Irradiation of Cultured Human Lymphocytes under the Optimum Irradiation Time Scheme

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ABSTRACT

Human lymphocytes exposed to low doses of X-rays, become less susceptible to the induction of chromosome aberrations by subsequent exposure to high doses of X-rays. This has been termed the radioadaptive response. One of the most important questions in the adaptive response studies was that of the possible existence of an optimum adapting dose. Early experiments indicated that this response could be induced by low doses of X-rays from 1 cGy to 20 cGy. Recently, it has been interestingly shown that the time scheme of exposure to adapting and challenge doses plays an important role in determination of the magnitude of the induced adaptive response. In this study, using the optimum irradiation time scheme (24-48), we have monitored the cytogenetic endpoint of chromosome aberrations to assess the magnitude of adaptation to ionizing radiation in the cultured human lymphocytes. Lymphocytes were pre-exposed to an adapting dose of 1-20 cGy at 24 hours, before an acute challenge dose of 1 or 2 Gy at 48 hours. Cells were fixed at 54 hours. Lymphocytes, which were pretreated with 5 as well as 10 cGy adapting doses, had significantly fewer chromosome aberrations. In spite of the fact that lymphocytes of some of our blood donors which were pre-treated with 1 or 20 cGy adapting doses, showed an adaptive response, the pooled data (all donors) indicated that such an induction of adaptive response can not be observed in these lymphocytes. The overall pattern of the induced adaptive response, indicated that in human lymphocyte (at least under the above mentioned irradiation scheme), 5 cGy and 10 cGy adapting doses are the optimum doses.

Key words: optimum adapting dose, adaptive response, ionizing radiation, human lymphocytes, and Chromosome aberrations.

INTRODUCTION

It is now clearly known that when organisms as diverse as bacteria, animals and plants are exposed to a variety of DNA damaging stresses such as UV, alkylating or oxidizing agents and heat, adaptive responses may be induced which render them resistant to the killing and mutagenic impacts (1-2). The results of many studies indicated that when cells are exposed to low doses of these agents, they often become less sensitive to the harmful effects of a subsequent higher dose. This type of induced repair is called adaptive response (AR). Samson and Carins first reported the induction of adaptive response. Using Escherichia Coli, they showed that bacterial cells, which had been exposed to low levels of alkylating agents, became less susceptible to the subsequent high doses of the same agent or other similar compounds (1).

Later it was first found that in vitro pretreatment of human lymphocytes with tritiated thymidine, caused these cells become less susceptible to cytogenetic damage of a subsequent high dose X-ray (3). They reported that surprisingly the frequency of chromatid aberrations were up to 50% less than expected. During the last years, many other researchers (4-8) and we (9-11) have been investigating the ability of cultured human lymphocytes to adapt to different radiation exposures in a wide variety of culture and irradiation conditions. In addition, considering the possible implications of induced radio-protective mechanisms for human health (12), we have also been testing the effects of the X-ray photon energy (diagnostic radiology range) in an attempt to determine
whether or not the energy of X-ray photons within this range can change the magnitude of radioadaptive response (11). In these experiments we also found a young healthy donor who showed no adaptive response in all of separate experiments. Furthermore, his cultured lymphocytes showed a severe hypersensitivity to radiation (extraordinary synergistic effect) after a common adapting dose in all of our experiments performed during a six month follow-up study (9).

One of the most important questions in the adaptive response studies was that of the possible existence of an optimum adapting dose in different cells. Despite the fact that there are a number of reports indicating lack of such an optimum adapting dose in some cells such as HeLa cells (13), investigators have found the optimum dose in a wide variety of cells from Vibrio Cholerae (14), to HE22 fibroblasts (13) and m55 mouse embryonic skin cell line (15). In this study we have attempted to find a correlation between the magnitude of adapting dose and the magnitude of the induced adaptive response.

As early as 1987, it was indicated that doses of X-rays as low as 0.5 or 1 cGy are capable of rendering human lymphocytes less susceptible to chromosomal damage induced by high X-ray doses. In addition, it was indicated that doses of X-rays greater than 20 cGy did not lead to significant reductions (16). Interestingly, in 1998, Ryabchenko and his co-workers showed that the time scheme of exposure to adapting and challenge doses plays an important role in determination of the magnitude of the induced adaptive response (17). They showed that the most effective schemes are those schemes that the challenge dose delivered at G2 stage.

Furthermore, they compared the adaptive response coefficient of each scheme. It was indicated that at least for aberrant cell frequency, the 24-48 scheme (adapting dose at 24 hours and challenge dose at 48 hours) had the greatest magnitude of adaptive response (the least adaptive response coefficient) among 0-48, 24-48 and 30-48 irradiation schemes. On the other hand, for aberrant cell frequency as well as aberrations per cell, the 30-48 irradiation time scheme had the least magnitude of adaptive response (the highest adaptive response coefficient; approximately 1 for aberrant cell frequency). Since Shadley and Wolff had used the 32-48 irradiation time scheme, the coefficient of the induced adaptive response was possibly much lower than the reported optimum irradiation time scheme (24-48). In this study, using this optimum irradiation time scheme, we have monitored the cytogenetic endpoint of chromosome aberrations to assess the magnitude of adaptation to ionizing radiation in the cultured human lymphocytes.

2-MATERIALS AND METHODS

Blood samples were taken from 40 healthy, non-smoker donors of both sexes, aged from 18 to 40 years. Blood donors had no alcohol or drug consumption, history of irradiation or viral infection. Blood donors were divided into four groups (to test four different adapting doses), consisting 10 donors in each group. In the next experiment we decided to eliminate the possible effects of inter-individual variations, so using the lymphocytes of four blood donors, we studied the magnitude of induced adaptive response by each adapting dose. In other words, in this experiment the lymphocytes of each donor exposed to all four adapting doses and/or the challenge dose.

We used standard condition for cell cultivation and analysis of chromosome aberrations (18-20). Separate blood cultures were set up from each blood sample, using 0.5 ml blood in 5 ml RPMI 1640 medium, containing 20% fetal calf serum and 2.5% PHA (Gibco). The lymphocytes were cultured in dark at 37°C. The cells were exposed to the adapting dose of 5 or 10 cGy X-ray (Shimadzu radioscopy machine, 80 kVp, 2 mA, dose rate 34 mGy/min) at 24 hours and/or to the challenge dose of 1 or 2 Gy Gamma rays (AECL, model 780, Canada) at 48 hours. Exposure rates were measured using a Farmer-2570 radiometer. After the challenge dose the culture flasks were returned to the incubator for a further incubation of 6 hours.

Colcemid was added 2 hours before harvesting (52 h after stimulation) at a final concentration of 0.05 µg/ml to arrest the dividing lymphocytes in mitosis. After harvesting cells exposed to 0.075 M KCl for 10 min at 37°C and fixed with methanol-acetic acid (3:1 v/v). The fixed cells were dropped onto wet slides, air dried and stained with Giemsa. For each data point/donor, 100 well-spread metaphases were examined for chromosome aberrations. The number of chromosome aberrations was determined. Gaps (aphromatic lesions smaller than the width of a chromatid) were excluded. Using mean chromosome aberration per cell (MCAPC), the expected numbers of aberrations in cells irradiated with adapting dose as well as challenge dose were calculated as follows:

\[
\text{Expected MCAPC} = (\text{MCAPC}_{CD} + \text{MCAPC}_{AD} - \text{MCAPC}_{CONT})
\]

MCAPC: Mean Chromosome Aberrations per Cell
ACDC: Cells Irradiated with both Adapting and Challenge Dose
AD: Cells Irradiated only with both Adapting Doses  
CD: Cells Irradiated only with Challenge Dose  
CONT: Cells Neither Irradiated with Adapting nor Challenge Dose  

Also the coefficient of induced adaptive response (k) in each experiment was calculated as follows:

\[
k = \frac{AD_{MCAPC}}{AD_{MCAPC} + CD_{MCAPC} - CONT_{MCAPC}}
\]

Obviously, if \( k=1 \), this means that there is a simple additivity effect. When this coefficient is significantly less than 1, it can be concluded that a positive adaptive response is induced. Finally, when there is a \( k \) which is significantly greater than 1, this means that a synergistic effect is induced.

The statistical significance of increased or decreased frequencies of chromosome aberrations was evaluated using two-tailed student’s t-test.

3-RESULTS

The frequency of chromosome aberrations induced by a challenge dose of 1 Gy X-rays in lymphocytes of 40 donors pre-exposed to four different adapting doses (1, 5, 10 and 20 cGy) are summarized in Table 1. As we expected, the frequency of chromosome aberrations in the cells that were irradiated with challenge dose alone was higher than the cells exposed to an adapting dose before the challenge dose. These differences were significant in the cells, which were exposed to adapting doses of 5 cGy (P<0.00001) and 10 cGy (P<0.00001) and not significant in the cells, which were exposed to adapting doses of 1 cGy and 20 cGy. In order to determine the magnitude of the induced adaptive response in each group, we calculated the coefficients of adaptive response. The adaptive response coefficient was 0.77, 0.56, 0.57, and 0.87 for the first (1 cGy), second (5 cGy), third (10 cGy) and fourth group (20 cGy), respectively (Table 2).

In the next experiment, the possible effect of inter-individual variability was completely eliminated. The frequency of chromosome aberrations induced by a challenge dose of 1 or 2 Gy X-rays in lymphocytes of 4 donors pre-exposed to previous four different adapting doses (1, 5, 10 and 20 cGy) are summarized in Tables 3 and 4, respectively. Again, the frequencies of chromosome aberrations in the cells which were irradiated with 1 Gy challenge dose alone, were higher than the cells which were exposed to an adapting dose before the challenge dose (Table 3), but these differences were statistically significant only in the cells which were exposed to adapting doses of 5 cGy (P<0.001) and 10 cGy (P<0.001). The magnitude of the coefficient of adaptive response was 0.88, 0.59, 0.60, and 0.83 for the first (1 cGy), second (5 cGy), third (10 cGy) and fourth group (20 cGy), respectively.

Again, the frequencies of chromosome aberrations in the cells which were irradiated with 2 Gy challenge dose alone, were higher than the cells which were exposed to an adapting dose before the challenge dose (Table 4), but these differences were statistically significant only in the cells which were exposed to adapting doses of 5 cGy (P<0.001) and 10 cGy (P<0.001). The magnitude of the coefficient of adaptive response was 0.84, 0.58, 0.61, and 0.88 for the first (1 cGy), second (5 cGy), third (10 cGy) and fourth group (20 cGy), respectively. The magnitude of the coefficients of adaptive responses by four different adapting doses in three separate experiments are compared in Fig. 1.

4- DISCUSSION

Our results on the existence of a window for adapting doses confirm the early findings of Shadley and Wolff (16), however we propose that this window of dose is smaller than that these investigators, indicated. Our preliminary results indicate that under the optimum irradiation time scheme of 24-48, the optimum window for induction of a significant adaptive response is from 5 to 10 cGy. Interestingly, when we eliminated the effect of inter-individual variations (using lymphocytes of each donor to test all of the adapting doses simultaneously), the overall pattern of the magnitude of the induced adaptive response by different adapting doses was the same as that we had before elimination of inter-individual variations (using different groups, each one consisting 10 donors, to test adapting doses). This finding is consistent with the results of Gadhia (21). He indicated that the magnitude of the adaptive response varies between blood samples from different donors, but the differences were not statistically significant. However, Bosi and Olivieri (22), analysed blood samples of 18 donors aged from 27 to 51 years and found inter-individual variations. Considering the existence of an optimum adapting dose range
(5-10 cGy) observed in all of our three separate experiments, it can be concluded that 5 cGy and 10 cGy adapting doses (under the optimum irradiation time scheme [24-48], used in our experiments) are the optimum adapting doses in cultured human lymphocytes.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. Tizmaghz and Mrs. Ghaem-maghami of the Cancer Institute, Imam Khomeini Hospital, Tehran University of Medical Sciences for irradiating blood samples and Mrs. Sepehri of the Standard Dosimetry Department for her excellent technical assistance in dose measurements.

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Table 1. The frequency of chromosome aberrations induced by a challenge dose of 1 Gy X-rays in human lymphocytes pre-exposed to four different adapting doses.

<table>
<thead>
<tr>
<th>Cells Scored</th>
<th>Adapting Dose (cGy)</th>
<th>Challenge Dose (cGy)</th>
<th>Mean Chromosome Aberrations per Cell (MCAPC)</th>
<th>P @</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>-</td>
<td>1 Gy</td>
<td>0.196 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>1 cGy</td>
<td>1 Gy</td>
<td>0.157 ± 0.01</td>
<td>NS (0.052)</td>
</tr>
<tr>
<td>1000</td>
<td>-</td>
<td>1 Gy</td>
<td>0.215 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>5 cGy</td>
<td>1 Gy</td>
<td>0.138 ± 0.01</td>
<td>&lt;0.00001 a</td>
</tr>
<tr>
<td>1000</td>
<td>-</td>
<td>1 Gy</td>
<td>0.223 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>10 cGy</td>
<td>1 Gy</td>
<td>0.153 ± 0.01</td>
<td>&lt;0.00001 a</td>
</tr>
<tr>
<td>1000</td>
<td>-</td>
<td>1 Gy</td>
<td>0.257 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>20 cGy</td>
<td>1 Gy</td>
<td>0.285 ± 0.02</td>
<td>NS (0.071)</td>
</tr>
</tbody>
</table>

10 donors aged from 18 to 45 years of both sexes.
@ Two-tailed student’s t-test.
a Significantly different from expected values (expected values are not shown).
b Mean ± SE
NS: Non-significant
Table 2. The coefficient of the induced adaptive response by a challenge dose of 1Gy X-rays in human lymphocytes pre-exposed to four different adapting doses.

<table>
<thead>
<tr>
<th>Adapting and Challenge Doses</th>
<th>AD (1 cGy) ⇓ CD (1 Gy)</th>
<th>AD (5 cGy) ⇓ CD (1 Gy)</th>
<th>AD (10 cGy) ⇓ CD (1 Gy)</th>
<th>AD (20 cGy) ⇓ CD (1 Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaptive Response Coefficient (k)</td>
<td>0.77</td>
<td>0.56</td>
<td>0.57</td>
<td>0.87</td>
</tr>
</tbody>
</table>
Table 3. The frequency of chromosome aberrations induced by a challenge dose of 1 Gy X-rays in human lymphocytes pre-exposed to four different adapting doses (all experiments are performed with the same blood donors to prevent the effects of inter-individual variations).

<table>
<thead>
<tr>
<th>Cells Scored</th>
<th>Adapting Dose (cGy)</th>
<th>Challenge Dose (cGy)</th>
<th>Mean Chromosome Aberrations per Cell&lt;sub&gt;a&lt;/sub&gt; (MCAPC)</th>
<th>P&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>-</td>
<td>1 Gy</td>
<td>0.24 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>1 eGy</td>
<td>1 Gy</td>
<td>0.21 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>400</td>
<td>-</td>
<td>1 Gy</td>
<td>0.24 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>5 eGy</td>
<td>1 Gy</td>
<td>0.16 ± 0.02</td>
<td>&lt;0.001&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>400</td>
<td>-</td>
<td>1 Gy</td>
<td>0.24 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>10 eGy</td>
<td>1 Gy</td>
<td>0.18 ± 0.02</td>
<td>&lt;0.001&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>400</td>
<td>-</td>
<td>1 Gy</td>
<td>0.24 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>20 eGy</td>
<td>1 Gy</td>
<td>0.29 ± 0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

4 donors aged from 25 to 35 years (2 male and 2 female).

<sup>a</sup> Two-tailed student’s t-test.

<sup>b</sup> Significantly different from expected values (expected values are not shown).

<sup>c</sup> Mean ± SE

NS: Non-significant
Table 4. The frequency of chromosome aberrations induced by a challenge dose of 2 Gy X-rays in human lymphocytes pre-exposed to four different adapting doses (all experiments are performed with the same blood donors to prevent the effects of inter-individual variations).

<table>
<thead>
<tr>
<th>Cells Scored</th>
<th>Adapting Dose (cGy)</th>
<th>Challenge Dose (cGy)</th>
<th>Mean Chromosome Aberrations per Cell&lt;sub&gt;a&lt;/sub&gt; (MCAPC)</th>
<th>P&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>-</td>
<td>2 Gy</td>
<td>0.38 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>1 cGy</td>
<td>2 Gy</td>
<td>0.32 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>400</td>
<td>-</td>
<td>2 Gy</td>
<td>0.38 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>5 cGy</td>
<td>2 Gy</td>
<td>0.24 ± 0.03</td>
<td>&lt;0.001&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>400</td>
<td>-</td>
<td>2 Gy</td>
<td>0.38 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>10 cGy</td>
<td>2 Gy</td>
<td>0.27 ± 0.03</td>
<td>&lt;0.001&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>400</td>
<td>-</td>
<td>2 Gy</td>
<td>0.38 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>20 cGy</td>
<td>2 Gy</td>
<td>0.43 ± 0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly different from expected values (expected values are not shown).

4 donors aged from 25 to 35 years (2 male and 2 female).

<sup>b</sup> Mean ± SE

NS: Non-significant
Figure 1. Comparison of the adaptive response coefficients of four different adapting doses in three separate experiments.