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Adaptive Response to Ionizing Radiation Induced by Low Dose of Gamma Ray in Human Hepatoma Cell Lines

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When cells are exposed to a low dose of a mutagenic or clastogenic agent, they often become less sensitive to the effects of a higher dose administered subsequently. Such adaptive responses were first described in Escherichia coli. Studies on mammalian cells have been limited to human lymphocytes exposed to low doses of an alkylating agent. In this study, the adaptive response to 1 cGy of gamma rays was investigated in human tumor cells using two human hepatoma cell lines, Hep G_2 and Hep 3B. Experiments were carried out by delivering 1 cGy followed by 50 cGy of gamma radiation and chromatid breaks were scored as an endpoint. The results of this study indicate that prior exposure to 1 cGy of gamma rays reduces the number of chromatid breaks induced by subsequent higher doses (50 cGy). The time necessary for the expression of the adaptive response was determined by varying the time interval between the two doses from 1 hour to 72 hours. In G_2 chromatids, the adaptive response was observed both at short time intervals, as early as 1 hour, and at long time intervals. In S chromatids, however, the adaptive response was shown only at long time intervals. When 3-aminobenzamide, an inhibitor of poly (ADP-ribose) polymerase, was added after 50 cGy, adaptive responses were abolished in all the experimental groups. Therefore, it is suggested that the adaptive response can be observed in human hepatoma cell lines, which is first documented through this study. The elimination of the adaptive response with 3-aminobenzamide is consistent with the proposal that this adaptive response is the result of the induction of a certain chromosomal repair mechanism.

Key Words: Adaptive response, human hepatoma cells, chromatid breaks, gamma rays

In most studies on mutagenesis, proliferating cells were exposed to a large dose of mutagens for a short period. In a natural environment, however, cells are probably exposed more often to a low concentration of mutagens for long periods. Samson and Cairns (1977) first studied the effect of low doses of a mutagen, alkylating agent, in Escherichia coli and they showed that the cells became less sensitive to the mutagen by previous exposure to low doses of a mutagen. Such adaptive responses were also described in mammalian cells to low doses of an alkylating agent (Samson and Schwartz, 1980; Kaina, 1982).

Although an adaptive response can occur with various chemical mutagens, the concept of adaptive response has been much less clear in ionizing radiations. The amount of energy deposited by low doses of radiation is just too small to bring about the physiological effects that can lead to stimulation.

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Consequently, to account for the effects of low-level radiation, it has been necessary to look for a system within the cell which not only is sensitive to radiation, but also is capable of magnifying an individual lesion so that it can have a physiological effect.

The genetic apparatus, the genes and chromosomes in the nuclei, represent such a target for radiation. Radiation can induce mutations, occasionally by inducing some random base changes but mainly by breaking chromosomes, which then can result in the broken pieces being deleted or rearranged, and these effects can have a profound influence on the cell.

In the experiments on the induction of chromatid breaks in irradiated human lymphocytes, Olivieri *et al.* (1984) showed that prior exposure to low doses of endogenous radiation from incorporated tritiated thymidine ([^{3}H]dThd) led to a reduction in cytogenetic damage induced by a subsequent high dose of X-rays. These experiments led to the conclusion that low doses of radiation from the decay of the tritium induced a chromosome break repair mechanism that made the lymphocytes less susceptible to subsequent exposure to X-rays.

However, this phenomenon is still a matter of controversy with many questions which remains unanswered. One of the major questions is whether the adaptive response to ionizing radiation is a universally occurring phenomenon or restricted to very specific conditions and cell systems. Many results remain equivocal even with lymphocytes. Some authors could not observe any adaptation to low doses of X-ray applied in the G_0 stage of the lymphocytes (Shadley *et al.* 1987; Moquet *et al.* 1987), while others reported the contrary results (Tuschl *et al.* 1980; Sanderson *et al.* 1986; Cai *et al.* 1990; Sankaranarayanan *et al.* 1989). Similarly, the amplitude of the protective effect triggered by the adapting dose is very heterogeneous (Bauchinger *et al.* 1989) and while some individuals seem to be genetically deficient for the adaptive response (Shadley, 1991), in others the expression of adaptive response appears to depend on their physiological state and the lymphocyte culture conditions (Purnell and Whish, 1980).

In this study, we analysed whether the adaptive response could be induced in two human hepatoma cell lines. The time necessary for the expression and disappearance of the adaptive response was also investigated. Some authors have found that this adaptive response resulted from an unknown chromosomal repair mechanism involving the poly (ADP-ribosyl)ation process through the experiments on human lymphocytes in the presence of 3-aminobenzamide (3-AB), a potential inhibitor of poly (ADP-ribose) polymerase (Wiencke *et al.* 1986; Heartlein and Preston, 1985). To examine if the same result could be reproduced in this experimental systems, the experiments with 3-AB were carried out. In all experiments chromatid breaks were scored as endpoints.

MATERIALS AND METHODS

Human hepatoma cell lines, Hep G2 and Hep 3B, were provided by Dr. J. H. Kim (Yonsei Cancer Center, Seoul, Korea). The cells were grown in Rosewell Park Memorial Institute 1640 medium, supplemented with 10 % heat inactivated fetal calf serum (Gibco, Grand Island, NY), penicillin 100 units/ml (Gibco, Grand Island, NY), streptomycin 100 μg/ml (Gibco, Grand Island, NY), and L-glutamine 2 mM (Sigma, St. Louis, MO), at 37℃ in a 5% CO_2 incubator.

Exponentially growing cells were placed in a 25 cm^2 flask at a seeding density of 3×10^6 cells in 10 ml complete culture medium 24 hours prior to the experiment. Gamma irradiation was performed with a Cobalt-60 irradiator; 1 cGy was delivered at a 160 cm distance from the source with a dose rate of 0.76 cGy /minutes, which was possible by passing gamma rays through a 6.5 cm-thick cerrobend block and then 50 cGy was delivered at an 80 cm distance from the source with a dose rate of 154 cGy/minutes.

Four experimental groups were set up in each cell line: control, 1 cGy alone, 50 cGy alone, and 1 cGy followed by 50 cGy with various time intervals. For the experiments, 1 cGy of gamma rays were delivered to the cells and the cells were exposed to a subsequent 50 cGy of gamma rays. The time interval between the two doses varied from 1 hour to 72 hours. When it was used, 3-AB 2

mM (Sigma, St. Louis, MO) was added immediately after the 50 cGy exposure. For G_2 phase originated chromatid analysis, Colcemid 0.04 μg/ml (Gibco, Grand Island, NY), a mitotic arrestant, was added to the cultures 30 minutes after the 50 cGy exposure. For S phase originated chromatid analysis, Colcemid was added 6 hours after the 50 cGy exposure. The cells were treated with Colcemid for one hour and exposed to 1% sodium citrate for 10 minutes. Then the cells were fixed in Carnoy solution (a mixture of acetic acid and methanol with 3:1 ratio). The fixed cells were dropped onto wet glass slides and dried overnight. Slides were stained in Giemsa (Gurr, U.K.) for the scoring of chromatid aberrations. Since very few chromatid exchanges are found in cultures fixed up to 6 hours after the exposure to 50 cGy, the scoring was restricted to chromatid and isochromatid breaks. Gaps, where the apparent discontinuity was less than the width of a chromatid, were disregarded. One hundred cells were scored for each treatment. The statistical significance of reductions in chromatid breaks was determined with one-tailed t-test.

RESULTS

When the cells are pretreated with 1 cGy and followed by a subsequent 50 cGy, the expected number of chromatid breaks would be the sum of the 1 cGy effect and the 50 cGy effect minus the control. In this study, a prior exposure to 1 cGy significantly reduced

Table 1. Effects of 1 cGy pretreatment on 50 cGy-induced G_2 chromatid breaks

Cell lines	No. of chromatid breaks/100 cells				
	Control	1 cGy(D1)	50 cGy(D2)	D1-(1hr)-D2	Expected
Hep G2	1	4	110	63*	113
Hep 3B	2	4	125	73*	127

*$P<0.05$, one-tailed t-test, in difference with expected value

Table 2. Effects of 1 cGy pretreatment of 50 cGy-induced S chromatid breaks

Cell lines	No. of chromatid breaks/100 cells				
	Control	1 cGy(D1)	50 cGy(D2)	D1-(1hr)-D2	Expected
Hep G2	2	2	26	22	26
Hep 3B	2	2	31	23	28

Table 3. Effects of 1 cGy pretreatment on 50 cGy-induced G_2 chromatid breaks by the time interval between the two doses

Cell lines	No. of chromatid breaks/100 cells by time interval (hr)							
	Expectd	1	3	6	12	24	48	72
Hep G2	113	63*	85*	99	98	101	69*	59*
Hep 3B	127	73*	79*	91*	105	107	74*	54*

*$P<0.05$, one-tailed t-test, in difference with expected value

the number of chromatid breaks induced by the next higher dose of gamma rays (50 cGy) after 1 hour in the G_2 chromatid, as shown in Table 1. In the S chromatid, however, pretreatment with 1 cGy did not reduce the number of chromatid breaks induced by the subsequent 50 cGy (Table 2).

The time necessary for the expression and the disappearance of the adaptive response was determined by delivering 1 cGy followed by the 50 cGy at hourly intervals (1-6 hours), 12, 24, 48, and 72 hours; In the G_2 chromatid, the cell lines showed a significant reduction of chromatid breaks at the 1 hour and 3 hour intervals. This adaptive response disappeared at the 6 hour interval and it reappeared after 48 or 72 hours. It is summarized in Table 3 and Fig. 1. In the S chromatid, however, the adaptive response first appeared at the 72 hour interval (Table 4 and Fig. 2). The time course of the expression of the adaptive response related with the time interval between the two doses was similar either in the G_2 or S chromatid in the two cell lines.

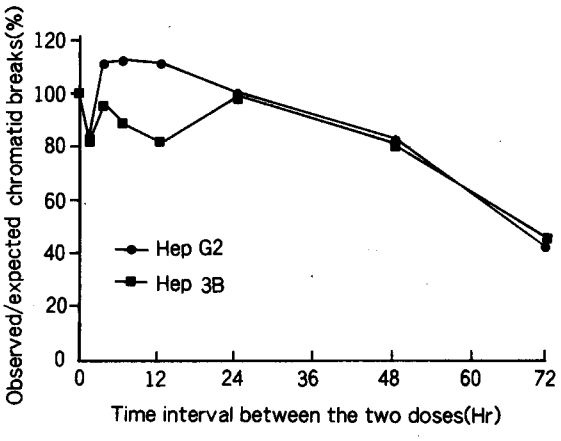

Fig. 2. Time course of expression of adaptive response in S chromatid.

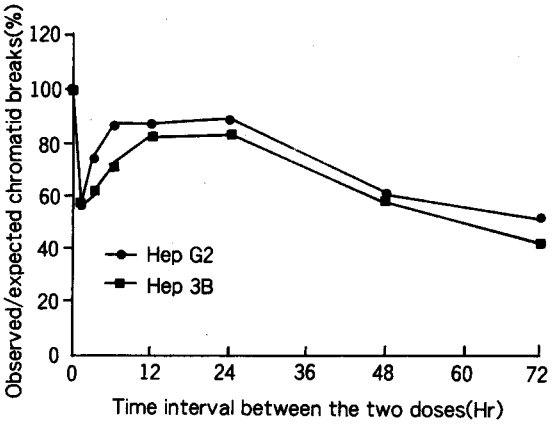

Fig. 1. Time course of expression of adaptive response in G_2 chromatid.

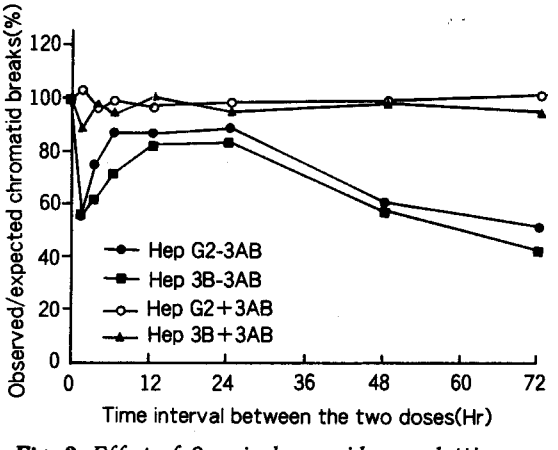

Fig. 3. Effect of 3-aminobenzamide on adaptive response of G_2 chromatid.

Table 4. Effects of 1 cGy pretreatment on 50 cGy-induced S chromatid breaks by the time interval between the two doses

Cell lines	No. of chromatid breaks/100 cells by time interval (hr)							
	Expectd	1	3	6	12	24	48	72
Hep G2	26	22	29	30	29	26	22	15*
Hep 3B	28	23	27	25	23	28	23	13*

*$P<0.05$, one-tailed t-test, in difference with expected value

Table 5. Effects of 3-aminobenzamide on adaptive response of G_2 chromatid breaks by the time interval between the two doses

Cell Lines			No. of chromatid breaks/100 cells by time interval (hr)							
			Expected		1	3	6	12	24	48	72
Hep	G2	no	3AB:	113	63*	85*	99	98	101	69*	59*
		with	3AB:	124	109	121	116	124	118	121	118
Hep	3B	no	3AB:	127	73*	79*	91*	105	107	74*	54*
		with	3AB:	125	129	121	124	121	122	124	127

*$P<0.05$, one tailed t-test, in difference with expected value

In the experiments with 3-AB, an inhibitor of poly (ADP-ribose) polymerase, the yield of chromatid breaks was almost the same as the sum of the individual effects of 1 cGy and 50 cGy as shown in Table 5 and Fig. 3. The addition of 3-AB eliminated the adaptive response in the tested cell lines, which suggested a role of this enzyme in the expression of the adaptive response.

DISCUSSION

We have previously shown that an acute low dose of gamma rays can adapt human lymphoblastoid cells to ionizing radiation, making them less susceptible to damage induced by a subsequent higher dose (Seong *et al.* in press). This response does not seem to be dependent upon the degree of damage induced by the high damaging dose because the cells from ataxia telangiectasia (AT) homozygote, which is known to be highly radiosensitive, still had a similar reduction in chromatid breaks to other tested cells after being adapted with low dose radiation. A similar result had been reported by Shadley *et al.* (1987) and they observed that the lymphocytes from an AT patient still showed a similar reduction of chromatid aberrations after being adapted with a low level of tritiated thymidine to those in cells from a normal individual.

The data presented here, which observed chromatid breaks as endpoints, demonstrate that an acute low dose of gamma irradiation can adapt human hepatoma cells to ionizing radiation so that the cells become less susceptible to damage induced by a subsequent higher dose. Since most experiments relating to adaptive response have been performed on human lymphocytes, which have a limited life span, the results in this study extend our understanding of the presence of adaptive response to the immortalized tumor cells. Although more experiments should be carried out in various cell systems, these results implicate that the adaptive response is not a phenomenon limited to a specific cell system.

There have been some studies on the time course of the adaptive response regarding the interval between the adapting and challenging dose. Shadley *et al.* (1987) reported that the interval between the adapting and challenging dose should be at least 4~6 hours in his experiments with human lymphocytes. Ikushima (1989) observed similar results in Chinese hamster cells. It has been suggested that during 4 hours protein synthesis is required for full expression of the adapting effect (Youngblom *et al.* 1989).

In this study the experiments were also carried out to determine the life span of the adaptive response; In both G_2 and S chromatids, the adaptive response was exhibited after 48 hours. In G_2 chromatids, however, the adaptive response was also observed at short time intervals as early as 1 hour. This difference in time course of the adaptive response according to the cell cycle phase was first observed in this study. Particularly, the adaptive response as early as the 1 hour interval in G_2 chromatids suggests that the mechanism of this adaptive response involves a certain process other than protein synthesis although they aim at the same result, adaptation. This problem remains unsolved at

present. Elucidation of the underlying mechanism of the adaptive response seems to be crucial.

For the mechanism of the adaptive response it was postulated that it might be mediated through the induction of an enzyme involved in chromosomal repair. Because poly (ADP-ribose) polymerase, which has been implicated in repair (Skidmore *et al.* 1979; Durkacz *et al.* 1980), increased by DNA strand breaks of the type produced by ionizing radiation (Benjamin and Gill, 1980), the possibility that poly (ADP-ribosyl)ation might be involved in the adaptive response was also raised. To test this, adaptation studies were carried out in the presence of 3-AB, a potent inhibitor of poly (ADP-ribose) polymerase; Wiencke *et al.* (1986) showed that the adaptive response in human lymphocytes was abolished when 3-AB was present during the entire culture period or just after the second irradiation while 3-AB treatment before the second radiation did not affect the adaptive response. Their results clearly showed that inhibition of poly (ADP-ribose) polymerase by 3-AB treatment after irradiation reverses the adaptive response.

In this study the presence of 3-AB inhibited the expression of the adaptive response in human hepatoma cells. It inhibited the expression of the adaptive response in both short and long time intervals. The result indicates that in human hepatoma cells, a low level of gamma radiation induces a chromosome repair mechanism whose activity can be eliminated by an inhibitor of poly (ADP-ribose) polymerase.

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