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Adaptive Response to Ionizing Radiation Induced by Low Doses of Gamma Rays in Human Lymphoblastoid Cell Lines

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=Abstract=

When cells are exposed to low doses of a mutagenic or clastogenic agents, they often become less sensitive to the effects of a higher dose administered subsequently. Such adaptive responses were first described in Escherichia coli and mammalian cells to low doses of an alkylating agent.

Since most of the studies have been carried out with human lymphocytes, it is urgently necessary to study this effect in different cellular systems. Its relation with inherent cellular radiosensitivity and underlying mechanism also remain to be answered. In this study, adaptive response by 1 cGy of gamma rays was investigated in three human lymphoblastoid cell lines which were derived from ataxia telangiectasia homozygote, ataxia telangiectasia heterozygote, and normal individual. 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 indicate that prior exposure to 1 cGy of gamma rays reduces the number of chromatid breaks induced by subsequent higher dose (50 cGy). The expression of this adaptive response was similar among three cell lines despite of their different radiosensitivity. When 3-aminobenzamide, an inhibitor of poly (ADP-ribose) polymerase, was added after 50 cGy, adaptive responses were abolished in all the tested cell lines.

Therefore it is suggested that the adaptive response can be observed in human lymphoblastoid cell lines, which was first documented through this study. The expression of adaptive response was similar among the cell lines regardless of their radiosensitivity. The elimination of the adaptive response by 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, Lymphoblastoid cells, Gamma rays

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INTRODUCTION

When cells are exposed to low doses of mutagenic or clastogenic agents, they often become less sensitive to the effects of a higher dose administered subsequently. Such adaptive responses were first described in Escherichia coli[1] and mammalian cells[2,3] to low doses of an alkylating agent.

In the experiments on the induction of chromatid breaks in X-irradiated human lymphocytes, Olivieri et al.[4] 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. This reduction in chromatid aberration was attributable to neither different cell stage radiosensitivity nor the selective killing of a radiosensitive population of lymphocytes that had incorporated [^{3}H]dThd[5]. 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.

Since most of the studies have been carried out in vitro and with human lymphocytes, one of the major questions is whether the adaptive response is a universally occurring phenomenon or whether it is restricted to very specific conditions and cell systems. Many results remain equivocal even with lymphocytes. Some authors could not observe any adaptation to low adapting doses applied in G_0 stage of the lymphocytes[6,7], while others reported the contrary[8-11]. Similarly, the amplitude of the protective effect triggered by the adapting dose is very heterogeneous[12] and while some individuals seem to be genetically deficient for adaptive response[13], in others the expression of the effect appears to depend on their physiological state and the lymphocyte culture conditions[14].

The basic mechanism, however, may apply to other systems and may have a considerable impact on radiation risk. Thus, it is urgently necessary to study this effect in different cellular systems and examine various endpoints.

In this study, we chose three human lymphoblastoid cell lines with different radiosensitivity as experimental systems: The lymphoblastoid cell lines are derived from ataxia telangiectasia (AT) homozygote, which is highly radiosensitive, AT heterozygote, and normal individual, respectively.

The aims of this study were to analyse whether the adaptive response could be induced in human lymphoblastoid cell lines and the relationship between the chromosomal radiosensitivity and the expression of the adaptive response. 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 a unknown chromosomal repair mechanism involving poly (ADP-ribosyl)ation through the experiments on human lymphocytes in the presence of 3-aminobenzamide (3-AB), a potential inhibitor of poly (ADP-ribose) polymerase[5,15]. To examine if the same result could be reproduced in our experimental systems, the experiments with 3-AB were carried out. In all experiments chromatid breaks were scored as endpoints.

MATERIALS AND METHODS

The Ebstein-Barr virus transformed lymphoblastoid cell lines from AT homozygote (GM 1526), AT heterozygote (GM 3382), and normal individual (3402P) were provided by Dr. T. C. Hsu (M. D. Anderson Cancer Center, Houston, Texas). The cells were grown in suspension 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 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 Cobalt-60 irradiator; 50 cGy was delivered at 80 cm distance from the source with a dose rate of 154 cGy/minutes at 80 cm distance from the source and 1 cGy was delivered at 160 cm distance from the source with a dose rate of 0.76 cGy/minutes, which was possible by passing gamma rays through 6.5 cm-thick cerrobend block.

For the experiments, 1 cGy of gamma rays were delivered to the cells and the cells were exposed to subsequent 50 cGy of gamma rays. The time interval between the two doses varied from 1 hour to 72 hours. When used, 3-AB 2 mM (Sigma, St. Louis, MO) was added immediately after the 50 cGy exposure according to Wiencke et al's suggestion[5]. 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 and for the 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

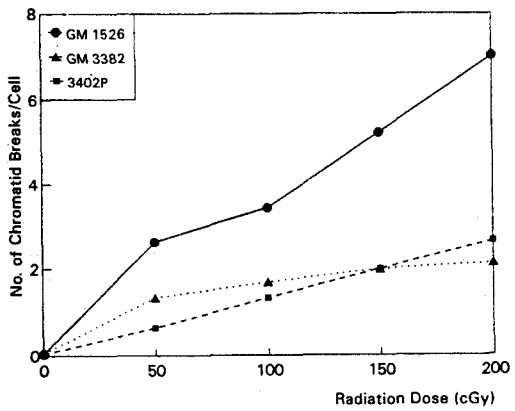

Fig. 1. Radiation induced G_2 chromatid breaks in the tested cell lines

(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 also disregarded. One hundred cells were scored for each treatment. The statistical significance of reductions in chromatid breaks was determined with a one-tailed t-test.

RESULTS

Radiosensitivity of the three cell lines were tested for G_2 chromatid radiosensitivity as a function of dose. As shown in Fig. 1, GM 1526, an AT cell line, showed the highest frequency of G_2 chromatid breaks by radiation dose among the tested cell lines, which means the highest sensitivity to radiation. G_2 chromatid radiosensitivity of GM 3382, an AT heterozygous cell line, and 3402P, cells from normal individual, was in similar range, which was much lower than that of GM 1526.

When the cells are pretreated with 1 cGy and followed by subsequent 50 cGy, the expected number of chromatid breaks would be the sum of 1 cGy effect and 50 cGy effect minus the control. In G_2 chromatid, a prior exposure to 1 cGy significantly reduced the number of chromatid breaks induced by next higher dose of gamma rays (50 cGy) after 1 hour in all the tested cell lines as shown in Table 1. There was no difference in the expression of this adaptive response

Table 1. Effects of 1 cGy Pretreatment on 50 cGy-induced G_2 Chromatid Breaks.

Cell lines	No. of chromatid breaks/100 cells				
	control	D_1 (1 cGy)	D_2 (50 cGy)	D_1-(1hr)-D_2	Expected
GM 1526	4	33	263	219*	292
GM 3382	5	19	134	103*	148
3402P	4	9	64	42*	69

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

among the cell lines. In S chromatid, however, pretreatment with 1 cGy did not reduce the number of chromatid breaks induced by subsequent 50 cGy after 1 hour in all the tested cell lines (Table 2).

Table 2. Effects of 1 cGy Pretreatment on 50 cGy-induced S Chromatid Breaks.

Cell lines	No. of chromatid breaks/100 cells				
	Control	D_1 (1 cGy)	D_2 (50 cGy)	D_1-(1hr)-D_2	Expected
GM 1526	4	27	69	80	92
GM 3382	4	9	19	19	23
3402P	4	5	24	24	25

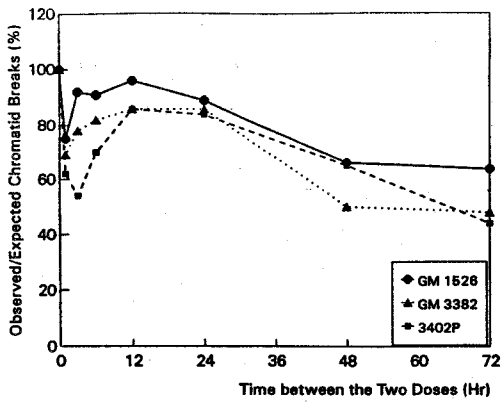

Fig. 2. Time course of expression of adaptive response in G_2 chromatid

The time necessary for the expression and the disappearance of the adaptive response was determined by delivering 1 cGy at hourly intervals (1–6hours), 12, 24, 48, and 72 hours before the 50 cGy dose; In G_2 chromatid, all the tested cell lines showed significant reduction of chromatid breaks at 1 hour and 3 hour interval. Except 3402P, adaptive response disappeared at 6 hour interval and it reappeared after 48 or 72 hours. It is summarized in Table 3 and Fig. 2. In S chromatid, however, adaptive response first appeared from 72 hour interval except AT cells, which showed the adaptive response from 48 hours interval (Table 4 and Fig. 3). This relationship between the expression of the adaptive response and time interval between the two doses was similar either in G_2 or S chromatid among the three cell lines.

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. 4. The addition of 3-AB eliminated the adaptive response in all the tested cell lines, which suggested a role of this enzyme, poly (ADP-ribose) polymerase, in the expression of the adaptive response.

Table 3. Effects of 1 cGy pretreatment on 50 cGy-induced G_2 Chromatid Breaks by Time Interval between the Two Doses

Cell lines	No. of chromatid breaks/100 cells by time interval (hour)							
	Expected	1	3	6	12	24	48	72
GM 1526	282	219*	269	266	282	262	174*	188*
GM 3382	148	103*	116*	121	127	127	75*	71*
3402P	69	42*	37*	48*	59	57	44*	30*

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

Table 4. Effects of 1 cGy Pretreatment on 50 cGy-induced S Chromatid Breaks by Time Interval between the Two Doses

Cell lines	No. of chromatid breaks/100 cells by time interval (hours)							
	Expected	1	3	6	12	24	48	72
GM 1526	92	80	100	87	101	91	62*	58*
GM 3382	23	19	19	21	21	23	19	12*
3402P	25	26	22	22	25	26	21	9*

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

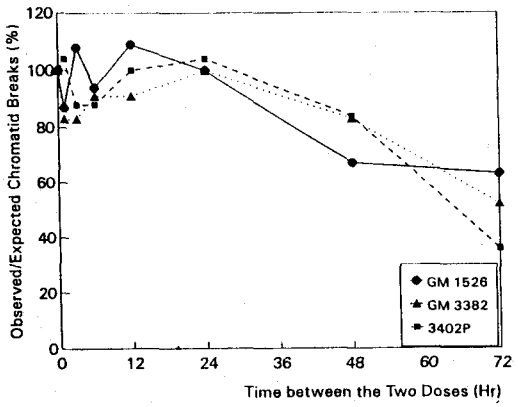

Fig. 3. Time course of expression of adaptive response in S chromatid

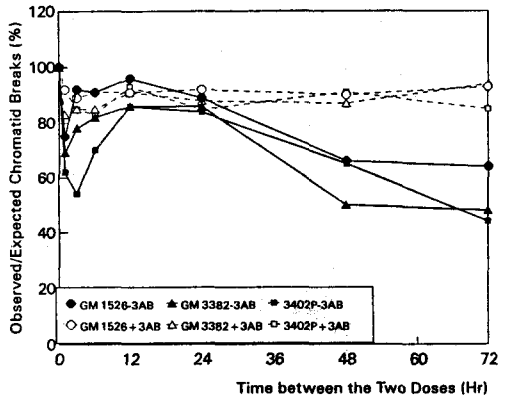

Fig. 4. Effect of 3-aminobenzamide on adaptive response of G_2 chromatid

Table 5. Effects of 3-aminobenzamide on Adaptive Response of G_2 Chromatid Breaks by Time Interval between the Two Doses

Cell lines		No. of chromatid breaks/100 cells by time interval (hour)							
		Expected	1	3	6	12	24	48	72
GM 1526	—3AB:	292	219*	269	266	282	262	174*	188*
	+3AB:	298	274	266	271	271	274	269	277
GM 3382	—3AB:	148	103*	116*	121	127	127	75*	71*
	+3AB:	156	130	132	131	142	138	136	146
3402P	—3AB:	69	42*	37*	48*	59	57	44*	30*
	+3AB:	75	69	64	62	70	64	68	64

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

DISCUSSION

Although an adaptive response can occur with pharmacologic agents, the situation has been much less clear with ionizing radiation, which produce random lesions within the cells. The amount of energy deposited by low doses of radiation is just too small to bring about the physiological effects that could lead to stimulation. Consequently, to account for the effects of low-level radiation, it has been necessary to look for a system within the cell that 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 nucleus, represents 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.

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

phenomenon to a specific cell system.

Sankaranarayan et al.[12] and Bauchinger et al.[13] advocated that adaptive response is very heterogenous in the amplitude and some individuals seem to be genetically deficient for adaptive response. However, in the study by Shadley et al.[6], the lymphocytes from AT patient still showed reduction of chromatid aberrations after being adapted with low levels of tritiated thymidine and similar reductions were observed in cells from normal individual. Also in this study, adaptive response does not seem to be dependent upon the inherent cellular radiosensitivity in that the cells from AT homozygote, which is extremely radiosensitive, still had similar reduction in chromatid breaks to other tested cells after being adapted with low dose radiation. These results suggest that a mechanism of adaptive response might have no relation with cellular radiosensitivity although more investigation should be pursued.

In most studies cells were harvested 6 hours after irradiation, which means that they observed S phase chromosomes, and adaptive response could be observed from 4 hours interval.[4-7,14] In this study, cells were harvested at both G_2 and S phase and the expression of adaptive response appeared differently depending on cell cycle phases that were given 50 cGy. The expression of adaptive response at 48 or 72 hours after adapting dose was a common feature regardless of cell cycle phase. However, early expression of adaptive response in G_2 chromatids from 1 hour interval was quite different from S chromatids. The reason of this difference is not well understood at present. The expression of adaptive response from 1 hour interval in G_2 chromatids and that in 48 or 72 hour interval might not be the same mechanism and this question remains to be answered. Elucidation of underlying mechanism of adaptive response seems to be essential.

It is already known that the enzyme poly (ADP -ribose) polymerase activity is required for the efficient repair of chromosomal damage[16]. Wiencke et al[5] 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 reverse the adaptive response. In our study with lymphoblastoid cells, the loss of adaptive response in the experiments with 3-AB strongly suggests that mechanisms of adaptive response involve the induction of chromosomal repair system.

Therefore it is suggested that the adaptive response can also be observed in human lymphoblastoid cell lines, which was first documented through this study. The elimination of the adaptive response with 3-AB is consistent with the proposal that this adaptive response is the result of the induction of a certain unknown chromosomal repair mechanism.

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=국문초록=

인체임파양세포에서 저선량의 감마선에 의해서 유도되는 적응 반응

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성 진 실·서 창 옥·김 귀 언

미량의 변이 유발소에 노출된 세포는 그 다음에 투여되는 다량의 변이 유발소에 대하여 내성을 갖게 된다. 이같은 적응 반응(adaptive response)은 대장균에서 처음으로 밝혀진 이후 주로 인체 말초 혈액 임파구에서 연구가 진행되어 왔다. 그러나 적응 반응이 세포 종류를 막론하고 존재하는 일반적인 현상인지에 대하여, 또한 세포의 고유한 방사선 감수성과의 관계나 그 기전 등에 대하여도 규명되어야 할 필요가 있다.

본 연구는 이같은 의문에 보다 접근하기 위하여 방사선에 매우 민감한 ataxia telangiectasia homozygote, ataxia telangiectasia heterozygote, 그리고 정상인에서 유래한 인체 임파양세포주를 대상으로 1 cGy의 감마선을 조사하고 일정 시간이 지난 후 다시 50 cGy의 감마선을 조사하여, 감마선에 의해 유도되는 염색체 손상을 측정하였다.

그 결과 1 cGy 전처치시 그 다음 50 cGy에 의한 염색체 손상이 50 cGy 단독 대조군에 비하여 의의있게 감소하여 적응 반응이 존재함을 알 수 있었다. 세가지 세포주의 방사선 감수성이 각기 달랐으나 적응 반응의 표현 양상은 이와 무관하게 유사하였다. 또한 염색체 손상의 복구에 필수적인 poly(ADP-ribose) polymerase를 억제하는 3-aminobenzamide를 50 cGy 직후에 투여한 실험에서는 적응 반응이 완전히 소실됨을 관찰하여 적응 반응이 어떤 종류의 방사선 손상의 복구 기전과 관계 있음을 추측케하였다.

따라서 임파양세포에서도 적응 반응이 존재함을 본 연구를 통하여 최초로 알 수 있었다. 이는 세포의 방사선 감수성과는 무관한 것으로 나타났으며 그 기전에 있어서는 아직 잘 알려지지 않은 손상 복구 기전을 유도하는 것으로 생각된다.