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HIGH INCIDENCE OF SISTER CHROMATID EXCHANGES AND CHROMATID INTERCHANGES IN THE CONDITIONS OF LOWERED ACTIVITY OF POLY(ADP-RIBOSE)POLYMERASE

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SUMMARY: The effect of lowering the activity of poly(ADP-ribose)polymerase on chromosome stability has been examined. Chinese hamster ovary cells, CHO-K1 grown in a nicotinamide-free medium exhibited an increased frequency of sister chromatid exchanges in a time-dependent manner. Furthermore, addition of m-aminobenzamide which is known to be a strong inhibitor of poly-(ADP-ribose)polymerase caused a manyfold increase in the frequency of both sister chromatid exchanges and non-sister chromatid interchanges. These results suggest that appropriate levels of NAD and the activity of poly-(ADP-ribose)polymerase are required for maintaining chromosome stability.

INTRODUCTION

Poly(ADP-ribose)polymerase is a chromatin-bound enzyme of eukaryotic cells that catalyzes the synthesis of poly(ADP-ribose) from the ADP-ribose moiety of NAD (See for review, 1, 2). Recent studies on the physiological roles of poly(ADP-ribose) suggest that the polymer may be involved in DNA metabolism, particularly in the process of cellular recovery from DNA damage. It is observed that DNA damaging agents such as alkylating agents (3-6), radiations(7-11), and other carcinogens(12) lower intracellular NAD levels by stimulating the specific activity of poly(ADP-ribose)polymerase. In addition the inhibition of the enzyme activity prevents the drop in NAD levels and enhances the cytotoxicity caused by DNA damaging agent.

In the present work, the effect of lowering the activity of poly(ADP-ribose)polymerase on chromosome stability was examined by assaying for SCEs and chromosome aberrations. Without damaging chromosomal DNA directly, lowering the activity of poly(ADP-ribose)polymerase caused a manyfold

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increase in the frequency of chromatid recombinations between sister and non-sister chromatids.

MATERIALS AND METHODS

Chemicals : Nicotinamide was purchased from Wako Pure Chemicals Industries, Osaka, Japan; m-aminobenzamide, α-picolinamide and N'-methylnicotinamide were from Tokyo Co., Tokyo, Japan.
Cells and culture conditions : Chinese hamster ovary cells (CHO-K1) were maintained as monolayers at 37°C in Eagle's MEM (Nissui Seiyaku Co., Tokyo, Japan) supplemented with 7.5% dialyzed fetal bovine serum (Gibco Grand Island, N.Y.) in atmosphere of 5% CO_2 and 95% air. The nicotinamide-free medium was prepared with nicotinamide-free Eagle's MEM (specially prepared by Nissui Seiyaku Co., Tokyo, Japan) supplemented with 7.5% dialyzed fetal bovine serum.
Analysis of sister chromatid exchanges (SCEs) and chromosome aberrations : Cells (2-5 x10^5) were grown in the presence of 5-bromodeoxyuridine (BrdUrd, 10μM) for about two cell cycles, 38-42 hours. Metaphase cells were arrested with colchicine (0.2μg/ml) for 2 hours before harvesting and chromosome spreads were made by the air drying method. The sister chromatids were differentially stained by a modified technique of Perry and Wolff (13). At 24 hours after preparation, the slides were stained with Hoechst 33258 (1μg/ml) in Sörensen's phosphate buffer (pH6.8) for 9 min, and mounted in the buffer under a coverslip. The slides were then exposed to a super-mercury lamp (Osram HBO, 200W) for 5 min at a distance of 6cm from the slide and stained with 2% Giemsa solution for 15 min. For scoring of SCEs, the second-division metaphases with diploid chromosome complements were selected and 50 metaphases were analyzed. For scoring of chromosome aberrations, metaphase spreads from the same samples were stained with conventional Giemsa staining and 100 metaphases were examined.

RESULTS

Increased frequency of SCEs in a nicotinamide-free medium : Since poly-(ADP-ribose)polymerase requires NAD for substrate, lowering of the NAD content should decrease the enzyme activity. Cellular NAD levels may be lowered by growing cells in a nicotinamide-free medium (14). Table I shows that when cells were cultured in the nicotinamide-free medium, the frequency of SCEs increased in a time-dependent manner. In these culture conditions, the cells continued to divide but with a slightly slower rate as judged by the rate of appearance of the second-division metaphases after BrdUrd addition. Addition of 10^{-2}mM nicotinamide, however, restored the SCE frequency to the spontaneous level. This concentration of nicotinamide is nearly equivalent to that in normal medium (0.82 x 10^{-2}mM). However, addition of higher concentration of nicotinamide resulted in a

Table I : Frequency of SCEs in a nicotinamide-free medium

Culture media	Preculture time (hours)	SCEs ± S.D. per cell
Normal	--	9.2 ± 1.7
Nicotinamide-free	0	12.7 ± 2.4
	24	16.7 ± 3.4
	48	21.4 ± 4.2
	72	26.4 ± 5.1
	72*	10.8 ± 2.3
	72**	12.2 ± 2.5

Cells were precultured for different periods of time and after addition of BrdUrd(10μM), the cultures were continued for about two cell cycles in the same medium. * and **; Nicotinamide (*, 10^{-2}mM; **, 10^{-1}mM) was added at the same time of BrdUrd addition.

significant increase of SCEs, as reported by others (15) and described below.

Induction of SCEs by inhibitors of poly(ADP-ribose)polymerase in nicotinamide-free medium : Table II compares the effects of m-aminobenzamide, a strong inhibitor of poly(ADP-ribose)polymerase (16), on the induction of SCEs in normal and nicotinamide-free medium. In normal medium, addition of various concentrations of the inhibitor resulted in dose-dependent increase in SCE frequencies, as previously reported (16). In nicotinamide -free medium, however, the effect of m-aminobenzamide in inducing SCEs was more enhanced. The interaction between effect of m-aminobenzamide and of nicotinamide-free medium appears to be synergistic.

Similar effects can be seen for other inhibitors of poly(ADP-ribose) synthesis (Table III). Both nicotinamide and α-picolinamide induced SCEs significantly. Nicotinamide is a well-known inhibitor of poly(ADP-ribose)-polymerase (1). α-picolinamide, an isomer of nicotinamide was recently reported to be just as strong an inhibitor of poly(ADP-ribose)polymerase

Table II : Frequency of SCEs induced by m-aminobenzamide in normal and nicotinamide-free medium.*

m-aminobenzamide	Culture media	
mM	Normal	Nicotinamide-free
0	9.5 ± 1.8	12.4 ± 3.0
1.25	25.3 ± 3.7	42.0 ± 8.2
2.5	33.0 ± 6.4	49.7 ± 9.0
5	51.1 ± 7.7	79.7 ± 9.2
10	73.4 ± 13.5	122.4 ± 23.6

* Values are SCEs ± S.D. per cell. 50 cells were examined.

as nicotinamide in rat pancreatic islet cells (18). It can also be seen in Table III that the inhibitors were even more effective in inducing SCEs in nicotinamide-free medium. N'-methylnicotinamide, a very weak inhibitor (17) induced SCE only slightly and no enhancement in nicotinamide-free medium was detected.

Table III: Frequency of SCEs induced by other inhibitors of poly(ADP-ribose) polymerase.*

Inhibitors	Doses	Culture media	
	mM	Normal	Nicotinamide-free
α-picolinamide	5	20.8 ± 4.4	27.4 ± 3.7
	10	26.6 ± 6.7	37.6 ± 5.8
Nicotinamide	5	18.1 ± 3.1	20.0 ± 3.4
	10	28.0 ± 3.8	36.6 ± 6.9
N'-methylnicotinamide	5	10.0 ± 1.9	12.3 ± 3.5
	10	10.6 ± 2.3	12.6 ± 2.6

* Values are SCEs ± S.D. per cell. 50 cells were examined.

Table IV : Chromosome aberrations induced by m-aminobenzamide and N-metyl-N'-nitro-N-nitrosoguanidine (MNNG).

Chemicals (dose)		Control		m-aminobenzamide (10mM)		MNNG (0.25ug/ml)	
Culture media *		+	-	+	-	+	-
Number of cells examined		100	100	100	100	100	100
Number of aberrant cells		3	10	22	58	57	79
Chromatid-type aberrations							
Chromatid gaps		2	4	5	7	29	26
Chromatid breaks		0	1	4	9	43	48
Isochromatid gaps		1	0	1	0	2	3
Isochromatid breaks		0	0	0	1	7	11
Exchanges	Triradials	0	3	11	32	27	28
	Quadriradials	0	0	6	11	7	11
	Complex **	0	0	2	9	6	16
Chromosome-type aberrations							
	Dicentrics	0	2	6	11	4	7
	Rings	0	1	2	4	0	5
Total		3	11	37	87	127	155

* +, Normal medium; -, Nicotinamide-free medium.

** Complex exchanges incolved more than three chromosome arms.

Chromosome aberrations induced by m-aminobenzamide in nicotinamide-free medium : The results of the analysis on chromosome aberrations are shown in Table IV. Addition of m-aminobenzamide in normal medium resulted in a significant increase in the frequency of exchange-type aberrations, but only a slight increase in the number of simple chromatid gaps or breaks. This effect was further enhanced in the nicotinamide-free medium. The majority of triradials and quadriradials induced were the results of re-joining between non-homologous chromosomes.

As a positive control, the effect of a direct DNA damaging agent, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) on chromosome stability was examined. The dose of MNNG used was 0.25μg/ml which induced 62.9±1.1 and 80.2±1.3 SCEs per cell in normal and nicotinamide-free medium, respectively. In contrast to the effect of m-aminobenzamide, MNNG induced many simple chromatid breaks, accompanied with exchange type aberrations.

DISCUSSION

The results presented in this communication indicate that without damaging DNA directly, chromosome instability was induced under cellular conditions in which the activity of poly(ADP-ribose)polymerase was lowered or inhibited. When cells were grown in a nicotinamide-free medium, the frequency of SCEs was increased significantly in a time-dependent fashion (Table I). In these cells, NAD may become limiting for polyADP-ribosylation reactions during continuous growth in nicotinamide-free medium. This conclusion is supported by the observation that addition of nicotinamide (10^{-2}mM) to the nicotinamide-free medium resulted in the restoration of normal levels of SCEs.

Recently, Oikawa and co-workers (17) reported a possitive correlation between the inhibitory effects of benzamide and related compounds on poly(ADP-ribose)polymerase and their effects in inducing SCEs. The present results confirmed that m-aminobenzamide, a strong inhibitor, is a strong inducer of SCEs. In addition, a combined treatment with m-aminobenzamide in nicotinamide-free medium resulted in manyfold increase not only in the frequency of SCEs (Table II), but also in the frequency of chromosome aberrations (Table IV). The present results strongly suggest that appropriate levels of cellular NAD and the activity of poly(ADP-ribose)polymerase are required for maintaining chromosome stability.

Although SCEs occur spontaneously and can be induced by a variety of DNA damaging agents, it is possible that SCEs may arise by different

mechanisms, some of which may depend upon the induction of DNA lesions (19). The mechanism by which SCEs are induced by lowering the activity of poly(ADP-ribose)polymerase is unknown, but two possibilities may be considered. One possibility would be that the inhibition of poly(ADP-ribose) synthesis alters chromatin structure and results in a retardation in discontinous DNA replication (20, 21). There is circumstantial evidence that DNA lesions which retard the rate of DNA chain elongation are more responsible for the induction of SCEs than those which inhibit the initiation of replicons or replicon clusters (22). A second possibility may be that the lowering the activity of poly(ADP-ribose)polymerase induces DNA strand breaks indirectly. It is known that a Ca^{2+}, Mg^{2+} dependent endonuclease is polyADP-ribosylated and that this modification of the enzyme causes a reversible inhibition of the nuclease activity (23). Therefore, it is possible that lesions which lead to SCEs are strand breaks induced by an activation of a Ca^{2+}, Mg^{2+} dependent endoculease via inhibition of poly-ADP-ribosylation.

It may be noteworthy that a combined treatment with m-aminobenzamide and nicotinamide-free medium induced a variety of exchange type aberration (Table IV). It has been suggested in many studies on induced SCEs that the lesions which lead to SCEs may be quite different from those that lead to chromosome aberrations, although both events apparently share certain common features (24). In the case of lowered activity of poly(ADP-ribose)-polymerase studied here, the lesion(s) induced, whatever the nature of the lesion, appears to be involved in the process of double strand recombination between sister chromatids and non-sister chromatids, rather than leading to the induction of simple chromatid breaks.

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