Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 997-1002

5-METHYLNICOTINAMIDE-RESISTANT VARIANT OF MOUSE LYMPHOMA L1210 CELLS

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Received July 11,1980

SUMMARY

5-methylnicotinamide is an inhibitor of poly(ADP-ribose) synthetase, and it enhances the cytotoxicity of alkylating agents and of radiation in mouse lymphoma L1210 cells. We have isolated a spontaneous variant L1210 cell which shows increased resistance to 5-methylnicotinamide and has a reduced potentiation of cell-killing by methylnitrosourea and by γ-radiation. This observation is further evidence in support of the participation of $(ADP\text{-}ribose)_n$ in DNA excision repair and in cell survival. This variant may be of use in the molecular analysis of this component of DNA repair.

Introduction.

The activity of some chromatin proteins is regulated by their chemical modification; one of these post-synthetic, covalent modifications is effected by the enzyme poly(ADP-ribose) synthetase, which uses the coenzyme NAD^+ to ADP-ribosylate chromatin proteins (1,2). The modification consists largely of mono (ADP-ribose), but long, homopolymer chains of (ADP-ribose) are also present. Various physiological functions have been suggested for $(ADP\text{-}ribose)_n$, and it seems likely that it fulfills several different regulatory functions in a way similar to protein phosphorylation.

There is very persuasive evidence that one function of $(ADP\text{-}ribose)_n$ is to participate in DNA excision repair (3-20).

0006-291X/80/190997-06$01.00/0

However, the possibility of involvement in other chromatin functions remains open.

Considerable use has been made of specific enzyme inhibitors. Four classes of inhibitors of poly(ADP-ribose) synthetase are known; nicotinamides (such as 5-methylnicotinamide) (21-23), methylxanthines (12,15,24,25), thymidine (21,26) and aromatic amides such as benzamides (26,28,20).

Inhibitors of poly(ADP-ribose) synthetase increase the toxicity of monofunctional alkylating agents and of γ-radiation (20,29).

A HeLa cell variant partially resistant to 5-methylnicotinamide has been described by Kidwell & Burdette (30). The variant clone was able to grow in the presence of 10mM 5-methylnicotinamide at 70% of the rate of cells in the absence of the inhibitor. The enzyme in isolated nuclei from the variant was slightly less sensitive to the inhibitor than in wild type nuclei.

We have begun a genetic analysis of ADP-ribose metabolism by seeking variants in poly(ADP-ribose) synthetase. We describe the isolation and some properties of a mouse lymphoma variant that is partially resistant to 5-methylnicotinamide; we show that this has marked biological consequences.

Materials and Methods.

5-methylnicotinamide (lot no.405-476-42) was a gift from Lilly Research Laboratories, Indianapolis, U.S.A. Methylnitrosourea (lot no.M4484) was purchased from Cambrian Chemicals Ltd., Croydon, England. Noble agar was from Difco Laboratories Ltd.

The L1210 mouse lymphoma cell line, originally derived from the ascites fluid of a DBA/2 mouse, was supplied by Flow Laboratories, Glasgow, U.K. It was grown in suspension culture in RPMI 1640 medium.

The γ-radiation source was ^{60}Co. The samples were irradiated in air at a dose rate of approximately 2 Krad/min.

(a) Treatment of cells with cytotoxic agent.

10ml of a cell suspension (in complete medium) were treated with the indicated concentration of N-methyl-N-nitrosourea with or without the enzyme inhibitor for 1 hour at 37°C. The cells were then washed twice with prewarmed, complete medium by centrifugation at 1000 rpm for 10 minutes. Inhibitor was present during the washing step when appropriate. For γ-irradiation, two cell suspensions at room temperature were simultaneously irradiated, one with and one without the enzyme inhibitor.

(b) Cell survival assay.

The fraction of viable cells was estimated by their ability to form colonies in agar (29). When used, the inhibitor was present during exposure to the cytotoxic agent as well as during washing and cloning.

(c) Isolation of variant.

Mouse lymphoma L1210 cells were cloned as described above in toxic concentrations of 5-methylnicotinamide. 0.25, 0.5 and 1.0 x 10^6 cells were seeded into 35 mm plates in the presence of 6 mM, 7 mM or 8 mM of the inhibitor. Any colony that formed in the agar was isolated and grown in suspension culture at 37°C.

Results and Discussion.

Only one colony appeared in all the plates examined. This colony was found in a plate containing 6 mM 5-methylnicotinamide. The total number of cells examined was 11.3 x 10^6. This low frequency is not surprising since we did not mutagenize the cells.

The variant and the wild type grew with similar doubling times of about 12 hours. The cloning efficiency in soft agar of the two cell types was similar at 97 ± 10%. There is a clear difference in the response to the cytotoxic effects of 5-methylnicotinamide; the variant is more resistant to the enzyme inhibitor than the wild-type (Fig.1).

We have previously shown that in wild type L1210 cells the enzyme inhibitor, 5-methylnicotinamide, increases MNU cytotoxicity by a large amount and increases γ-radiation toxicity by a small amount (29). We therefore examined the sensitivity of the variant cell to MNU and γ-radiation in the presence and absence

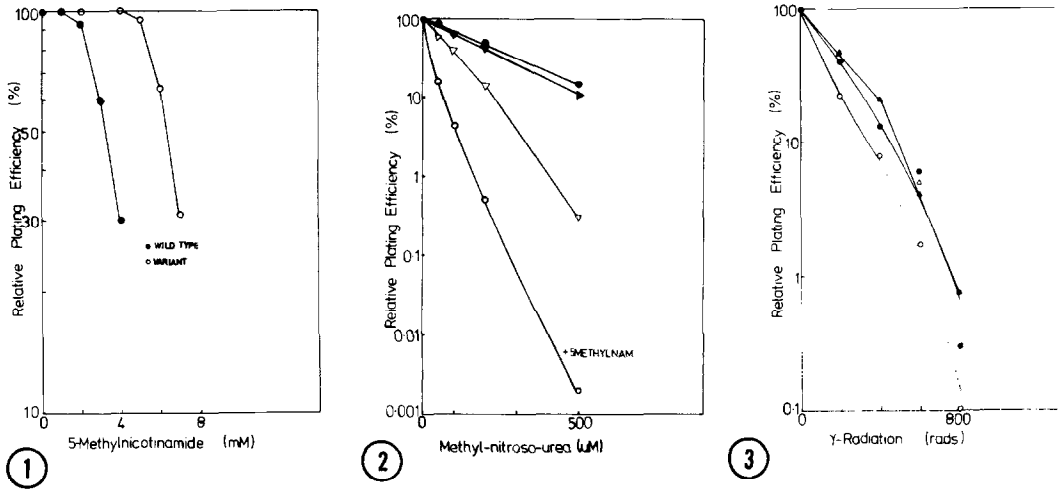

Figure 1. Cytotoxicity of 5-methylnicotinamide to wild type and variant mouse lymphoma L1210 cells.

Cells were plated in soft agar in the presence of increasing concentrations of 5-methylnicotinamide. The survival (relative plating efficiency) is expressed relative to the survival of wild-type cells in the absence of 5-methylnicotinamide, which was 90 ± 10% (100 determinations). ●, wild type; ○, variant.

Figure 2. Potentiation of methylnitrosourea cytotoxicity by 5-methylnicotinamide.

Cells were exposed to methylnitrosourea for 1 hour and then plated in soft agar in the presence or absence of 2 mM 5-methylnicotinamide. The survival (relative plating efficiency) is expressed relative to the survival of wild type cells in the absence of both 5-methylnicotinamide and methylnitrosourea. Open symbols, cells were plated in the presence of 5-methylnicotinamide. ● ○, wild type cells; ▲ △, variant cells.

Figure 3. Potentiation of γ-radiation cytotoxicity by 5-methylnicotinamide.

Cells were irradiated at room temperature at the indicated doses then plated in soft agar with or without 2 mM 5-methylnicotinamide. Open symbols, cells were plated with 5-methylnicotinamide. ● ○, wild type; ▲ △, variant cells.

of 5-methylnicotinamide. The variant cell shows a significant reduction of the potentiation of cytotoxicity by 5-methylnicotinamide (Fig.2). The small, but significant potentiation of killing by γ-radiation is abolished in the variant (Fig.3).

We have apparently isolated a spontaneous variant of the mouse lymphoma L1210 cell that is more resistant to the cytotoxicity of 5-methylnicotinamide and also shows a reduced potentiation of cell killing by this enzyme inhibitor. We have previously shown that $(ADP\text{-}ribose)_n$ biosynthesis is required for efficient excision repair and survival, following damage with monofunctional alkylating agents (20). The possibility that this variant has an altered poly(ADP-ribose) synthetase activity is consistent with the observations. The properties of this variant offer further evidence for the involvement of $(ADP\text{-}ribose)_n$ metabolism in DNA repair and cell survival.

Acknowledgements

This work was supported by the SRC, MRC and Cancer Research Campaign.

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