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Delayed DNA Synthesis Induced by 3-Aminobenzamide in Partially Hepatectomized Liver of Rats

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The possibility of poly(ADP-ribosyl)ation playing a role during liver regeneration induced by partial hepatectomy (PH) *in vivo* was examined. When rats were given an i.p. injection of 3-aminobenzamide (ABA) at a dose of 600 mg/kg body weight 12 h after PH, the levels of DNA synthesis at 20 h after PH were significantly reduced. The time course of DNA synthesis in regenerating liver was significantly delayed in the ABA-treated group. Enzymatic assay revealed the activity of poly-(ADP-ribose)polymerase (PADPRP) in controls to be increased in parallel with the increase of DNA synthesis induced by PH. This increase in PADPRP activity was delayed and very much weaker after ABA treatment. The results thus suggested that poly(ADP-ribosyl)ation might play an important role in DNA synthesis during liver regeneration *in vivo*.

Key words: 3-Aminobenzamide — Poly(ADP-ribosyl)ation — DNA synthesis — Rat

Poly(ADP-ribose)polymerase (PADPRP) plays various roles in biological processes, including DNA repair, cell differentiation, cell proliferation and malignant transformation.[1-6] Recently, the importance of cell proliferation for initiation and promotion of carcinogenesis has been pointed out.[7-9] It has been shown that mRNA, protein synthesis and enzymatic activity of PADPRP increased in phytohemagglutinin-stimulated human lymphocytes *in vitro*,[10,11] and also DNA synthesis increased in rat hepatocarcinogenesis.[12,13] We have been studying the role of PADPRP in liver and pancreatic carcinogenesis in rats or hamsters using various PADPRP inhibitors including 3-aminobenzamide (ABA).[14-16] Recently, we reported an inhibitory influence of ABA on the promoting activity of phenobarbital (PB) in rat hepatocarcinogenesis, suggesting a possible connection between delay in the cell proliferation and poly(ADP-ribosyl)ation.[17] In the present study, we investigated the effects of ABA on DNA synthesis and PADPRP activity during liver regeneration induced by partial hepatectomy (PH) in rats.

MATERIALS AND METHODS

Animals and chemicals A total of 50–60 male Fischer 344 rats (Shizuoka Laboratory Animal Center, Shizuoka), 6 weeks old, were used. ABA (Tokyo Kasei Kogyo, Co. Ltd., Tokyo) was dissolved in dimethylsulfoxide (DMSO) (Sigma Chemical Co., St. Louis, MO) at a concentration of 30 mg/ml immediately prior to administration.

Treatment Rats were housed in an air-conditioned room at 24°C with a 12 h light-dark cycle. PH was performed under ether anesthesia, two-thirds of the liver being routinely removed. To detect the effects of ABA on the first wave of DNA synthesis, five rats were given an intraperitoneal (i.p.) injection of 600 mg/kg body wt. ABA at −18, −6, 0, 6, 12 or 20 h after PH and all rats were killed at 22 h. The solvent DMSO was injected at the same time points into control rats. In addition, for detection of the effects of ABA on the time course of DNA synthesis, all rats were given an i.p. injection of 600 mg/kg body wt. ABA at 12 h after PH and five rats were killed at 18, 22, 26, 28 or 34 h. Controls received an injection of saline 12 h after PH. All experiments were repeated at least twice.

BrdU labeling and immunohistochemical studies Rats received bromodeoxyuridine (BrdU) (Sigma Chemical Co.), at a dose of 30 mg/kg body wt. by i.p. injection 2 h before death. Immediately after the animals were killed, livers were sliced, fixed in 95% ethanol containing 1% acetic acid at 4°C for 2 h and then overnight in ethanol at 4°C, and routinely processed to paraffin sections. Immunohistochemically, BrdU was detected by the avidin-biotin peroxidase complex (ABC) method (Vectastain ABC kit, Vector Labs, Burlingame, CA) using a monoclonal anti-BrdU antibody (Becton-Dickinson, Mountain View, CA). Labeling indices were independently scored by two different investigators.[18]

Rat liver protein extraction For the protein extraction, crude cell extracts from livers were obtained by homogenization using a polytron homogenizer and extraction buffer containing 0.6 *M* NaCl, 50 m*M* Tris-HCl (pH 8.0), 1 m*M* EDTA, 0.5 m*M* dithiothreitol (DTT), 10

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m*M* $NaHSO_3$, 1 m*M* $PhMeSO_2F$ and 1 μM pepstatin. Cellular suspensions were sonicated and centrifuged at 15,000 rpm for 10 min at 4°C using a microcentrifuge.

Activity gel analysis The activity gel method for the detection of the PADPRP activity was applied as described by Scovassi *et al.*[19] Crude cell extract (10 μg) was separated on 7.5% SDS-PAGE containing 100 μg/ml sonicated salmon testis DNA. After electrophoresis, the intact gel was incubated with [^{32}P]NAD, washed with trichloroacetic acid and visualized by autoradiography.

RESULTS

The effects of ABA given at various time points before or after PH on DNA synthesis at 22 h are shown in Table I. The labeling index in hepatocytes of rats given ABA at 12 h after PH was significantly reducd as compared to the respective DMSO control value ($P<0.001$).

Based on this result, in the second experiment to detect the effects of ABA on the time course of DNA synthesis during liver regeneration, rats received ABA at 12 h after PH and were killed at various time intervals thereafter (Fig. 1). The peak of DNA synthesis in the saline-administered group was approximately between 20 and 24 h after PH. In contrast, the peak in ABA-treated rats was delayed and seemed to be present between 26 and 32 h.

To examine the effects of ABA on the enzymatic activity of PADPRP in regenerating livers of rats given ABA at 12 h after PH, activity gel analysis was used (Fig. 2). The levels of PADPRP activity increased in

Table I. Labeling Indices of Hepatocytes 22 Hours after Partial Hepatectomy in Rats Receiving 3-Aminobenzamide at Various Time Points

Treatment	Labeling index (%)					
	Time of ABA administration (h)					
	−18	−6	0[a]	6	12	20
DMSO	32.4±1.8[b]	33.9±1.8	34.3±5.5	34.9±6.1	30.7±3.5	35.2±3.4
ABA	34.1±8.2	34.4±4.0	34.6±6.0	34.9±4.5	11.1±4.5*	31.3±2.4

a) Time of performance of partial hepatectomy.
b) Each value represents mean±SD for 5 rats in which 1000 hepatocytes were randomly counted.
* Significantly different from DMSO-treated group, $P<0.001$.

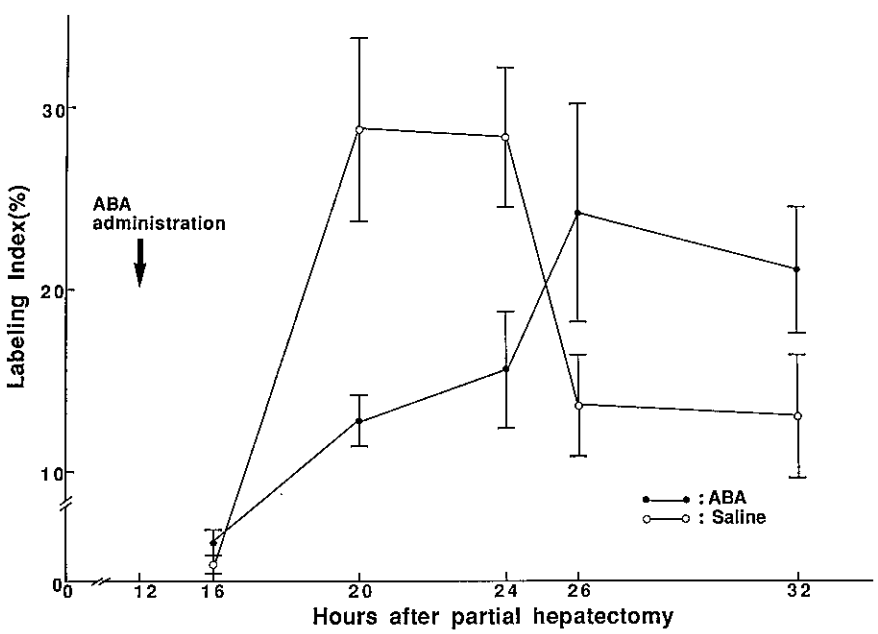

Fig. 1. Effects of ABA on the time course of DNA synthesis induced by PH in rats. ABA (600 mg/kg body wt.) was injected 12 h after PH and rats were killed at various time intervals thereafter.

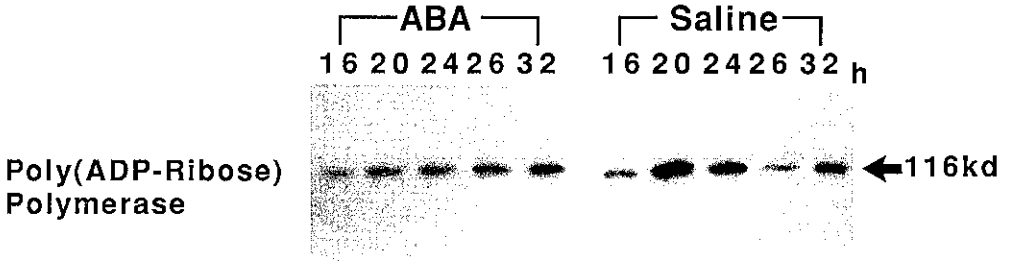

Fig. 2. Activity gel analysis of PADPRP in regenerating rat livers. ABA was given 12 h after PH and 5 g aliquots of protein extracts from regenerating liver at different time points were separated by 7.5% SDS-PAGE containing activated salmon testis DNA. The enzymatic assay was performed as described in "Materials and Methods."

parallel with DNA synthesis in the saline-administered group, the highest activity being found at 20 h after PH. However, the increase of activity in the ABA-treated group was only weak and the peak was delayed to between 26 and 32 h.

DISCUSSION

In the present investigation, ABA exerted a significant delaying effect on the cell cycle during regeneration induced by PH. Since both DNA synthesis and enzymatic activity of PADPRP were reduced in parallel, the results indicate a possible involvement of poly(ADP-ribosyl)-ation in cell proliferation in the rat liver after PH.

We reported previously on the half-life and liver concentration of ABA in rats,[20] showing that an effective concentration of ABA given intraperitoneally remained in the liver 4 h after administration. Therefore, the present results may reflect the action of ABA on DNA synthesis in the liver at about 16 h after PH, because ABA was given 12 h after the operation.

It was earlier reported that PADPRP activity increased after PH in rat liver[21,22] and that levels of DNA synthesis correlate with PADPRP activity in human lymphocytes.[23] Moreover, other investigators reported an increase of PADPRP activity at the onset of DNA replication in human lymphocytes.[19] In the present study, the peak in PADPRP activity was similarly shown to parallel DNA synthesis induced by PH, while ABA delayed both the increase in DNA synthesis and the enzymatic activity of PADPRP. The results thus indicate that the activation of PADPRP directly corresponds with elevated DNA synthesis during rat liver regeneration.

Our findings showed high activity of PADPRP in the G_1-S phase after PH. On the other hand, it has been reported that the activity of PADPRP in S phase is very low in transformed hamster lung cells,[24] and that activity levels throughout the cell cycle do not show any change in synchronized HeLa cells.[11] This would suggest that the relation between enzyme activity and the cell cycle depends upon the species of cell and the tissue.

Recent reports have documented increased levels of PADPRP mRNA in proliferating human T lymphocytes[10] and in rat liver during growth induced by PH and a mitogen.[21] Therefore, the possibility that our previous finding of an inhibitory effect of ABA on PB-promotion in the liver of rats initiated with diethylnitrosamine (DEN) might depend on a delayed cell cycle warrants further investigation.

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REFERENCES

1) Shall, S. ADP-ribose in DNA repair: a new component of DNA excision repair. *Adv. Radiat. Biol.*, **11**, 1–69 (1984).
2) Schwartz, J. L., Morgan, W. F. and Weichselbaum, R. Different efficiencies of interaction between 3-aminobenzamide and various monofunctional alkylating agents in the induction of sister chromatid exchanges. *Carcinogenesis*, **6**, 699–704 (1985).
3) Althaus, F. R., Lawrence, S. D., He, Y.-Z., Sattler, G. L., Tsukada, Y. and Pitot, H. C. Effects of altered (ADP-ribose) metabolism on expression of fetal functions by

adult hepatocytes. *Nature,* **300,** 366–368 (1982).
4) Farzaneh, F., Zalin, R., Brill, D. and Shall, S. DNA strand breaks and ADP-ribosyl transferase activation during cell differentiation. *Nature,* **300,** 362–366 (1982).
5) Borek, C., Morgan, W. F., Ong, A. and Cleaver, J. E. Inhibition of malignant transformation *in vitro* by inhibitors of poly(ADP-ribose) synthesis. *Proc. Natl. Acad. Sci. USA,* **81,** 243–247 (1984).
6) Cesarone, C. F., Scovassi, A. I., Scarabelli, L., Izzo, R., Orunesu, M. and Bertazzoni, U. Depletion of adenosine diphosphate-ribosyl transferase activity in rat liver during exposure to N-2-acetylaminofluorene: effect of thiols. *Cancer Res.,* **48,** 3581–3585 (1988).
7) Yamagami, T., Miwa, A., Takasawa, S., Yamamoto, H. and Okamoto, H. Induction of rat pancreatic B-cell tumor by the combined administration of streptozotocin or alloxan and poly(adenosine diphosphate ribose) synthetase inhibitors. *Cancer Res.,* **45,** 1845–1849 (1985).
8) Romano, F., Menapace, L. and Armato, U. Inhibitors of ADP-ribosyl transferase suppress the mitogenic actions exerted by tumour promoters, but not those evoked by peptide mitogens, in primary neonatal rat hepatocytes. *Carcinogenesis,* **9,** 2147–2154 (1988).
9) Nakajima, K., Utsunomiya, J. and Ishikawa, T. Inhibition of methylazoxymethanol acetate initiation of colon carcinogenesis in rats by treatment with the poly(ADP-ribose) polymerase inhibitor 3-aminobenzamide. *Carcinogenesis,* **9,** 1167–1171 (1988).
10) Menegazzi, M., Gelosa, F., Tommasi, M., Uchida, K., Miwa, M., Sugimura, T. and Suzuki, H. Induction of poly(ADP-ribose)polymerase gene expression in lectin-stimulated human T lymphocytes is dependent on protein synthesis. *Biochem. Biophys. Res. Commun.,* **156,** 995–999 (1988).
11) Scovassi, A. I., Stefanini, M., Lagomarsini, P., Izzo, R. and Bertazzoni, U. Response of mammalian ADP-ribosyl transferase to lymphocyte stimulation, mutagen treatment and cycling. *Carcinogenesis,* **8,** 1295–1300 (1987).
12) Schwarze, D. E., Pettersen, E. O., Shoaib, C. and Seglen, P. O. Emergence of a population of small, diploid hepatocytes during hepatocarcinogenesis. *Carcinogenesis,* **5,** 1267–1275 (1984).
13) Saeter, G., Schwarze, P. E., Nesland, J. M., Juul, N., Pettersen, E. O. and Seglen, P. O. The polyploidizing growth pattern of normal rat liver is replaced by divisional, diploid growth in hepatocellular nodules and carcinomas. *Carcinogenesis,* **9,** 939–945 (1988).
14) Takahashi, S., Nakae, D., Yokose, Y., Emi, Y., Denda, A., Mikami, S., Ohnishi, T. and Konishi, Y. Enhancement of DEN initiation of liver carcinogenesis by inhibitors of NAD^+ADP ribosylation in rats. *Carcinogenesis,* **5,** 901–906 (1984).
15) Tsujiuchi, T., Tsutsumi, M., Denda, A., Amanuma, T., Kondoh, S., Kamino, K. and Konishi, Y. Effects of 3-aminobenzamide on induction of multiorgan carcinogenesis by N-nitrosobis(2-hydroxypropyl)amine in hamsters. *Jpn. J. Cancer Res.,* **82,** 793–799 (1991).
16) Tsujiuchi, T., Mizumoto, K., Tsutsumi, M., Denda, A., Amanuma, T., Kondoh, S. and Konishi, Y. Effects of 3-aminobenzamide on the post-initiation phase of N-nitrosobis(2-oxopropyl)amine induced pancreatic carcinogenesis in Syrian hamsters. *Cancer Lett.,* **61,** 61–66 (1991).
17) Tsujiuchi, T., Tsutsumi, M., Denda, A., Kondoh, S., Nakae, D., Maruyama, H. and Konishi, Y. Possible involvement of poly ADP-ribosylation in phenobarbital promotion of rat hepatocarcinogenesis. *Carcinogenesis,* **11,** 1783–1787 (1990).
18) Schutte, B., Reynders, M. M. J., Bosman, F. T. and Blijham, G. H. Effect of tissue fixation on anti-bromodeoxyuridine immunohistochemistry. *J. Histochem. Cytochem.,* **35,** 1343–1345 (1987).
19) Scovassi, A. I., Stefanini, M. and Bertazzoni, U. Catalytic activities of human poly(ADP-ribose) polymerase from normal and mutagenized cells detected after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.,* **259,** 10973–10977 (1984).
20) Uchida, K., Takahashi, S., Fujiwara, K., Ueda, K., Nakae, D., Emi, Y., Tsutsumi, M., Shiraiwa, K., Ohnishi, T. and Konishi, Y. Preventive effect of 3-aminobenzamide on the reduction of NAD levels in rat liver following administration of diethylnitrosamine. *Jpn. J. Cancer Res.,* **79,** 1094–1100 (1988).
21) Menegazzi, M., De Prati, A. C., Ledda-Columbano, G. M., Columbano, A., Uchida, K., Miwa, M. and Suzuki, H. Regulation of poly(ADP-ribose) polymerase mRNA levels during compensatory and mitogen-induced growth of rat liver. *Arch. Biochem. Biophys.,* **279,** 232–236 (1990).
22) Alvarez-Gonzalez, R. and Ringer, D. P. Nuclear matrix associated poly(ADP-ribose) metabolism in regenerating rat liver. *FEBS Lett.,* **236,** 362–366 (1988).
23) Rochette-Egly, C., Ittel, M. E., Bilen, J. and Mandel, P. Effects of nicotinamide on RNA and DNA synthesis and on poly(ADP-ribose) polymerase activity in normal and phytohemagglutinin stimulated human lymphocytes. *FEBS Lett.,* **120,** 7–11 (1980).
24) Miwa, M., Sugimura, T., Inui, N. and Takayama, S. Poly-(adenine diphosphate ribose) synthesis during the cell cycle of transformed hamster lung cells. *Cancer Res.,* **33,** 1306–1309 (1973).