Jpn. J. Cancer Res. 82, 793–799, July 1991

Effects of 3-Aminobenzamide on Induction of Multiorgan Carcinogenesis by N-Nitrosobis(2-hydroxypropyl)amine in Hamsters

Toshifumi Tsujiuchi,[1] Masahiro Tsutsumi,[1] Ayumi Denda,[1] Toshihiro Amanuma,[1] Satoshi Kondoh,[1] Kenji Kamino[2] and Yoichi Konishi[1,3]

[1]*Department of Oncological Pathology, Cancer Center, Nara Medical College, 840 Shijo-cho, Kashihara, Nara 634, Japan and* [2]*Department of Experimental Pathology, Hannover Medical School, Konstanty-Gutschow-Str. 8, 3000 Hannover 61, Germany*

The effects of an inhibitor of poly(ADP-ribose)polymerase, 3-aminobenzamide (ABA), on N-nitrosobis(2-hydroxypropyl)amine (BHP)-induced pancreas, liver, gallbladder and lung carcinogenesis in Syrian golden hamsters were investigated. Animals were given either BHP alone, by subcutaneous injection at a dose of 500 mg/kg body weight, or in combination with an intraperitoneal injection of ABA 30 min after the BHP at a dose of 300 or 600 mg/kg body weight once a week for 5 weeks, and then killed 35 weeks after the commencement of the experiment. ABA exerted inhibitory effects on pancreas and lung carcinogenesis induced by BHP, with mean numbers of lesions (including hyperplasias and carcinomas) being significantly decreased compared with the BHP-alone group values, while no significant effect was observed on liver or gallbladder carcinogenesis. These results suggest that the effects of ABA on carcinogenesis depend on the target organ as well as the chemical carcinogen examined.

Key words: Multiorgan carcinogenesis — Poly(ADP-ribose)polymerase — 3-Aminobenzamide — N-Nitrosobis(2-hydroxypropyl)amine — Hamster

Poly(ADP-ribose)polymerase, a nuclear enzyme activated by nicked DNA, catalyzes the transfer and covalent binding of ADP-ribose units to various acceptor proteins, including the enzyme itself, resulting in the synthesis of homopolymers of poly(ADP-ribose) from nicotinamide adenine dinucleotide (NAD[4]).[1–5] Recently, evidence has accumulated suggesting that poly(ADP-ribosyl)ation is involved in structural changes in chromatin, notably in unfolding of DNA.[6,7] Thus, poly(ADP-ribosyl)ation has been postulated to play roles in a wide variety of important biological processes including DNA repair,[8,9] cell differentiation,[10,11] cell proliferation,[12] heat shock[13] and malignant transformation.[14,15] So far, various inhibitors of poly(ADP-ribose)polymerase, including 3-aminobenzamide (ABA),[16–18] have been used as tools for elucidating the involvement of poly(ADP-ribose)polymerase in various biological processes.

We have been studying the possible relevance of poly(ADP-ribosyl)ation to carcinogenesis *in vivo* using various inhibitors, including ABA.[19–22] Co-administration of the inhibitors with carcinogens in the initiation stage enhanced the induction of preneoplastic and in some cases neoplastic lesions by N-nitrosodiethylamine (DEN) and benzo[*a*]pyrene but not by N-methyl-N-nitrosourea (MNU) and N-nitrosobis(2-hydroxypropyl)amine (BHP) in rat liver.[19–22] Moreover, co-administration of inhibitors with phenobarbital (PB) exerted inhibitory effects on the promoting activity of this xenobiotic in rat liver.[23] Possible involvement of poly(ADP-ribosyl)ation in carcinogenesis has also been reported by other investigators, who found that inhibitors showed enhancing effects on pancreas islet cell carcinogenesis induced by streptozotocin and alloxan in rats,[24,25] on liver tumorigenesis by methylazoxymethanol acetate (MAM) in a fish, medaka (*Oryzias latipes*)[26] and on oral carcinogenesis by dimethylbenz[*a*]anthracene in the hamster cheek pouch,[27] but an inhibitory effect on colon carcinogenesis by MAM in rats.[28] Thus, the influence appears to vary depending upon the carcinogen and target organ.

In the present study, in order to cast further light on tissue specificity, we have adopted a multiorgan carcinogenesis model system where the effects of inhibitors of poly(ADP-ribose)polymerase can be analyzed in many different target organs in the same animal. Thus, the influence of ABA on multiorgan carcinogenesis in hamsters induced by BHP, a potent carcinogen having a wide spectrum of target organs in both rats[29,30] and hamsters,[31,32] was investigated.

MATERIALS AND METHODS

Animals A total of 68 male Syrian golden hamsters (Shizuoka Labolatory Animal Center, Shizuoka), 6 weeks old, weighing approximately 90–110 g, were used.

[3] To whom reprint requests should be sent.

[4] Abbreviations: NAD, nicotinamide adenine dinucleotide; ABA, 3-aminobenzamide; BHP, N-nitrosobis(2-hydroxypropyl)amine; DMSO, dimethylsulfoxide; DEN, N-nitrosodiethylamine; MNU, N-methyl-N-nitrosourea; MAM, methylazoxymethanol acetate; PB, phenobarbital.

The animals were housed, five per plastic cage, in an air-conditioned room at 23°C and 60% humidity under a daily cycle of alternating 12 h periods of light and darkness, and given a commercial stock diet, Oriental MF (Oriental Yeast Co. Ltd., Tokyo) and water *ad libitum*.

Chemicals BHP (Tokyo Kasei Kogyo, Co. Ltd., Tokyo) was dissolved in 0.9% NaCl at a concentration of 250 mg/ml, and ABA (Tokyo Kasei Kogyo, Co. Ltd.) was dissolved in dimethylsulfoxide (DMSO)(Sigma Chemicals Co., USA) at a concentration of 300 mg/ml.

Experimental protocol Hamsters were divided into five groups according to the treatment, as shown in Fig. 1. Group 1 served as a non-initiated control, receiving subcutaneous (s.c.) injections of 0.9% NaCl once a week for 5 weeks. Groups 2 and 3 respectively received s.c. injections of 500 mg/kg body weight of BHP alone or intraperitoneal (i.p.) injections of 600 mg/kg of ABA alone at the same time points. Groups 4 and 5 similarly received s.c. injections of 500 mg/kg of BHP in combination with i.p. injections of ABA at doses of, respectively, 300 or 600 mg/kg body weight 30 min after the BHP administration. Animals were killed under ether anesthesia, after being starved for 16 h, 35 weeks after the beginning of the experiment, and the pancreas, liver, gallbladder and lung tissues were immediately removed.

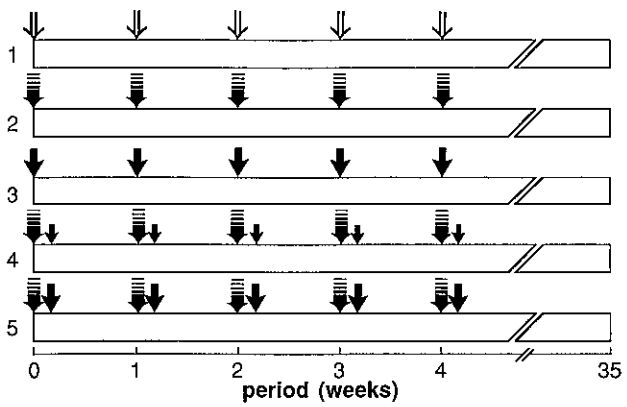

Fig. 1. Experimental protocol. ⇓: 0.9% NaCl, s.c. ⇓: 500 mg/kg BHP, s.c. ↓: 600 mg/kg ABA, i.p. ↓: 300 mg/kg ABA, i.p.

Histopathological examination The pancreas was divided into splenic, gastric and duodenal lobes and fixed with 95% ethanol containing 1% acetic acid for 2–3 h followed by absolute ethanol overnight at 4°C. The liver and gallbladder were removed together and 2–3 slices with gallbladder were taken from each lobe, and fixed in 10% neutral formalin. The lungs were cut serially at 1 mm thickness and fixed in 10% neutral formalin. All tissues were routinely processed and paraffin-enbedded sections were stained with hematoxylin and eosin.

Pancreatic lesions were diagnosed as described previously,[33-35] liver lesions according to the criteria published by the Institute of Laboratory Animal Resources[36] and lung lesions after Boorman.[37] Quantitative data were statistically analyzed using the χ^2 test and Student's t test.

RESULTS

Numbers of animals, body weights and organ weights Animal numbers, body weights, and pancreas, liver and

Table I. Experimental Details

Group No.	Treatment	Number of hamsters		Body weight (g)[a]		Organ weight (g) (g/100 g body wt.)[a]		
		Initial	Effective	Initial	Final	Pancreas	Liver	Lung
1	Saline	10	10	106.3±4.5	145.8±26.1	0.58±0.16(0.40±0.10)	7.31±1.52(5.04±0.43)	1.20±0.25(0.82±0.11)
2	BHP	13	13	107.5±5.8	130.3±22.6	0.61±0.10(0.47±0.06)	6.94±1.80(5.21±0.62)	1.15±0.16(0.90±0.11)
3	ABA (600)	10	10	109.3±6.1	142.9±29.7	0.71±0.27(0.49±0.12)	6.27±2.51(4.84±0.64)	1.17±0.32(0.83±0.20)
4	BHP+ ABA (300)	15	15	106.6±6.9	126.1±16.8	0.59±0.17(0.47±0.11)	7.25±1.12(5.76±0.48)[d,f]	1.26±0.25(1.00±0.19)[e]
5	BHP+ ABA (600)	20	20	106.4±8.4	133.5±18.2	0.58±0.13(0.43±0.08)	7.59±1.26(5.73±0.41)[e,g]	1.29±0.28(0.98±0.19)[b]

a) Data shown are mean±SD values.
b) Significantly different from group 1, $P<0.05$.
c) Significantly different from group 1, $P<0.02$.
d) Significantly different from group 1, $P<0.01$.
e) Significantly different from group 1, $P<0.001$.
f) Significantly different from group 2, $P<0.02$.
g) Significantly different from group 2, $P<0.01$.

Table II. Incidences and Numbers of Pancreatic Lesions

Group No.	Treatment	Effective number of hamsters	Incidence (%)				Mean number of pancreatic lesions[a, b]
			Total pancreatic lesions[a]	Hyperplasias	Atypical hyperplasias	Carcinomas	
1	Saline	10	0 (0)	0 (0)	0 (0)	0 (0)	0.00±0.00
2	BHP	13	13 (100.0)	12 (92.3)	8 (61.5)	5 (38.5)	3.92±2.63
3	ABA (600)	10	0 (0)	0 (0)	0 (0)	0 (0)	0.00±0.00
4	BHP+ABA (300)	15	13 (86.7)	11 (73.3)	4 (26.7)	6 (40.0)	2.60±1.59
5	BHP+ABA (600)	20	18 (90.0)	14 (70.0)	8 (40.0)	5 (25.0)	2.25±1.71[c]

a) Pancreatic lesions comprise hyperplasia, atypical hyperplasia and carcinoma categories.
b) Data shown are mean±SD values.
c) Significantly different from group 2, $P<0.05$.

Table III. Incidences and Numbers of Hepatic and Gallbladder Lesions

Group No.	Treatment	Effective number of hamsters	Incidence of hepatocellular lesions (%)			Mean number of hepatocellular lesions[a, c]	Incidence of cholangiocellular lesions (%)			Mean number of cholangiocellular lesions[b, c]	Incidence of gallbladder lesions or polyps (%)
			Total hepatocellular lesions[a]	Hyperplastic nodules	Hepatocellular carcinomas		Total cholangiocelluar lesions[b]	Ductal proliferations	Cholangiocarcinomas		
1	Saline	10	0 (0)	0 (0)	0 (0)	0.00±0.00	0 (0)	0 (0)	0 (0)	0.00±0.00	0 (0)
2	BHP	13	2 (15.4)	2 (15.4)	0 (0)	0.15±0.38	3 (23.1)	3 (23.1)	0 (0)	0.23±0.44	0 (0)
3	ABA (600)	10	0 (0)	0 (0)	0 (0)	0.00±0.00	0 (0)	0 (0)	0 (0)	0.00±0.00	0 (0)
4	BHP+ ABA (300)	15	4 (26.7)	2 (13.3)	1 (6.7)	0.27±0.59	3 (20.0)	2 (13.3)	1 (6.7)	0.20±0.41	0 (0)
5	BHP+ ABA (600)	20	6 (30.0)	5 (25.0)	1 (5.0)	0.30±0.57	2 (10.0)	2 (10.0)	0 (0)	0.10±0.31	3 (15.0)

a) Hepatocellular lesions comprise hyperplastic nodule, and hepatocellular carcinoma categories.
b) Cholangiocellular comprise ductal proliferation and cholangiocarcinoma categories.
c) Data shown are mean±SD values.

Table IV. Incidences and Numbers of Lung Lesions

Group No.	Treatment	Effective number of hamsters	Incidence (%)				Mean number of lung lesions[a, b]
			Total lung lesions[a]	Hyperplasias	Adenomas	Adenocarcinomas	
1	Saline	10	0 (0)	0 (0)	0 (0)	0 (0)	0.00±0.00
2	BHP	13	11 (84.6)	11 (84.6)	0 (0)	1 (7.7)	2.23±1.24
3	ABA (600)	10	0 (0)	0 (0)	0 (0)	0 (0)	0.00±0.00
4	BHP+ABA (300)	15	10 (66.7)	9 (60.0)	1 (6.7)	0 (0)	1.13±0.92[c]
5	BHP+ABA (600)	20	9 (45.0)	9 (45.0)	1 (5.0)	0 (0)	0.75±0.97[d]

a) Lung lesions comprise hyperplasia, adenoma and adenocarcinoma categories.
b) Data shown are mean±SD values.
c) Significantly different from group 2, $P<0.02$.
d) Significantly different from group 2, $P<0.001$.

lung weights are given in Table I. BHP treatment with or without ABA caused a slight retardation of growth, although this was not statistically significant. ABA itself exhibited no effect on animal growth. Liver weights in groups 4 and 5 were significantly increased in absolute values and relative to body weight, as compared with either group 1 or 2, in line with tumor development.

Effects of ABA on the induction of pancreatic, liver and lung lesions by BHP Histopathology results are summarized in Tables II, III and IV. BHP induced pre-

neoplastic and neoplastic lesions in the pancreas, liver and lung, in line with previous reports,[31, 32] while ABA itself induced no lesions in these organs. Neither 300 nor 600 mg/kg body weight of ABA exerted significant effects on the incidences of pancreatic ductal cell hyperplasia, atypical hyperplasia or adenocarcinomas induced by BHP, but the 600 mg/kg body weight dose was associated with a decrease in the mean number of total pancreatic lesions. The 300 mg/kg body weight dose also exhibited a similar tendency. ABA exerted no significant effects on the incidences or mean numbers of hepatocellular lesions, including hyperplastic nodules and carcinomas, and cholangiocellular lesions, including ductal proliferations and carcinomas, induced by BHP. BHP itself induced no gallbladder lesions, but in combination with the high dose of ABA induced polyps. ABA dose-dependently decreased the mean total numbers of lung lesions, including hyperplasias, adenomas and adenocarcinomas, induced by BHP, without exerting any significant effects on their incidences.

DISCUSSION

In the present investigation, ABA exerted inhibitory effects on the induction by BHP of preneoplastic and neoplastic lesions in the pancreas and lung in hamsters, with the mean total numbers of lesions, but not their incidences, being significantly decreased. No significant effects were exerted regarding hepatocellular or cholangiocellular lesions. The present results thus indicate that the influence of ABA on tumorigenesis depends upon the target organ, even when a single chemical carcinogen is employed.

Since, in the present study, ABA was given 30 min after BHP administration, when DNA repair was presumably taking place, it is conceivable that ABA affected the repair process. Although BHP has been reported to induce mainly methylation of DNA including 7-methylguanine and O^6-methylguanine,[38] DNA adducts formed in different target organs have not been fully analyzed. Another BHP-related β-oxidized derivative of di-*n*-propylnitrosamine, N-nitrosobis(2-oxopropyl)-amine, reportedly induces both qualitatively and quantitatively different amounts of DNA adducts, mainly methylation products, in the pancreas, liver and lung target organs in hamsters.[39] Thus, the present differential effects of ABA on the different target organs might partly be ascribed to the extent or type of DNA adducts formed, raising the question of whether involvement of poly(ADP-ribosyl)ation depends on the particular DNA repair process. Thus, ABA might have exerted differential modulations of either initiating or promoting activity of BHP in different target organs, since in the present experiment BHP was repeatedly administered.

An involvement of poly(ADP-ribosyl)ation in DNA repair processes has long been postulated, based upon evidence that inhibitors of poly(ADP-ribose)polymerase enhance repair replication, DNA single strand break frequency, sister chromatid exchange, cytotoxicity, mutation and transformation by DNA-damaging agents.[8-15] Nevertheless, it still remains unclear which types of DNA damage require poly(ADP-ribosyl)ation for their repair, since the effects of the inhibitors on these phenomena vary depending on the DNA-damaging agents and cell types used.[19-28, 40] The involvement of poly(ADP-ribosyl)-ation in repair has been postulated to depend upon production of nicks in DNA. Recently, direct evidence of a role in resealing DNA strand breaks has been provided by cells overexpressing poly(ADP-ribose)polymerase after cDNA recombination.[12] Further, it has been shown that depletion of poly(ADP-ribose) in cultured hepatocytes is associated with inhibition of N-acetoxy-2-acetylaminofluorene DNA adduct excision along with a disturbance in refolding of DNA, though there is no effect on the repair patch synthesis.[41] However, in spite of wide support for the hypothesis that poly(ADP-ribose)polymerase is involved in repair of monoalkyl ating agent-induced DNA damage,[8, 9] no direct evidence for this has been reported so far. In our previous study in Fischer 344 and Wistar strains of rat liver, ABA exerted enhancing effects on initiation by DEN, an ethylating agent, and benzo[*a*]pyrene, which forms bulky DNA adducts, but had no effect on that by the methylating agents, MNU and BHP. With another methylating agent, 1,2-dimethylhydrazine (DMH), a strain difference was observed, enhancement occurring in the Wistar rat but not in the Fischer rat.[19-22] Thus, although the present finding of no significant effect of ABA on the development of hepatocellular lesions is in line with our previous report,[21] the involvement of poly(ADP-ribosyl)ation in repair in the case of methylating agents remains unclear.

A recent report that effects of inhibitors, including ABA, on poly(ADP-ribose)polymerase in cultured cells vary depending on the concentration used, being inhibitory in the μM range but stimulatory in the nM range,[42] might provide an explanation for the conflucting results concerning DNA repair. The lack of any increased induction of poly(ADP-ribose)polymerase gene expression following X-ray, UV or methylating agent exposure in cultured cells provides support for the view that existing enzyme activity rather than *de novo* expression of the enzyme is important for the repair process.[43] Previously we reported that i.p. administration of ABA at a dose of 600 mg/kg body weight gave rise to approximately 4.5 and 1.3 mM concentrations of ABA in the rat liver after 5 min and 4 h, respectively.[44] Further studies on the intracellular concentration of ABA in different target organs in hamsters are warranted.

Since, in contrast to our previous study, in the present experiment BHP and ABA were repeatedly administered over 5 weeks, ABA might have affected the promotion stage, in which initiated cells grow into preneoplastic lesions. In this context, the recent report of involvement of poly(ADP-ribosyl)ation in cell proliferation is worth noting, expression of poly(ADP-ribose)polymerase gene having been found to increase in mid to late S phase.[12] ABA reportedly exerts inhibitory effects on DNA synthesis in malignant cells.[45] Further, 1,2-benzopyrone and benzamide can inhibit tumorigenic growth of transformed cells, transfected with the steroid-inducible EJ-*ras* gene, accompanied with an inhibition of poly(ADP-ribose)polymerase.[46] Moreover, evidence has been accumulating of an involvement of poly(ADP-ribosyl)ation in gene expression and differentiation, probably through effects on structural changes in chromatin, notably unfolding of DNA.[6, 7, 12] Thus, potential for influence during the promotion stage is considerable. Reccent success in cloning of cDNA of poly(ADP-ribose)polymerase has made possible a more precise and mechanistic approach to the poly(ADP-ribosyl)ation research field.[12] Thus, differential extents of expression of poly(ADP-ribose)polymerase gene in different organs in mice and rats have been reported.[47, 48]

In conclusion, repeated co-administration of ABA, an inhibitor of poly(ADP-ribose)polymerase, with BHP, a potent carcinogen with a wide spectrum of target organs, exerts differential effects on the induction of preneoplastic and neoplastic lesions depending upon the organ, inhibitory effects being observed in the hamster pancreas and lung but not liver.

ACKNOWLEDGMENTS

We thank H. Megumi and Y. Kawai for their assistance in the preparation of the manuscript. The work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, and by a Grant-in-Aid from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control, Japan.

(Received February 8, 1991/Accepted April 30, 1991)

REFERENCES

1) Sugimura, T. Poly(adenosine-diphosphate-ribose). *Prog. Nucleic Acid Res. Mol. Biol.*, **13**, 127–151 (1973).
2) Hilz, H. and Stone, P. R. Poly(ADP-ribose) and ADP-ribosylation of proteins. *Rev. Physiol. Biochem. Pharmacol.*, **76**, 1–59 (1976).
3) Hayaishi, O. and Ueda, K. Poly(ADP-ribose) and ADP ribosylation of proteins. *Annu. Rev. Biochem.*, **46**, 95–116 (1977).
4) Purnell, M. R., Stone, P. R. and Wish, W. J. D. ADP-ribosylation of nuclear proteins. *Biochem. Soc. Trans.*, **8**, 215–217 (1980).
5) Sugimura, T. and Miwa, M. Poly(ADP-ribose) and cancer research. *Carcinogenesis*, **4**, 1503–1506 (1983).
6) Althaus, F. R., Mathis, G., Alvarez-Gonzalez, R., Loetscher, P. and Mattenberger, M. ADP-ribosylation and chromatin function. *In* "ADP-Ribose Tranfer Reactions. Mechanisms and Biological Significance," ed. M. K. Jacobson and E. L. Jacobson, pp. 151–157 (1989). Springer-Verlag, New York.
7) Sauermann, G. and Wesierska-Gadek, J. A proposed molecular mechanism of nucleosome unfolding. Effect of poly(ADP-ribose) on DNA-histone H4 interaction. *In* "ADP-Ribose Transfer Reactions. Mechanisms and Biological Significance," ed. M. K. Jacobson and E. L. Jacobson, pp. 179–183 (1989). Springer-Verlag, New York.
8) Shall, S. ADP-ribose in DNA repair: a new component of DNA excision repair. *Adv. Radiat. Biol.*, **11**, 1–69 (1984).
9) 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).
10) 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).
11) Farzaneh, F., Zalin, R., Brill, D. and Shall, S. DNA strand break and ADP-ribosyl transferase activation during cell differentiation. *Nature*, **300**, 362–366 (1982).
12) Smulson, M., Alkhatib, H., Bhatia, K., Chen, D., Cherney, B., Notario, V., Tahhourdin, C., Dritschilo, A., Hensley, P., Breiyman, T., Stein, G., Pommier, Y., McBride, O. W., Bustin, M. and Giri, C. The cloning of the cDNA and the gene for human poly(ADP-ribose)polymerase: status on the biological function(s) using recombinant probes. *In* "ADP-Ribose Transfer Reactions. Mechanisms and Biological Significance," ed. M. K. Jacobson and E. L. Jacobson, pp. 463–477 (1989). Springer-Verlag, New York.
13) Duran-Torres, G., Juarez-Salinas, H. and Jacobson, M. K. Alterations of poly(ADP-ribose) metabolism induced by hyperthermia. *Fed. Proc.*, **43**, 2064 (1984).
14) 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).
15) 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).
16) Purnell, M. R. and Whish, W. J. D. Novel inhibitors of poly(ADP-ribose) synthetase. *Biochem. J.*, **185**, 775–777 (1980).
17) Ueda, K., Kawaichi, M. and Hayaishi, O. Poly(ADP-ribose) synthetase. *In* "ADP-Ribosylation Reactions," ed. O. Hayaishi and K. Ueda, pp. 117–155 (1982). Academic Press, New York.
18) Banasik, M., Komura, H., Saito, I., Abed, N. A. N. and Ueda, K. New inhibitors of poly(ADP-ribose) synthetase. *In* "ADP-Ribose Transfer Reactions. Mechanisms and Biological Significance," ed. M. K. Jacobson and E. L. Jacobson, pp. 130–133 (1989). Springer-Verlag, New York.
19) Takahashi, S., Ohnishi, T., Denda, A. and Konishi, Y. Enhancing effect of 3-aminobenzamide on induction of γ-glutamyltranspeptidase-positive foci in rat liver. *Chem.-Biol. Interact.*, **39**, 363–368 (1982).
20) 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).
21) Denda, A., Tsutsumi, M., Yokose, Y., Eimoto, H. and Konishi, Y. Effect of 3-aminobenzamide on the induction of γ-glutamyltranspeptidase-positive foci by various chemicals in rat liver. *Cancer Lett.*, **39**, 29–36 (1988).
22) Konishi, Y., Denda, A., Tsutsumi, M., Nakae, D. and Takahashi, S. Effect of 3-aminobenzamide on the induction of γ-glutamyltranspeptidase positive foci in the liver of rats treated with various chemical carcinogens. *In* "ADP-Ribose Transfer Reactions. Mechanisms and Biological Significance," ed. M. K. Jacobson and E. L. Jacobson, pp. 242–244 (1989). Springer-Verlag, New York.
23) 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).
24) Rakieten, N., Gordon, B. S., Beaty, A., Cooney, D. A., Davis, R. D. and Schein, P. S. Pancreatic islet cell tumors produced by the combined action of streptozotocin and nicotinamide. *Proc. Soc. Exp. Biol. Med.*, **137**, 280–283 (1971).
25) Yamagami, T., Miwa, A., Takasawa, S., Yamamoto, H. and Okamoto, H. Induction of rat pancreatic B-cell tumors by the combined administration of streptozotocin or alloxan and poly(adenosine diphosphate ribose)synthetase inhibitors. *Cancer Res.*, **45**, 1845–1849 (1985).
26) Miwa, M., Ishikawa, T., Kondo, T., Takayama, S. and Sugimura, T. Enhancement by 3-aminobenzamide of methylazoxymethanol acetate-induced hepatoma of the small fish "Medaka" (*Oryzias latipes*). *In* "ADP-Ribosylation of Proteins," ed. F. R. Althaus, H. Hilz and S. Shall, pp. 480–483 (1985). Springer-Verlag, Berlin-Heidelberg-New York-Tokyo.
27) Miller, E. G., Rivera-Hidalgo, F. and Binnie, W. H. 3-Methoxybenzamide, a possible initiator for DMBA-induced carcinogenesis. *In* "ADP-Ribose Transfer Reactions. Mechanisms and Biological Significance," ed. M. K. Jacobson and E. L. Jacobson, pp. 287–290 (1989). Springer-Verlag, New York.
28) Nakagawa, 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).
29) Konishi, Y., Denda, A., Kondo, H. and Takahashi, S. Lung carcinomas induced by oral administration of N-bis-(2-hydroxypropyl)nitrosamine in rats. *Gann*, **67**, 773–780 (1976).
30) Konishi, Y., Ikeda, T., Kawabata, A., Yoshimura, H. and Mikami, R. Induction of rat lung carcinoma by a single injection of N-bis(2-hydroxypropyl)nitrosoamine. *Gann*, **69**, 855–856 (1978).
31) Pour, P., Kruger, F. W., Althoff, J., Cardesa, A. and Mohr, U. Effect of beta-oxidized nitrosamines on Syrian hamsters. III. 2,2′-Dihydroxy-di-n-propylnitrosamine. *J. Natl. Cancer Inst.*, **54**, 141–146 (1975).
32) Gingell, R., Wallcave, L., Nagel, D., Kupper, R. and Pour, P. Metabolism of the pancreatic carcinogens N-nitroso-bis(2-oxopropyl)amine and N-nitroso-bis(2-hydroxypropyl)amine in the Syrian hamsters. *J. Natl. Cancer Inst.*, **63**, 181–190 (1976).
33) Pour, P. M. and Wilson, R. B. Experimental tumors of the pancreas. *In* "Tumors of the Pancreas," ed. A. R. Mossa, pp. 37–158 (1980). Williams & Wilkins, Baltimore.
34) Scarpelli, D. G., Rao, M. S. and Subbarao, V. Augmentation of carcinogenesis by N-nitrosobis(2-oxopropyl)amine administered during S phase of the cell cycle in regenerating hamster pancreas. *Cancer Res.*, **43**, 611–616 (1983).
35) Mizumoto, K., Kitazawa, S., Eguchi, T., Tsutsumi, M., Ito, S., Denda, A. and Konishi, Y. Modulation of N-nitrosobis(2-hydroxypropyl)amine induced carcinogenesis by clofibrate in hamsters. *Carcinogenesis*, **9**, 1421–1425 (1988).
36) Squire, R. A. and Levitt, M. H. Report of a workshop on classification of specific hepatocellular lesions in rats. *Cancer Res.*, **35**, 3214–3223 (1975).
37) Boorman, G. A. Bronchiolar/alveolar carcinomas. *In* "Respiratory System," ed. T. C. Jones, U. Mohr and R. D. Hund, pp. 112–116 (1985). Springer-Verlag, Berlin.
38) Lijinsky, W., Saavedra, J. E. and Kovatch, R. M. Carcinogenesis and nucleic acid alkylation by some oxygenated nitrosamines in rats and hamsters. *Chem.-Biol. Interact.*, **66**, 37–47 (1988).
39) Lawson, T. A., Gingell, R., Nagel, D., Hines, L. A. and Ross, A. Methylation of hamster DNA by the carcinogen N-nitrosobis(2-oxopropyl)amine. *Cancer Lett.*, **11**, 251–255 (1981).
40) Althaus, F. R. and Richter, C. H. Poly-ADP-ribosylation

in the recovery of mammalian cells from DNA damage. *In* "ADP-Ribosylation of Proteins," pp. 66–92 (1987). Springer-Verlag, Berlin-Heidelberg.
41) Mathis, G. and Althaus, F. R. Uncoupling of DNA excision repair and chromatin rearrangement in poly-(ADP-ribose)-depleted cells. *In* "ADP-Ribose Transfer Reactions. Mechanisms and Biological Significance," ed. M. K. Jacobson and E. L. Jacobson, pp. 218–221 (1989). Springer-Verlag, New York.
42) Jones, J., Patel, B. N. and Skidmore, C. J. Benzamide can stimulate as well as inhibit the activity of nuclear ADP-ribosyltransferase. *Carcinogenesis*, **9**, 2023–2026 (1988).
43) Bhatia, K., Pommier, Y., Fornace, A., Breitman, T., Cherney, B., Giri, C. and Smulson, M. The biological function(s) of poly(ADP-ribose)polymerase as observed by transient expression of the cDNA and transcriptional regulation of the gene. *In* "ADP-Reactions. Mechanisms and Biological Significance," ed. M. K. Jacobson and E. L. Jacobson, pp. 494–501 (1989). Springer-Verlag, New York.
44) 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).
45) Lonn, U. and Lonn, S. Accumulation of 10-kilobase DNA replication intermediates in cells treated with 3-aminobenzamide. *Proc. Natl. Acad. Sci. USA*, **82**, 104–108 (1985).
46) Tseng, A., Jr., Lee, W. M. F., Kirsten, E., Hakam, A., McLick, J., Buki, K. and Kun, E. Prevention of tumorigenesis of oncogene-transformed rat fibroblasts with DNA site inhibitors of poly(ADP-ribose) polymerase. *Proc. Natl. Acad. Sci. USA*, **84**, 1107–1111 (1987).
47) Agemori, M., Kagamiyama, H., Nishikimi, M. and Shizuta, Y. Purification and properties of poly(ADP-ribose) synthetase from mouse testicle. *Arch. Biochem. Biophys.*, **215**, 621–627 (1982).
48) Ogura, T., Takenouchi, N., Yamaguchi, M., Matsukage, A., Sugimura, T. and Esumi, H. Striking similarity of the distribution patterns of the poly(ADP-ribose)polymerase and polymerase β among various mouse organs. *Biochem. Biophys. Res. Commun.*, **172**, 377–384 (1990).