Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 243–247, January 1984
Medical Sciences

Inhibition of malignant transformation *in vitro* by inhibitors of poly(ADP-ribose) synthesis

(3-aminobenzamide/alkylating agents/ultraviolet light/x-rays/DNA breaks)

CARMIA BOREK*, WILLIAM F. MORGAN†, AUGUSTINUS ONG*, AND JAMES E. CLEAVER†

*Radiation Research Laboratory, Department of Radiology, Columbia University College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10031; and †Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143

Communicated by Roscoe O. Brady, August 29, 1983

ABSTRACT **Malignant transformation *in vitro* of hamster embryo cells and mouse C3H 10T½ cells by x-rays, ultraviolet light, and chemical carcinogens was inhibited by benzamide and by 3-aminobenzamide at concentrations that are specific for inhibition of poly(ADP-ribose) formation. These compounds slow the ligation stage of repair of x-ray and alkylation damage but not of ultraviolet light damage. At high concentrations they also inhibited *de novo* synthesis of DNA purines and DNA methylation by *S*-adenosylmethionine. The suppression of transformation by the benzamides is in striking contrast to their reported effectiveness in enhancing sister chromatid exchange, mutagenesis, and killing in cells exposed to alkylating agents. Our results suggest that mechanisms regulating malignant transformation are different from those regulating DNA repair, sister chromatid exchange, and mutagenesis and may be associated with changes in gene regulation and expression caused by alterations in poly(ADP-ribosyl)ation.**

Malignant transformation of cells *in vitro* by radiation or chemical carcinogens is a reproducible quantitative system for studying the mechanisms of carcinogenesis (1–7). Although fixation of transformation and mutagenesis involves DNA metabolism and cell replication (2, 4–6), the numbers and kinds of genes involved are not known. Poly(ADP-ribose) is an important cellular regulatory molecule (8, 9) and its synthesis can be inhibited by 3-aminobenzamide (3ABzA) and benzamide (BzA). We therefore investigated the effects of these inhibitors on transformation by a variety of DNA-damaging agents with careful comparison to their effects on DNA repair and nucleotide precursor pathways over a range of concentrations. These agents, at concentrations below those at which side effects were evident, inhibited malignant transformation *in vitro*, in contrast to their reported enhancement of sister chromatid exchange (10–12) and mutagenesis (13) in cells exposed to radiation and chemical carcinogens. We have therefore identified the biochemical nature of a common regulatory step in radiation and chemical carcinogenesis that is subject to experimental manipulation and that may aid in defining mechanisms of carcinogenesis.

MATERIALS AND METHODS

Transformation. Minced midterm whole embryos from golden hamsters (Lakeview, Wilmington, MA) were used as a source of normal cells (1–4). Primary cultures were established by progressive dissociation of minced fresh tissue. For transformation experiments 10^3 cells were cloned onto 60-mm Petri dishes on x-irradiated (40 Gy; 1 Gy = 1 J/kg = 100 rads) syngeneic feeder cells (2) in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (50 units/ml), and streptomycin (50 μg/ml; GIBCO) (1–4).

Mouse embryo fibroblast cells C3H 10T½ (clone 8), originally obtained from C. Heidelberger (University of Southern California, Los Angeles), were treated as described (3, 7). Stock cultures were maintained at 37°C, aerated with 5% CO_2 in air, in Eagle's basal medium containing 10% heat-inactivated fetal calf serum, penicillin (50 units/ml), and streptomycin (50 μg/ml).

At 24 hr after plating, cells were irradiated with a Siemens 300-kVp constant-potential generator with an additional filter of 0.2 mm of Cu at room temperature with 3 or 4 Gy of x-rays at a dose rate of 0.322 Gy/min and incubated at 37°C in 5% CO_2/95% air with weekly changes of medium. After a 10-day incubation period for the hamster cells (1, 2) and 6 weeks for the 10T½ cells (2, 3, 7), cultures were fixed and stained with Giemsa stain. Assays for cell survival, cloning efficiency, and transformation were carried out as described previously for the hamster cells (1–4) and for the C3H 10T½ cells (2, 3, 7). For experiments with UV light, C3H 10T½ cells were exposed to 10–13 J/m^2 (254 nm, dose rate of 0.15–1.3 J/m^2·sec).

For experiments with alkylating agents, 24-hr cloned cultures of hamster embryo C3H 10T½ cells were treated with 3.4–34 μM *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG; Aldrich) for 30 min to 4 hr or with 0.15 mM methyl methanesulfonate ($MeSO_2OMe$; ICNS Pharmaceutical, Plainview, NY) for 1 hr. After treatment, cells were washed and refed with fresh medium. Survival and transformation were evaluated for both cell systems as described.

Stock solutions of BzA and 3ABzA (Sigma) were prepared in ethanol or water and added to cultures to give a final concentration of 1 mM or more. Each agent was added promptly after exposure of cells to x-rays or UV or immediately after the removal of the alkylating agents. In some experiments cytosine arabinonucleoside (20 μM) was added after exposure to alkylating agents. Cells were maintained in medium containing BzA or 3ABzA for 1 week, after which the medium was replaced with fresh medium.

In hamster embryo cells a differential count was made of normal and transformed colonies. Transformed colonies were identified by their irregular growth pattern and their tendency to form multilayers as compared to controls (1–4). The relationship between this morphology and the malignant nature of the cells has been well documented (1, 2). Both normal and transformed colonies were scored so that both transformation frequency and cell survival could be assessed within the same experiment. In C3H 10T½ cells transformation was scored as described (7). Both type II and type III foci were scored as transformed.

Labeling Studies. The incorporation of various precursors into DNA in the presence of 3ABzA was determined by growing cells continuously for 3 days in [^{14}C]thymidine (0.01 μCi/ml; specific activity 56 μCi/mmol; 1 Ci = 37 GBq) and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BzA, benzamide; 3ABzA, 3-aminobenzamide; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; $MeSO_2OMe$, methyl methanesulfonate.

Table 1. Transformation of mouse C3H 10T½ cells irradiated with x-rays or UV light or exposed to alkylating agents and grown in BzA (1 mM) or 3ABzA (1 mM) for 7 days, starting immediately after exposure

Damaging agent	Drug	Surviving cells*	Transformation frequency × 10^4
None	None	(15.0 ± 0.2%)	0
	BzA	0.98 ± 0.08	0
	3ABzA	0.96 ± 0.07	0
X-rays, 4 Gy	None	0.70 ± 0.11	10.3 ± 2.1
	BzA	0.60 ± 0.11	4.91 ± 0.6
	3ABzA	0.62 ± 0.12	0.584 ± 0.4
UV light, 8.6 J/m^2	None	0.68 ± 0.13	9.97 ± 2.17
	BzA	0.72 ± 0.10	2.41 ± 1.07
	3ABzA	0.75 ± 0.11	2.83 ± 1.15
$MeSO_2OMe$, 0.15 mM	None	0.59 ± 0.14	5.90 ± 1.43
	BzA	0.67 ± 0.11	1.94 ± 0.79
	3ABzA	0.61 ± 0.18	1.09 ± 0.62
MNNG, 3.4 μM	None	0.47 ± 0.13	10.9 ± 2.0
	BzA	0.41 ± 0.10	3.00 ± 1.06
	3ABzA	0.39 ± 0.10	2.83 ± 1.06

Results are given as mean ± SEM.
*Number of colonies counted/(number of cells plated × plating efficiency). Plating efficiency (shown in parentheses for no damaging agent and no drug) = (number of colonies counted/number of cells plated) × 100.

then labeling cells for 4 hr in [^{3}H]thymidine (1 μCi/ml; 80 Ci/mmol), [^{3}H]hypoxanthine (10 μCi/ml; 3 Ci/mmol), [^{3}H]methionine (10 μCi/ml; 80 Ci/mmol), or *S*-adenosyl-[^{3}H]methionine (100 μCi/ml; 80 Ci/mmol). In another series of experiments, unlabeled cultures were grown for 4 hr in [^{14}C]glucose (0.1 μCi/ml, 0.35 Ci/mol). DNA was isolated as described (14) and the specific activity of the DNA was determined by the ratio of ^{3}H to either ^{14}C or A_{260}. The effect of various concentrations of 3ABzA was expressed as the specific activity of the labeled DNA relative to that obtained without incubation with 3ABzA.

Synthesis of poly(ADP-ribose) in [^{14}C]thymidine-labeled cells damaged by $MeSO_2OMe$ (5 mM) was measured by gentle permeabilization with lysophosphatidylcholine (0.3 mg/ml in 150 mM sucrose/80 mM KCl/35 mM Tris·HCl, pH 7.4, for 10 min at 4°C) followed by resuspension in [^{3}H]nicotinamide adenine dinucleotide (5 μCi/ml, 2 mCi/mmol) in 100 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$/2.5 mM dithiothreitol/0.5 mM phenylmethanesulfonyl fluoride containing various concentrations of 3ABzA for 30 min at 37°C. At the end of the incubation, the cells were fixed in 10% perchloric acid and the ^{3}H/^{14}C ratios were determined from protein-bound radioactivity collected on glass fiber filters. The effect of 3ABzA on poly(ADP-ribose) synthesis was calculated from changes in the ^{3}H/^{14}C ratios expressed as a percentage of the value obtained in the absence of 3ABzA. Measuring poly(ADP-ribose) synthesis in this way favors the stable histone-bound ADP-ribose residues and may underestimate the ephemeral long polymer (8, 9).

DNA Repair. Repair replication was measured by 5-bromodeoxyuridine (BrdUrd; 10 μM) substitution on alkaline isopycnic gradients as described (14).

For alkaline elution (15) approximately 4 × 10^5 [^{14}C]thymidine-labeled cells, treated as described, were mixed with an equal number of [^{3}H]thymidine-labeled cells (the internal standards) and collected on 25-mm-diameter, 2-μm pore size polyvinylchloride filters (Millipore) and washed twice with cold phosphate-buffered saline. The [^{3}H]thymidine-labeled cells contained a known number of single-strand breaks that resulted from irradiation with 1.5 Gy of x-rays from a General Electric Maxitron 300 (300 kVp; 20 mA; nominal half value layer, 2.0 mm Cu). The cells were lysed on the filter with 5 ml of Sarkosyl/NaCl/EDTA lysis solution (pH 10.0) and elution was carried out in the dark with a solution of 0.02 M EDTA plus tetrapropylammonium hydroxide (Kodak, 10% in water), pH 12.2, at a flow rate of 2 ml/hr (0.03 ml/min), as previously described (15, 16).

Table 2. Transformation of Syrian hamster embryo cells exposed to x-rays or alkylating agents and grown in BzA (1 mM) or 3ABzA (1 mM) for 7 days, starting immediately after exposure

Damaging agent	Drug	Surviving cells*	Transformation frequency × 10^3
None	None	(4.80 ± 0.3%)	0
	BzA	0.97 ± 0.08	0
	3ABzA	0.96 ± 0.08	0
X-rays, 3 Gy	None	0.66 ± 0.13	11.2 ± 0.10
	BzA	0.56 ± 0.14	2.1 ± 0.16
	3ABzA	0.59 ± 0.11	1.2 ± 0.09
MNNG, 3.4 μM	None	0.55 ± 0.14	8.8 ± 0.17
	BzA	0.50 ± 0.13	4.1 ± 0.10
	3ABzA	0.49 ± 0.13	1.9 ± 0.09

Results are given as mean ± SEM.
*Number of colonies counted/(number of cells plated × plating efficiency). Plating efficiency (shown in parentheses for no damaging agent and no drug) = (number of colonies counted/number of cells plated) × 100.

The results were presented as the fraction of ^{14}C-labeled DNA retained on the filter versus the fraction of ^{3}H-labeled DNA retained on the filter for each sample to obtain an elution profile. The elution curves were calibrated by obtaining a family of standard curves from ^{14}C-labeled cells irradiated with 1–10 Gy of x-rays; since strand breaks are a linear function of x-ray dose, an elution curve from cells exposed to experimental treatments could be expressed as equivalent to a number of Gy (16, 17).

RESULTS

Effect of BzA and 3ABzA on Transformation Frequencies. Transformation frequencies in mouse and hamster cells ex-

Table 3. Effect of 30-min exposure to various 3ABzA concentrations on the synthesis of poly(ADP-ribose) and the single-strand break frequencies in $MeSO_2OMe$-damaged C3H 10T½ cells

3ABzA, mM	Inhibition of poly(ADP-ribose) synthesis*	Strand break frequencies	
		Equivalent Gy†	Fractional effect‡
0	0.00	2.57 ± 0.05	0.00
0.05	0.31 ± 0.10	2.55 ± 0.09	0.01 ± 0.04
0.1	0.41 ± 0.06	2.88 ± 0.05	0.13 ± 0.02
0.5	0.57 ± 0.10	3.23 ± 0.12	0.28 ± 0.05
1.0	0.73 ± 0.11	3.48 ± 0.28	0.39 ± 0.12
2.0	0.93 ± 0.08	3.68 ± 0.14	0.47 ± 0.07
5.0	0.97 ± 0.03	4.93 ± 0.17	1.00

Results are given as mean ± SEM.
*Fraction by which [^{3}H]NAD incorporation is suppressed, when measured over a 30-min period in permeabilized cells.
†Determined from a family of elution curves generated by irradiation of C3H 10T½ cells with 1–9 Gy.
‡Calculated to allow the degree of inhibition of poly(ADP-ribose) synthesis compared to the effect on strand break frequencies. The full increase in strand break frequencies of 2.36 Gy is set at 1.00 and the fractional effect is calculated as the increase in observed strand breaks as a fraction of the maximal increase.

posed to radiation or chemicals at various doses were in the range of 10^{-4} to 10^{-2} foci per survivor and were higher in the hamster cells (Tables 1 and 2). BzA (1 mM) and 3ABzA (1 mM) had no influence alone but reduced transformation frequencies in both cell types for each damaging agent, with no significant effects on cell survival, unlike the increased toxicity seen in other cell types (18, 19) (Tables 1 and 2). BzA and 3ABzA had essentially the same effect on transformation frequencies except for C3H 10T½ cells irradiated with x-rays (Table 1), in which BzA was more effective. The concentration (1 mM) used in these experiments was carefully chosen to maximize the effects on poly(ADP-ribose) synthesis while minimizing other effects. This choice will be justified subsequently, where emphasis will be given to 3ABzA in view of its predominant use in poly(ADP-ribose) studies.

Effects of 3ABzA on Poly(ADP-ribose) Synthesis. With a dose of $MeSO_2OMe$ (5 mM) that we have previously found to maximally stimulate poly(ADP-ribose) synthesis by this form of damage (17), the effect of a range of doses of 3ABzA on poly(ADP-ribose) synthesis was determined (Table 3). Synthesis was reduced by 50% at approximately 0.25 mM, by 73% at 1 mM, and completely at ≥2 mM (Table 3). The values found with BzA were very similar; synthesis was reduced by 85% at 1 mM and eliminated completely at higher doses. These values resemble those reported in many cellular systems and define the effectiveness of 3ABzA and BzA in our experiments.

Effect of 3ABzA on DNA Repair. When cells are exposed to DNA-damaging agents, single-strand breaks are produced in the DNA. The frequency of breaks represents a dynamic balance between rates of direct and enzymatic strand breakage and rates of ligation (16–20). A representative example of strand break measurements in C3H 10T½ cells is shown in Fig. 1. At early times after exposure to x-rays and alkylating agents, a relatively high frequency of breaks was observed as a rapid rate of elution from filters in the alkaline elution assay (Fig. 1). Breaks associated with excision repair of UV damage were also observed, but at a lower frequency than we have observed in human cells (16, 17). When cells were incubated after exposure the break frequency declined as damaged sites were progressively excised from the DNA (Fig. 1).

These measurements were done at a high concentration of 3ABzA (5 mM), at which we observed a maximal increase in strand break frequencies in x-ray-damaged and alkylated cells, and they emphasize the absence of any effect of even these high concentrations of 3ABzA on strand breaks in UV-damaged cells. 3ABzA at a concentration of 1 mM had no effect on strand breakage frequencies in x-ray-damaged cells. The strand break frequencies in $MeSO_2OMe$-damaged

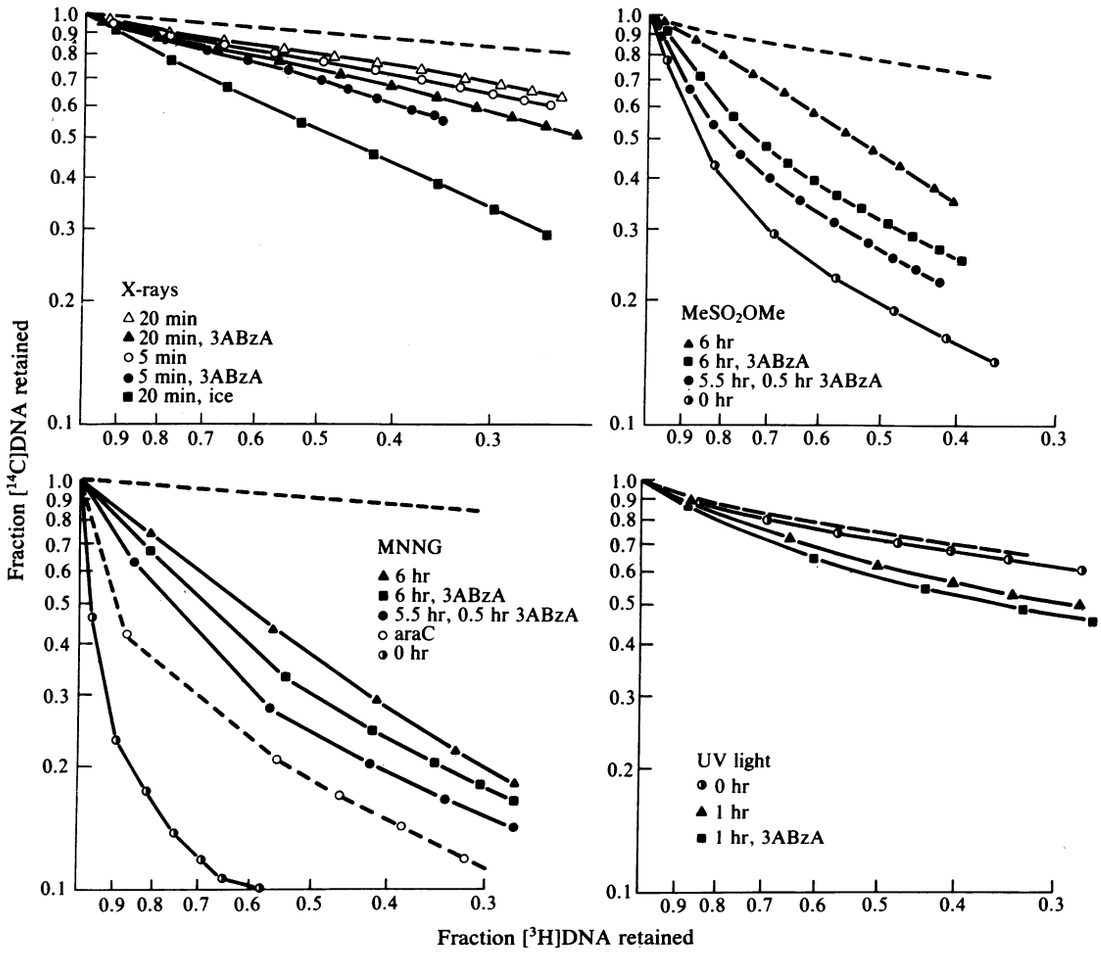

FIG. 1. Alkaline elution profiles of C3H 10T½ cells exposed to x-rays, $MeSO_2OMe$, MNNG, or UV light and grown for various periods in 3ABzA (5 mM whenever present). Broken lines are controls of cells not irradiated or exposed to carcinogens; controls with and without 3ABzA were the same. (*Upper Left*) Cells exposed to 5 Gy of x-rays and grown as follows: ○, 5 min; ●, 5 min with 3ABzA; △, 20 min; ▲, 20 min with 3ABzA; ■, 20 min held on ice to prevent break rejoining. (*Upper Right*) Cells exposed to 1 mM $MeSO_2OMe$ for 30 min and grown as follows: ◑, 0 hr; ▲, 6 hr; ■, 6 hr with 3ABzA; ●, 5.5 hr followed by 30 min with 3ABzA. (*Lower Left*) Cells exposed to 34 μM MNNG for 30 min and grown as follows: ◑, 0 hr; ▲, 6 hr; ■, 6 hr with 3ABzA; ●, 5.5 hr followed by 30 min with 3ABzA; ○, 6 hr with 20 μM cytosine arabinonucleoside. (*Lower Right*) Cells exposed to 13 J/m² of UV light and grown as follows: ◑, 0 hr; ▲, 1 hr; ■, 1 hr with 3ABzA.

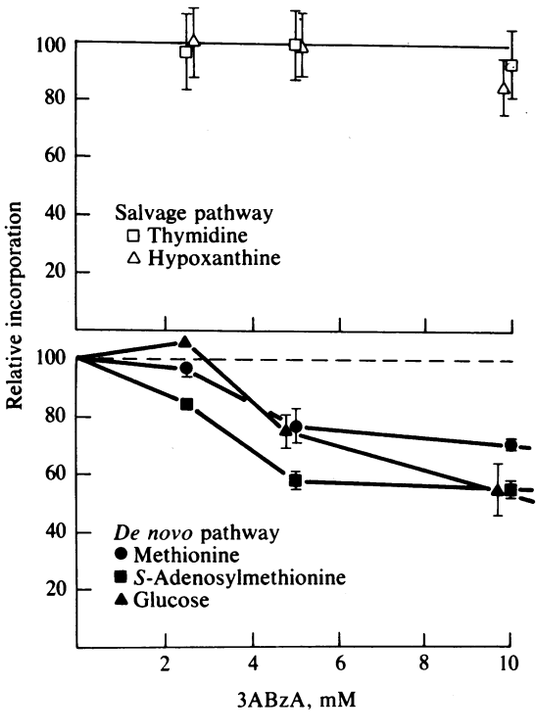

FIG. 2. Relative rate of incorporation of various precursors into DNA during a 4-hr period in the presence of 3ABzA. (*Upper*) Precursors labeling DNA via salvage pathways: □, thymidine; △, hypoxanthine. (*Lower*) Precursors labeling DNA via *de novo* pathways: ▲, glucose; ●, methionine; or via DNA methylation: ■, *S*-adenosylmethionine.

cells increased with increasing 3ABzA concentration, although to a lesser extent than the inhibition of poly(ADP-ribose) synthesis (Table 3). Poly(ADP-ribose) synthesis was completely suppressed by 2 mM 3ABzA, whereas strand breaks needed 5 mM for maximal increase. 3ABzA did not prevent repair completely, however; after x-irradiation the breaks rejoin in the presence of 3ABzA after a slight delay (Fig. 1). 3ABzA was also less effective in preventing repair than was cytosine arabinonucleoside, which holds a fraction of excision repair breaks open permanently (16).

Repair replication was determined in cells exposed to 50 or 200 Gy of x-rays, 1 or 5 mM $MeSO_2OMe$, 0.2 or 0.6 mM MNNG, and 13 or 26 J/m^2 of UV light. 3ABzA (5 mM) had no significant effect on repair replication in cells incubated for 6 hr after exposure to a damaging agent; values obtained with or without 3ABzA lay within ±10% of each other (results not shown), as we have observed in many other fibroblast cell types (17).

Effect of 3ABzA on Synthesis of DNA from *de Novo* or Salvage Pathways. Incorporation of thymidine and hypoxanthine into DNA pyrimidines and purines, respectively, which proceeds by the salvage pathways (21), was unaffected by 3ABzA at concentrations up to 10 mM (Fig. 2). In contrast, those precursors that label DNA by routes involving general cell metabolism (glucose and methionine) or by direct methylation of cytosine (*S*-adenosylmethionine) were inhibited up to 50% by 3ABzA (Fig. 2). This effect was evident at 2 mM 3ABzA for *S*-adenosylmethionine and at 5 mM 3ABzA for glucose and methionine. We have also found that 3ABzA inhibits glycolysis, measured by the production of $^{14}CO_2$ from [^{14}C]glucose, in lymphoid cells and fibroblasts (unpublished observations).

DISCUSSION

Malignant transformation *in vitro* by radiation and a variety of chemicals is a multistage process initiated by the induction of genetic (DNA) damage and the fixation of this damage by DNA and cellular replication (1, 2, 4). Later stages in transformation are associated with altered phenotypes that are expressed after an additional period of replication (1, 2, 4). During the period of expression a variety of events can promote the neoplastic state qualitatively and quantitatively (2).

Our earlier work has indicated that the expression of transformation after exposure of cells to radiation or chemical carcinogens can be inhibited by a variety of agents. These include protease inhibitors (3), which do not modify DNA metabolism (22), superoxide dismutase (23), specific retinoids, and selenium (24), which also inhibits promotion (23, 24). Transformation can also be inhibited in its initial stages by growing cells in medium depleted of thyroid hormone (25–27) before exposure to the DNA-damaging agent.

Our present work using mouse C3H 10T½ and Syrian hamster embryo cells indicates that the initiation of transformation by physical agents and alkylating chemicals can be inhibited by BzA and 3ABzA, at a concentration that inhibited poly(ADP-ribose) synthesis but was below that at which other side effects occurred (Table 3 and Fig. 2). This contrasts with observations that BzA and 3ABzA enhance mutagenesis (13) and sister chromatid exchanges (10–12), suggesting that those processes involve different regulatory pathways. Differentiation, however, is inhibited by 3ABzA (28), suggesting that this process involves steps in common with transformation.

A comparison of the known effects of the benzamides with our results on the inhibition of transformation permits a number of insights into possible relationships between poly(ADP-ribose) synthesis and transformation: (*i*) There may be a direct role for poly(ADP-ribosyl)ation in transformation; (*ii*) this role may be separate from its role in modulating strand break frequencies during DNA repair; (*iii*) the effect of benzamides on DNA methylation and DNA precursor pathways may have important effects on cellular regulation at high concentrations of the inhibitors.

Poly(ADP-ribose) synthesis is transient after exposure of cells to ionizing radiation but long lasting after exposure to alkylating agents and UV light, corresponding to the period during which DNA breaks are detectable (16, 18, 19). Despite the large difference in poly(ADP-ribosyl)ation, transformation is suppressed after exposure to each kind of agent (Tables 1 and 2), suggesting that transformation frequency is not directly dependent on the total amount and duration of poly(ADP-ribosyl)ation but more likely is dependent on specific proteins that are ADP-ribosylated. Fibroblasts from patients with many disorders that lead to abnormal carcinogen sensitivities or a high propensity for cancer, such as xeroderma pigmentosum (29), Cockayne syndrome (30), and Fanconi anemia (31), also exhibit abnormalities in the poly(ADP-ribose) synthesis pathway. A causal relationship between ADP-ribosylation activity and γ-glutamyl transpeptidase, an early marker of neoplasia, has also been reported (32).

Poly(ADP-ribose) formation may play an indirect role in transformation through its effect on the ligation rate of strand breaks during repair. This acts via the turnover of poly(ADP-ribose) chains on DNA ligase II, which regulates rates of ligation of certain kinds of repair sites, mainly those in cells exposed to alkylating agents (16–19, 33). However, 3ABzA concentrations of 5 mM are needed for maximal effect (Table 3), raising the possibility that side effects of 3ABzA may be involved. From our results, since 3ABzA has no effect on UV-induced DNA breaks (Fig. 1) but does suppress UV-induced transformation (Table 1) and has no effect on x-ray-induced strand breaks at 1 mM although transformation is suppressed, changes in DNA break frequency are unlikely to be involved in transformation.

A possibility that must always be entertained with inhibitors is the result of side effects on the system being investigated. 3ABzA may cause changes in gene regulation by inhibition of DNA precursor pathways, such as the *de novo* purine pathways, and DNA methylation, when used at concentrations above 2 mM (Fig. 2). Changes in precursor pathways can influence mutagenesis (34), and changes in DNA methylation can influence gene expression and differentiation (35). This does not apply to our results, however, because transformation was inhibited at 1 mM, a concentration at which poly(ADP-ribose) synthesis was inhibited (Table 3) but below which side effects were observed (Fig. 2). The contrast between the effect of the benzamides in inhibiting transformation but causing a stimulation of mutagenesis and sister chromatid exchange suggests that transformation involves specific ADP-ribosylation, whereas the other end points are mediated through DNA damage and repair. The results suggest a role in transformation for poly(ADP-ribosyl)ation during the induction of carcinogenic heritable changes in gene regulation or amplification (36–38).

Further investigation of the metabolic events affected by BzA and 3ABzA, particularly the critical proteins that may become poly(ADP-ribosyl)ated (8, 9), should serve to elucidate the mechanisms of *in vitro* transformation and carcinogenesis. If these experimental results can be reproduced *in vivo*, they might lead to a method to block carcinogenesis after exposure to chemical carcinogens. The practical importance of such a method cannot be overstated.

This work was supported by U.S. Department of Energy Contract DE-AM03-76-SF01012 and Grant CA12536-11 from the National Cancer Institute.

1. Borek, C., Hall, E. J. & Rossi, H. (1978) *Cancer Res.* **39,** 2997–3005.
2. Borek, C. (1982) *Adv. Cancer Res.* **37,** 159–231.
3. Borek, C., Miller, R., Pain, C. & Troll, W. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1800–1803.
4. Borek, C. & Sachs, L. (1966) *Nature (London)* **210,** 276–278.
5. Huberman, E., Magee, R. & Sachs, L. (1976) *Nature (London)* **264,** 360–361.
6. Barret, J. C. & T'so, P. O. P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3297–3301.
7. Reznikoff, C. A., Bertram, J. S., Brankow, D. W. & Heidelberger, C. (1973) *Cancer Res.* **33,** 3239–3249.
8. Sugimura, T., Miwa, T., Saito, H., Kanai, Y., Ikejima, M., Terada, M., Yamada, M. & Utakoji, T. (1980) *Adv. Enzyme Regul.* **18,** 195–220.
9. Haiyashi, O. & Ueda, K. (1977) *Annu. Rev. Biochem.* **46,** 95–116.
10. Morgan, W. F. & Cleaver, J. E. (1982) *Mutat. Res.* **104,** 361–366.
11. Hori, T.-A. (1981) *Biochem. Biophys. Res. Commun.* **102,** 38–45.
12. Oikawa, A., Tohda, H., Kanai, M., Miwa, M. & Sugimura, T. (1980) *Biochem. Biophys. Res. Commun.* **97,** 1131–1136.
13. Schwartz, J. L., Morgan, W. F. & Wolff, S. (1983) *Science*, in press.
14. Cleaver, J. E. (1977) in *Handbook of Mutagenicity Test Procedures*, ed. Kilbey, B. (Elsevier/North-Holland, Amsterdam), pp. 19–48.
15. Kohn, K. W., Ewig, R. A., Erickson, L. C. & Zwelling, L. A. (1981) in *DNA Repair: A Laboratory Manual of Research Procedures*, eds. Friedberg, E. C. & Hanawalt, P. C. (Dekker, New York), Vol. 1B, pp. 379–401.
16. Morgan, W. F. & Cleaver, J. E. (1983) *Cancer Res.* **43,** 3104–3107.
17. Cleaver, J. E., Bodell, W. J., Borek, C., Morgan, W. F. & Zelle, B. (1982) in *Proceedings of the 13th Princess Takamatsu Cancer Research Symposium*, eds. Miwa, O. H., Shall, M. & Takashi, S. (Japan Science Society Press, Tokyo), pp. 195–207.
18. James, M. R. & Lehmann, A. R. (1982) *Biochemistry* **21,** 4007–4013.
19. Durkacz, B. W., Omidiji, O., Gray, D. A. & Shall, S. (1980) *Nature (London)* **283,** 593–596.
20. Cleaver, J. E., Thomas, G. H., Trosko, J. E. & Lett, J. T. (1972) *Exp. Cell Res.* **74,** 67–80.
21. Cleaver, J. E. (1967) *Thymidine Metabolism and Cell Kinetics* (Elsevier/North-Holland, Amsterdam).
22. Borek, C. & Cleaver, J. E. (1981) *Mutat. Res.* **82,** 373–380.
23. Borek, C. & Troll, W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1304–1307.
24. Borek, C. (1982) in *Proceedings of the M. D. Anderson Symposium on Molecular Interrelations of Nutrition and Cancer*, ed. Arnott, M. (Raven, New York), pp. 337–350.
25. Guernsey, D. L., Ong, A. & Borek, C. (1980) *Nature (London)* **288,** 337–350.
26. Guernsey, D. L., Borek, C. & Edelman, I. S. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5708–5711.
27. Borek, C., Guernsey, D. L., Ong, A. & Edelman, I. S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5749–5752.
28. Farzaneh, F., Zalin, R., Brill, D. & Shall, S. (1982) *Nature (London)* **300,** 362–366.
29. Berger, N. A., Sikorski, H. W., Petzold, S. J. & Kurohara, K. K. (1980) *Biochemistry* **2,** 289–293.
30. Fujiwara, Y., Goto, K. & Kano, Y. (1982) *Exp. Cell Res.* **139,** 207–215.
31. Berger, N. A., Berger, S. J. & Catino, D. M. (1982) *Nature (London)* **299,** 271–272.
32. Althause, F. R., Lawrence, S. D., He, Y. Z., Sattler, G. L., Tsukuda, Y. & Pitot, H. C. (1982) *Nature (London)* **300,** 366–368.
33. Creissen, D. & Shall, S. (1982) *Nature (London)* **296,** 271–272.
34. Meuth, M. (1981) *Somatic Cell Genet.* **7,** 89–102.
35. Razin, A. & Riggs, A. D. (1980) *Science* **210,** 604–610.
36. Feinberg, A. P. & Vogelstein, B. (1983) *Nature (London)* **301,** 89–92.
37. Feinberg, A. P. & Vogelstein, B. (1983) *Biochem. Biophys. Res. Commun.* **111,** 47–54.
38. Schwab, M., Alitalo, K., Varmus, H. E., Bishop, J. M. & George, D. (1983) *Nature (London)* **303,** 497–501.