Quantitative Studies of Inhibitors of ADP-ribosylation in Vitro and in Vivo*

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The ADP-ribosyl moiety of NAD⁺ is consumed in reactions catalyzed by three classes of enzymes: poly(ADP-ribose) polymerase, protein mono(ADP-ribosyl)transferases, and NAD⁺ glycohydrolases. In this study, we have evaluated the selectivity of compounds originally identified as inhibitors of poly(ADP-ribose) polymerase on members of the three classes of enzymes. The 50% inhibitory concentration (IC₅₀) of more than 20 compounds was determined in vitro for both poly(ADP-ribose) polymerase and mono(ADP-ribose) polymerase in an assay containing 300 μM NAD⁺. Of the compounds tested, benzamide was the most potent inhibitor of poly(ADP-ribose) polymerase with an IC₅₀ of 3.3 μM. The IC₅₀ for benzamide for mono(ADP-ribosyl)transferase A was 4.1 mM, and similar values were observed for four additional cellular mono(ADP-ribosyl)transferases. The IC₅₀ for NAD⁺ glycohydrolase for benzamide was approximately 40 mM.

For seven of the best inhibitors, inhibition of poly(ADP-ribose) polymerase in intact C3H10T1/2 cells was studied as a function of the inhibitor concentration of the culture medium, and the concentration for 50% inhibition (culture medium IC₅₀) was determined. Culture medium IC₅₀ values for benzamide and its derivatives were very similar to in vitro IC₅₀ values. For other inhibitors, such as nicotinamide, 5-methylnicotinamide, and 5-bromodeoxyuridine, culture medium IC₅₀ values were 3-5-fold higher than in vitro IC₅₀ values. These results suggest that micromolar levels of the benzamides in the culture medium should allow selective inhibition of poly(ADP-ribose) metabolism in intact cells. Furthermore, comparative quantitative inhibition studies should prove useful for assigning the biological effects of these inhibitors as an effect on either poly(ADP-ribose) or mono(ADP-ribose) metabolism.

NAD⁺ is a cosubstrate in numerous hydride transfer reactions central to intermediary metabolism. It is also a substrate for a second distinct class of enzymes that catalyze the cleavage of the glycosylic linkage between nicotinamide and ribose and transfer of the ADP-ribosyl moiety to an acceptor. Enzymes that use proteins as acceptors include the nuclear enzyme poly(ADP-ribose) polymerase, which also catalyzes synthesis of polymers of ADP-ribose (1), and a second group of mono(ADP-ribosyl)transferases that transfer only a single ADP-ribose residue to acceptors (2). Although both poly(ADP-ribose) polymerase and mono(ADP-ribose) transferases can catalyze the hydrolysis of NAD⁺ to ADP-ribose and nicotinamide, they preferentially transfer ADP-ribose to proteins. A third class of enzymes, termed NAD⁺ glycohydrolases, preferentially catalyze the hydrolysis of NAD⁺ (3).

The biological functions of ADP-ribosylation reactions are poorly understood. Many studies concerned with elucidating their biological functions have used compounds originally identified as inhibitors of poly(ADP-ribose) polymerase (4, 5). Studies using these inhibitors have shown that they alter many processes including cellular recovery from DNA damage (6), sister chromatid exchange (8), malignant transformation (9-11), DNA replication (12), and cellular differentiation (13, 14). Whereas the effects of these inhibitors have been generally attributed to inhibition of poly(ADP-ribose) polymerase, the lack of absolute selectivity with regard to ADP-ribose-transferring enzymes or other enzymes makes the link to poly(ADP-ribose) polymerase a tenuous one. In this study, we have examined compounds originally identified as inhibitors of poly(ADP-ribose) polymerase for their relative inhibition of several classes of ADP-ribose-transferring enzymes and for their efficacy of inhibition of poly(ADP-ribose) polymerase in vivo.

EXPERIMENTAL PROCEDURES

Materials

The following were obtained from Sigma: benzamide, 3-methoxybenzamide, nicotinamide, thymidine, theophylline, pyrazinamide, caffeine, hypoxanthine, 1-methyl nicotinamide iodide, 8-methyl nicotinamide, benzamide, benzoxazole, 4-amino benzoate, nitric acid, theobromine, isonicotinate hydrate, and histone (type II, calf thymus). 2-Aminobenzamide, zinc chloride, and 3-isobutyl-1-methylxanthine were obtained from Aldrich. 3-Aminobenzamide and 3-amino benzoxazole were provided by Pfaltz & Bauer, Inc. (Stamford, CT), and 5-methyl nicotinamide was provided by Lilly. [32P]NAD⁺ (1140 Ci mmol⁻¹) and [2,3-3H]adenine (30-40 Ci mmol⁻¹) were purchased from ICN (Irvine, CA), and [4,5-3H]nicotinamide-labeled NAD⁺ (25 Ci mmol⁻¹) was obtained from Amersham Corp. Dihydroxy-Bio-Rex was synthesized as described by Jacobson et al. (16), and dihydroxy-Sepharose was synthesized as described by Jacobson et al. (16).

Methods

Determination of Poly(ADP-ribose) Polymerase Activity in Vitro—Poly(ADP-ribose) polymerase was partially purified from calf thymus as described previously (17). Different preparations had specific ac-

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tivities of 200–500 mg kg⁻¹ of protein. One unit is defined as the
amount of enzyme that converts 1 nmol of NAD⁺ to poly(ADP-
ribose)/min under the assay conditions described here. The enzyme
preparation was dependent upon exogenous DNA for activity, and
pBR322 DNA digested with *Hind*III endonuclease (17) was used as a
definitive source of activating DNA. Assays were performed in 100 mM
Tris/Cl, pH 7.5, 0.5 mM EDTA, and 10 mM *[3H]NAD⁺* (1000 cpm, 0.2
μg of DNA, and 1-2 units of enzyme. After incubation for 2 min at
37 °C, 3.0 ml of 20% trichloroacetic acid and 100 μg of bovine
serum albumin were added, and samples were mixed and placed on
ice. Acid-insoluble product was collected onto Whatman GF/A glass
fibers and washed three times with 10 ml of 5% trichloroacetic
acid and twice with 10 ml of 95% ethanol. Filters were air-dried, and
radiolabel was determined by liquid scintillation counting.

**Determination of Mono(ADP-ribose)-Transferase Activity in vitro—**
Mono(ADP-ribose)transferase A was purified to apparent homoge-
nenity as described previously (18). Other mono(ADP-ribose)-
transferases were purified as described (19). Transferase A had a
specific activity of 156 units mg⁻¹. Assays of 200 μl contained 100
mM Hepes, pH 7.5, 300 mM NaCl, 1.8 mg of histone, 300 μM *[14]NAD⁺* (1 × 10⁶ cpm), and approximately 1 milliunit of enzyme. After
incubation at 30 °C for 30 min, reactions were terminated with 3 ml
of 20% trichloroacetic acid and placed on ice. Samples were filtered,
and radiolabel was determined as described above.

**Determination of NAD⁺ Glycohydrolase Activity in vitro—** Partially
purified porcine brain NAD⁺ glycohydrolase was obtained from Sigma. For assay, 5 mg (0.055 unit) of acetone powder was added to 1.5
ml of medium, washing with phosphate-buffered saline, and addition of
ice bath. Insoluble material was removed by centrifugation for 10 min at
2000 g. The 200-μl assay contained 67 mM potassium phosphate
buffer, pH 6.0, containing 10 mM EDTA and immediately
applied to a 0.5-ml column of dihydroxy-Bio-Rex that had been
prewashed with 10 ml of the same buffer. The column flow-throug
containing the released nicotinamide was quantified by liquid scintil-
alation counting. The release of nicotinamide was linear with time
and did not exceed 10% of the total substrate. This enzyme preparation
was also examined for poly(ADP-ribose) polymerase or protein
(ADP-ribose)transferase activity utilizing NAD⁺ radiola-
beled in the adenine ring (21) and examining for the formation of an
acid-insoluble product. No acid-insoluble product was detected.

**Poly(ADP-ribose) Polymerase Activity in Vivo—** C3H1OT/2 cells were maintained as described previously (20). Con-
fuent cultures in 35-mm dishes were radiolabeled for 16 h with 1 ml
of medium containing 20 μCi of [*H]adenine and NAD⁺, and ADP-
ribose polymer layer polymers were determined using methodology whose
validation is described elsewhere (21). Medium was replaced, and
cells were incubated for 30 min in the presence of inhibitors and
incubation at 30 °C for 30 min, reactions were terminated with 3 ml
of 20% trichloroacetic acid and placed on ice. Samples were filtered,
and radiolabel was determined as described above.

**Comparison of Effect of Inhibitors on Poly(ADP-ribose)*
Polymerase and Mono(ADP-ribose)transferase A In Vitro—**
To examine inhibition patterns in vitro, a partially purified preparation of poly(ADP-ribose) polymerase from calf thymus
(17) was compared with homogeneous turkey erythrocyte
NAD⁺-arginine mono(ADP-ribose)transferase A (18). They
are referred to here as polymerase and transferase, respec-
tively. The NAD⁺ concentration of both assays was 300 μM,
which was selected because it approximates the estimated
intracellular concentration of NAD⁺ in cultured mouse cells
(22). Fig. 1 proves the rate of conversion of *[5P]*NAD⁺ into
acid-insoluble material for the polymerase (A) and transferase
(B) assays. Reaction periods of 2 and 30 min were chosen for
the inhibition studies for the polymerase and transferase,
respectively, since those periods were within the linear portion
of the time courses.

To evaluate each compound, varying concentrations were
competed against 300 μM NAD⁺. Fig. 2 shows representative
data of polymerase activity as a function of the concentration
of 3-methoxybenzamide, 3-aminobenzamide, nicotinamide,
nicotinate, and benzoate. The same amides have previously
been reported to inhibit the polymerase (4, 5), and dose-
dependent inhibition for each of these compounds was also
observed under the conditions used in this study. Nicotinate
and benzoate have been reported to be the most inhibitory
(4, 5), and they likewise did not show significant inhibition in these
assays. Data from multiple experiments such as those shown in
Fig. 2 were combined, and a best fit curve was generated by
computer analysis (23). From this analysis, a 50% inhibi-
tory concentration (IC₅₀), defined as the concentration which
inhibited enzyme activity by 50% under the assay conditions,
was determined. Table 1 lists IC₅₀ values for 23 compounds
examined. In addition, an IC₅₀ relative to that for benzamide
was calculated by dividing each IC₅₀ by the IC₅₀ for benzamide,
the most potent inhibitor. The most effective inhibitors were
the benzamides, with IC₅₀ values between 3 and 6 μM. The
best inhibitor of the polymerase which was not a benzamide
derivative was 5-bromodeoxyuridine with an IC₅₀ of 15 μM.
Nicotinamide, theophylline, and thymidine were also rela-
tively effective inhibitors with IC₅₀ values ranging from 31 to
46 μM.

**Fig. 3** shows representative data for inhibition of transfer-
ase A by 5-bromodeoxyuridine, 3-methoxybenzamide, 3-ami-
nobenzamide, and benzoic acid. Benzoic acid had no effect on
transferase activity, whereas dose-dependent inhibition was
evident for the three other compounds. The IC₅₀ values of 21
compounds examined with the transferase were calculated
using computer analysis and are shown in Table 2. The most
effective transferase inhibitors were the pyrimidine nucleo-
sides, 5-bromodeoxyuridine and thymidine, with IC₅₀ values

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1 S. J. Stanley and J. Moss, unpublished data.
2 The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piper-
azineethanesulfonic acid; MNNG, N-methyl-N-nitroso-N'-nitroagu-
ridine.
3 Portions of this paper (including Figs. 1–7 and Tables 1–4) are
presented in miniprint at the end of this paper. Miniprint is easily
read with the aid of a standard magnifying glass. Full size photocopies
are included in the microfilm edition of the Journal that is available
from Waverly Press.
of 590 and 1900 µM, respectively, followed by the benzamides, which yielded IC₅₀ values of between 2700 and 4100 µM. For many compounds tested, the IC₅₀ values were greater than 30 mM, and these compounds were designated as noninhibitory.

Comparison of Tables 1 and 2 reveals that IC₅₀ values of all compounds tested were much higher for transferase than for polymerase. This is illustrated in Fig. 4, which compares the in vitro inhibition curves for 3-aminobenzamide, nicotinamide, 5-methyl nicotinamide, and 5-bromodeoxyuridine. Table 3 lists ratios of the IC₅₀ values of transferase relative to polymerase for eight selected inhibitors. The largest difference in IC₅₀ values was seen for benzamide, in which the IC₅₀ value for the transferase was higher by more than three orders of magnitude. Such a marked quantitative difference in sensitivity is potentially useful for discriminating between effects of inhibitors on the polymerase and transferases in intact cells, providing that similar inhibition patterns are observed in vivo.

Inhibition Patterns of Poly(ADP-ribose) Polymerase in Vivo—The term in vivo is used here to refer to C3H10T1/2 cells maintained in culture. To study inhibition patterns in vivo, the effect of inhibitors on the accumulation of ADP-ribose polymers was examined following treatment of C3H10T1/2 cells with the alkylating agent MNNG. Previous studies have established that this treatment results in a rapid activation of poly(ADP-ribose) polymerase, resulting in elevated levels of ADP-ribose polymers (20, 23). Fig. 5 shows a time course of the NAD⁺ and poly(ADP-ribose) content of cells following treatment with 100 µM MNNG. These conditions resulted in a maximal accumulation of poly(ADP-ribose) at 30 min following treatment, and this treatment period was therefore chosen to study effects of the inhibitors.

Fig. 6 compares in vitro and in vivo inhibition curves for benzamide, nicotinamide, 3-aminobenzamide, 5-bromodeoxyuridine, 3-methoxybenzamide, and 5-methyl nicotinamide. The inhibitor concentrations shown represent the concentrations in the culture medium for the in vivo experiments. The concentration of inhibitor in the culture medium that inhibited the polymerase by 50% in vivo was calculated and termed culture medium IC₅₀. In the case of the benzamides, the in vivo and in vitro inhibition patterns derived were very similar. However, for nicotinamide, 5-bromodeoxyuridine, and 5-methylnicotinamide, the in vivo patterns were shifted to considerably higher concentrations. Table 4 shows calculated culture medium IC₅₀ values for the seven inhibitors and ratios of culture medium IC₅₀ values to in vitro IC₅₀ values. The ratio for all the benzamides tested was close to 1.0. However, the ratios for nicotinamide, 5-methyl nicotinamide, 5-bromodeoxyuridine, and theophylline ranged from 3.2 to 6.0.

Inhibition of Other Protein Mono(ADP-ribose)transferases and NAD⁺ Glycohydrolase in Vitro—Two results described here suggest that the benzamides may prove very useful in discriminating between effects on polymerase and transferases in intact cells. First, there are large quantitative differences in the relative sensitivity of the polymerase as compared to transferase A in vitro (Fig. 4). Second, there is a close correspondence between in vitro inhibition of the polymerase and the inhibition of poly(ADP-ribose) accumulation in vivo (Fig. 6). However, since multiple endogenous transferases are present in cells, the utility of this approach would depend upon whether the inhibition pattern of transferase A is representative of other endogenous transferases. Additionally, it was also of interest to determine the effects of these inhibitors on a third class of NAD⁺-consuming enzymes, NAD⁺ glycohydrolase. Thus, inhibition curves were generated for four additional transferases (19) and for NAD⁺ glycohydrolase.

Fig. 7 shows in vitro inhibition by benzamide of each of the five endogenous transferases and NAD⁺ glycohydrolase in relation to the polymerase. The data show that all of the transferases had similar inhibition patterns, whereas the NAD⁺ glycohydrolase was less sensitive to inhibition than the transferases. Additional experiments using 3-methoxybenzamide and 3-aminobenzamide also demonstrated equivalent responses by the five transferases (data not shown).

DISCUSSION

A number of studies have quantitatively evaluated the inhibition of poly(ADP-ribose) polymerase in vitro (4, 5, 29–31), but very little quantitative information concerning the inhibition of other classes of ADP-ribose-transfer enzymes or on the efficacy of inhibitors in intact cells has been reported. The existence of multiple endogenous mono(ADP-ribose)transferases (25) makes information concerning the selectivity of these inhibitors especially important with regard to assessing the scope of mono(ADP-ribose) metabolism in total cellular ADP-ribosylation and in assigning biological effects of these inhibitors. Whereas the use of inhibitors in intact cells has many limitations, the lack of genetic approaches to ADP-ribosylation in mammalian cells makes their use important in understanding the physiological functions of ADP-ribosylation. Whereas this study has focused on the selectivity of these inhibitors between different classes of ADP-ribose-containing enzymes, the possible action of the inhibitors on unrelated enzymes must also be a concern when evaluating biological effects of these compounds. For example, studies have suggested that the benzamides alter nucleotide synthesis (26, 27), although the studies of Hunting et al. (28) are most consistent with effects on nucleotide labeling that are secondary to effects on folate metabolism. In general, it has been necessary to employ medium concentrations of 5 mM and higher to observe effects on nucleotide labeling. Whereas the studies described here would argue that effects on nucleotide labeling are not likely due to inhibition of poly(ADP-ribose) polymerase, our data would be consistent with these effects being related to inhibition of mono(ADP-ribose)transferases or of unrelated enzymes.

The objective of the in vitro analyses described here was to generate data useful for interpreting the observed effects of inhibitors on intact cells rather than to provide a detailed kinetic analysis of each compound. Nevertheless, the IC₅₀ values reported here are similar to apparent Kᵢ values of several compounds previously reported for poly(ADP-ribose) polymerase. Purnell and Whish (4) previously reported apparent Kᵢ values for 3-aminobenzamide and 3-methoxybenzamide of 1.8 and 1.5 µM, respectively, whereas we report here corresponding IC₅₀ values of 5.4 and 3.4 µM. Furthermore, Clark et al. (29) and Niedergang et al. (30) have reported apparent Kᵢ values for nicotinamide in the range of 20–50 µM, whereas our studies observed an IC₅₀ of 31 µM. Stone and Shall (31) reported an apparent Kᵢ for thymidine of 33 µM, whereas our studies reported an IC₅₀ of 43 µM.

The results presented here are in general agreement with previous studies (4, 5) that have shown that benzamide and its derivatives are very potent inhibitors of poly(ADP-ribose) polymerase in vitro. This study has demonstrated that relatively low medium concentrations of these compounds effectively inhibit poly(ADP-ribose) polymerase in vivo. It is difficult to determine accurately the intracellular concentration of these compounds available to poly(ADP-ribose) polymerase due to several uncertainties including partitioning of the compound between aqueous and membrane phases and distribution between different intracellular compartments. How-
ever, the close correspondence between in vitro and in vivo inhibition curves for the polymerase is consistent with the possibility that the concentration of the benzamides in the nucleus closely approximates the extracellular concentration. However, our results are also noteworthy in that many of the inhibitors examined did not show a close correspondence between in vitro and in vivo inhibition curves. This lack of correlation could be due to several factors including membrane permeability, transport, metabolism, and intracellular compartmentalization. Nevertheless, such observations suggest that these inhibitors have a more limited utility in intact cells than the benzamides.

The results shown here have demonstrated that the benzamides are effective inhibitors of poly(ADP-ribose) polymerase in vitro at concentrations in the medium which are much lower than those generally used in studies designed to assess the effects of ADP-ribosylation inhibitors on biological responses (4–14). The large quantitative differences between the in vitro inhibition curves of poly(ADP-ribose) polymerase and the mono(ADP-ribosyl)transferases examined suggest that it may be possible to inhibit poly(ADP-ribose) polymerase with minimal effects on mono(ADP-ribosyl)transferases. However, it should be noted that the inhibition curves of endogenous mono(ADP-ribosyl)transferases shown here (Fig. 7) have all been generated with transferases specific for arginine residues. Endogenous transferases specific for cysteine (32) and modified histidine (33) residues have also been reported, and it remains to be determined whether or not these (ADP-ribosyl)transferases have similar inhibition properties.

The large quantitative differences between IC₅₀ values observed for poly(ADP-ribose) polymerase and mono(ADP-ribo)

by transferases may prove useful in assessing the mechanism of the biological effects of the benzamides. For example, benzamide and its derivatives have been shown to be relatively nontoxic to cells alone, but they enhance the cytotoxicity of DNA-alkylating agents (6, 7). Previous studies have used concentrations in the culture medium of 1 mM and higher to achieve an enhancement of cytotoxicity. In view of the results described here, we have re-examined the co-cytotoxic property of benzamide at lower concentrations and have observed maximal enhancement of the cytotoxicity of MNNG at micromolar concentrations. When quantitatively comparing the effects of inhibitors on biological end points such as cell survival, an exact correspondence between inhibition curves of the target enzyme and the biological effect is not necessarily expected since the process must be inhibited to the extent that it becomes rate-limiting for the end point measured. Nevertheless, the fact that benzamide was co-cytotoxic at similar concentrations to those that inhibited poly(ADP-ribose) synthesis in vitro, which is far below that where effects on other ADP-ribo-

transferring enzymes are expected, argues that poly(ADP-ribose) polymerase is the likely target for the co-cytotoxic effects of benzamide. Furthermore, it suggests that even relatively low levels in tissues may be effective in enhancing the cytotoxicity of chemotherapeutic DNA-damaging agents. A quantitative assessment of the effect of varying concentrations of the benzamides on other biological end points may prove valuable for assigning their physiological effects to poly(ADP-ribose) polymerase or mono(ADP-

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Quantitative Studies of ADP-ribosylation Inhibitors

SUPPLEMENTARY MATERIAL TO:
Quantitative Studies of Inhibition of ADP-Ribosylation In Vivo and In Vitro

by
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Table 1

Effects of Selected Compounds on Poly(ADP-Ribose) Polymerase In Vitro

| Compound                  | IC50 (uM) | Relative IC50 |
|---------------------------|-----------|---------------|
| Benzamide                 | 3.2 ± 0.28| 1.0           |
| 3-Methoxybenzamide        | 3.4 ± 0.31| 1.0           |
| 3-Aminobenzamide          | 5.4 ± 0.40| 1.0           |
| 5-Iododeoxyuridine        | 15 ± 1.3  | 4.5           |
| Nicotinamide              | 21 ± 3.9  | 9.5           |
| Thymidine                 | 43 ± 5.2  | 13            |
| Theophylline              | 46 ± 15   | 14            |
| 5-Methylcytosine          | 78 ± 13   | 21            |
| ZnCl2                     | 77 ± 21   | 23            |
| 3-Aminobenzamide          | 100 ± 26  | 30            |
| Theophylline              | 110 ± 19  | 33            |
| Pyrazinamide              | 130 ± 18  | 39            |
| 4-Aminobenzamide          | 400 ± 33  | 121           |
| Caffeine                  | 1,400 ± 240| 424          |
| Hypoxanthine              | 1,500 ± 190| 515          |
| 1-Methylcytosine          | 1,200 ± 250| 351          |
| 3-Isoxyl-1-methylbutamine| 3,000 ± 310| 1595         |
| Homocysteine hydrate      | 4,000 ± 370| 1454         |
| 8-Methylxanthine          | 1,600 ± 370| 2383         |
| Benzamide                 | 2,500 ± 290| 542          |
| 3-Aminobenzamide          | NI*       |               |
| 4-Aminobenzamide          | NI         |               |
| Nicotinamide              | NI         |               |

*IC50 >30,000uM

Table 2

Effect of Selected Compounds on Mono(ADP-Ribosyl) Transferase A In Vitro

| Compound                  | IC50 (uM) | Relative IC50 |
|---------------------------|-----------|---------------|
| 5-Iododeoxyuridine        | 290 ± 20  | 1.0           |
| Thymidine                 | 1,500 ± 300| 3.2          |
| 3-Methoxybenzamide        | 2,000 ± 250| 4.6          |
| Theophylline              | 2,000 ± 360| 4.7          |
| 3-Aminobenzamide          | 3,000 ± 900| 5.1          |
| Nicotinamide              | 3,000 ± 410| 5.8          |
| Benzamide                 | 4,000 ± 220| 7.0          |
| ZnCl2                     | 4,000 ± 140| 10.0         |
| 8-Methylxanthine          | 1,000 ± 1,000| 20           |
| Benzamide                 | 2,500 ± 8,500| 39          |
| 3-Aminobenzamide          | NI*       |               |
| 4-Aminobenzamide          | NI         |               |
| 2-Aminobenzamide          | NI         |               |
| Nicotinamide              | NI         |               |
| 5-Methylcytosine          | NI         |               |
| 1-Methylcytosine          | NI         |               |
| Pyrazinamide              | NI         |               |
| Homocysteine hydrate      | NI         |               |
| Caffeine                  | NI         |               |
| 3-Isoxyl-1-methylbutamine | NI         |               |

*IC50 >30,000uM

Table 3

IC50 Ratios for Mono(ADP-Ribosyl) Transferase A Relative to Poly(ADP-Ribose) Polymerase In Vitro

| Compound                  | IC50 Transferase | IC50 Polymerase |
|---------------------------|------------------|-----------------|
| Benzamide                 | 1,000            |                 |
| 3-Methoxybenzamide        | 550              |                 |
| 3-Aminobenzamide          | 550              |                 |
| Nicotinamide              | 550              |                 |
| ZnCl2                     | 550              |                 |
| Theophylline              | 550              |                 |
| 3-Methylcytosine          | 550              |                 |

Fig. 1. Time course of activity for poly(ADP-ribose) polymerase (Panel A) and mono(ADP-ribose) transferase A (Panel B).

Fig. 2. Activity of poly(ADP-ribose) polymerase in the presence of varying concentrations of L-methionine (.), 3-aminobenzamide (A), nicotinamide (B), nicotinic acid (D), and benzene acid (C).
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**Figure 3.** Activity of mono(ADP-ribosyl) transferase A in the presence of bromodeoxyuridine (●), 3-methoxybenzamide (▲), 3-aminobenzamide (●), and benzoic acid (○).

**Figure 6.** Comparison of inhibition curves for poly(ADP-ribose) polymerase obtained in vitro and in vivo. The open symbols represent in vitro data and the closed symbols represent in vivo data. Top Panel: Benzamide (●, ○) and nicotinamide (▲, ○). Middle Panel: 3-Aminobenzamide (●, ○) and 5-bromodeoxyuridine (▲, ○). Bottom Panel: 5-Methylnicotinamide (●, ○) and 3-methoxybenzamide (▲, ○).

**Figure 4.** Comparison of inhibition curves of poly(ADP-ribose) polymerase (●) and mono(ADP-ribosyl) transferase A (▲) for 3-aminobenzamide (Panel A), nicotinamide (Panel B), 3-aminobenzamide (Panel C), and 5-bromodeoxyuridine (Panel D).

**Figure 5.** NAD⁺ and poly(ADP-ribose) content of C3H10T1/2 cells following MNNG treatment. Cells were labeled with [14C]adenine and treated with MNNG as described in Methods. Cells were harvested at the indicated times and analyzed for radioactivity in NAD⁺ and poly(ADP-ribose) as described in Methods. The absolute NAD⁺ content of control controls was determined by a cycling assay described previously (21) and the specific radioactivity of the NAD⁺ pool was calculated and used to estimate the absolute quantitites of poly(ADP-ribose). The values shown are the means of duplicate determinations which agreed within a standard deviation of 10 percent of the mean. NAD⁺: (▲) poly(ADP-ribose).

**Figure 7.** Comparison of in vivo inhibition curves for poly(ADP-ribose) polymerase (●), mono(ADP-ribosyl) transferases A (▲, ○), A' (▲, ○), B (▲', ○), C (▲, ○), C' (▲, ○) and NAD⁺ glycohydrolase (●).