POLY(ADP-RIBOSE) POLYMERASE ACTIVITY IN HELA CELLS TREATED WITH VARIOUS ANTIBIOTICS

Sir:

Numerous compounds are being used for the treatment of human cancer[1]. Some of these compounds exert their anticancer action by causing DNA strand breaks and subsequently inhibiting DNA synthesis. There are many reports that DNA damage caused by alkylating agents, DNA damaging agents (such as streptozotocin[2], neocarzinostatin[3], and bleomycin[4]), ionizing radiation, and DNA endonuclease elicits a marked increase in the activity of poly(ADP-ribose) polymerase[5]. Poly(ADP-ribose) polymerase is DNA-dependent and utilizes NAD to form a homopolymer of ADP-ribose which is covalently bound to nuclear proteins in eukaryotes. Therefore, post-transcriptional modification, *i.e.*, poly(ADP-ribosylation), seemed to be a suitable parameter to use to evaluate compounds for their ability to damage DNA or cause changes in the chromatin structure in mammalian cells. HATA[6] reported that anticancer agents can be classified into five groups by determining the morphological changes they cause in HeLa cells. Here we report the changes in poly(ADP-ribose) polymerase activity in HeLa cells following treatment with established, purified inhibitors of mammalian cells. In addition, there is no rapid and direct assay for biologically active compounds while they are in fermentation culture. It might be possible to assay for compounds that affect nuclear processes in eukaryotes by measuring increases in poly(ADP-ribose) polymerase activity following the addition of culture broth directly to cells in culture. To determine if this technique is feasible, we have studied the changes in poly(ADP-ribose) polymerase activity in HeLa cells treated with authentic purified inhibitors of HeLa cells. This communication describes the affect of purified inhibitors on the activity of poly(ADP-ribose) polymerase. In addition, we also describe the increase in poly(ADP-ribose) polymerase activity in HeLa cells following the addition of the culture filtrates of *Cordyceps militaris*[7], containing the naturally occurring nucleoside antibiotic, cordycepin[8].

HeLa S3 cells were maintained at 37°C in suspension culture in MEM supplemented with 5% fetal calf and 5% calf serum. Asynchronously grown cells were diluted with fresh medium to 2.5×10^5 cells/ml. A 100 ml aliquot of culture was incubated with each compound. After 2.5 hours of treatment, 2 ml aliquots of culture were removed, washed once with Spinner buffer, and incubated with 1 ml of either [^{3}H]thymidine (1 μCi/ml, 20 Ci/mmole) or [^{3}H]uridine (1 μCi/ml, 30 Ci/mmole). DNA and RNA syntheses were measured by the [^{3}H]thymidine or [^{3}H]uridine incorporated into the acid-insoluble material. Poly(ADP-ribose) polymerase activity in nuclei was determined as described by LICHTENWALNER and SUHADOLNIK[9]. Briefly, 60 ml of culture was centrifuged ($3,000\times g$, 5 minutes) 3 hours after treatment; the cells were Dounce homogenized in buffer (10 mM tris-HCl, 1 mM EDTA, 4 mM $MgCl_2$, 6 mM 2-mercaptoethanol (pH 7.8)). The resulting nuclei were washed three times with the same buffer and centrifuged. The reaction mixture (100 μl) contained 5×10^6 nuclei, 100 μM [adenine-^{14}C]NAD (229 mCi/mmole), 10 mM $MgCl_2$, 200 mM sucrose, and 67 mM tris-HCl (pH 7.5). Poly(ADP-ribose) polymerase activity in permeabilized cells was determined as described[10]. Four ml aliquots of cells were removed, washed three times with phosphate buffered saline and permeabilized with a buffer composed of 10 mM tris-HCl, 1 mM EDTA, 4 mM $MgCl_2$, 1 mM DTT and 0.05% Triton X-100 (pH 7.3). Poly(ADP-ribose) glycohydrolase activity was determined using the cytoplasmic fraction of HeLa cells as described[11].

The reaction was terminated by adding 10% TCA containing 2% pyrophosphate. Radioactivity in the acid insoluble poly(ADP-ribose) was determined.

Bleomycin showed a 14.3 fold increase in poly(ADP-ribose) polymerase activity in permeabilized cells and a 2.5 fold increase in isolated nuclei (Table 1). Neocarzinostatin showed a 2.9 fold increase in permeabilized cells and a 1.5 fold increase in isolated nuclei. These two antibiotics have a radiomimetic sensitization effect on mammalian cells; they cleave double stranded DNA with a subsequent increase in poly(ADP-ribose) polymerase activity[3,4]. Actinomycin D and cordycepin stimulated poly(ADP-ribose) polymerase activity in both permeabilized cells and isolated nuclei. Actinomycin D interacts directly with DNA by intercalation or by a weaker external binding and interferes with RNA and

Table 1. *In vivo* effect of antibiotics on poly(ADP-ribose) polymerase activity.

Addition	Concentration (μg/ml)	*De novo* synthesis (% of control)		Poly(ADP-ribose) polymerase activity (% of control)		Cytoplasmic poly(ADP-ribose) glycohydrolase activity
		DNA	RNA	Permeabilized cells	Nuclei	
None	—	100[a]	100[b]	100[c]	100[d]	2.0[e]
Bleomycin	20	16	49	1,430	248	2.5
Neocarzinostatin	20	6	85	290	146	2.7
Actinomycin D	5	6	12	150	130	2.5
Cordycepin	20[f]	53	69	130	143	2.7
Sangivamycin	20	46	31	110	110	2.8
Rifampicin	40	67	107	76	96	2.5
Ara-A	40	4	87	59	108	1.6

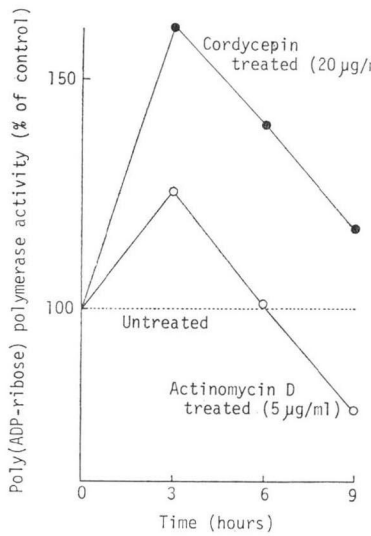
[a] Incorporation of [^{3}H]thymidine (10,600 cpm/1 $\times 10^6$ cells/30 minutes at 37°C)
[b] Incorporation of [^{3}H]uridine (2,600 cpm/1 $\times 10^6$ cells/30 minutes at 37°C)
[c] Incorporation of [adenine-^{14}C]NAD (3.8 pmoles/1 $\times 10^6$ cells/20 minutes at 25°C)
[d] Incorporation of [adenine-^{14}C]NAD (21 pmoles/1 $\times 10^6$ nuclei/20 minutes at 25°C)
[e] Hydrolysis of [^{14}C]poly(ADP-ribose), cpm/μg protein/30 minutes at 37°C. The data are an average of triplicate determinations ($\pm 5\%$). Protein was determined according to LOWRY *et al.*[12]
[f] The same stimulation of poly(ADP-ribose) polymerase was observed with 20 and 100 μg/ml.

DNA polymerase activity by steric hindrance[13]. Cordycepin inhibits RNA synthesis which has an indirect effect on DNA synthesis[14] (for review, see SUHADOLNIK[8]). Also, there is a report that actinomycin D and cordycepin do not act by cleaving DNA strands[15]. Thus, the increased poly(ADP-ribose) polymerase activity produced by actinomycin D and cordycepin occurs by a mechanism which differs from that of either bleomycin or neocarzinostatin. SMULSON and RIDEAU[16] reported that actinomycin D and cordycepin did not stimulate poly(ADP-ribose) polymerase activity in isolated nuclei from actinomycin D and cordycepin treated HeLa cells. LEHMANN *et al.*[17] observed that actinomycin D stimulated poly(ADP-ribose) activity in isolated nuclei from cordycepin treated L5178Y cells.

Sangivamycin, rifampicin and ara-A do not affect poly(ADP-ribose) polymerase activity in either permeabilized cells or isolated nuclei. RNA and DNA synthesis in antibiotic treated cells did not correlate with poly(ADP-ribose) polymerase activity.

To confirm our observation that actinomycin D and cordycepin treated HeLa cells showed an increased poly(ADP-ribose) polymerase activity, time course experiments were done (Fig. 1). Poly(ADP-ribose) polymerase activity in actinomycin D or cordycepin treated cells reached a maximum 3 hours after treatment and then the enzyme activity decreased rapidly. When the nucleoside, cordycepin, was preincubated with nuclei isolated from untreated cells, there was no change in poly(ADP-ribose) polymerase activity (data not shown). The exact mechanism by which

Fig. 1. *In vivo* effect of cordycepin and actinomycin D on poly(ADP-ribose) polymerase activity in isolated nuclei.
Experimental details are as described in the text.



actinomycin D and cordycepin stimulate poly-(ADP-ribose) polymerase activity is under investigation.

The fermentation broth of *Cordyceps militaris*[7] was also tested for poly(ADP-ribose) polymerase activity. A 20 ml aliquot of the broth (cordycepin content approximately 100 μg/ml) was incubated with HeLa cells (80 ml) for 3 hours. Poly(ADP-ribose) polymerase activity in isolated nuclei was increased to 134% of control.

The present study may provide a system suitable for future studies concerned with the screening of naturally occurring compounds for their ability to affect the nucleus or chromatin structure of mammalian cells.

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