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## Abstract

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**KEYWORDS:** DNA synthesis in vitro, polyamine, polyanion, daunomycin, neocarzinostatin, bleomycin

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# EFFECTS OF SOME POLYAMINES, POLYANIONS AND ANTITUMOR DRUGS ON REPLICATIVE DNA SYNTHESIS AND UNSCHEDULED DNA SYNTHESIS IN VITRO

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*Abstract.* The effects of various compounds on replicative DNA synthesis in permeable mouse ascites sarcoma cells and on unscheduled DNA synthesis in permeable cells or in isolated rat liver nuclei were studied. Polyamines such as spermidine, putrescine and cadaverine inhibited replicative DNA synthesis. Unscheduled DNA synthesis was inhibited by spermidine and cadaverine, but slightly stimulated by putrescine at low concentrations. Aurintricarboxylic acid, a low molecular weight polyanion, inhibited both replicative DNA synthesis and unscheduled DNA synthesis. Replicative DNA synthesis was inhibited by heparin, a high molecular weight polyanion, whereas unscheduled DNA synthesis was stimulated at low heparin concentrations. Antitumor drugs such as daunomycin, neocarzinostatin and bleomycin inhibited replicative DNA synthesis. Unscheduled DNA synthesis was inhibited by daunomycin, slightly induced by neocarzinostatin and highly induced by bleomycin. None of the drugs tested stimulated replicative DNA synthesis. The present system was thought to be useful for studying the separate effects of various drugs on either replicative DNA synthesis or unscheduled DNA synthesis *in vitro*.

*Key words:* DNA synthesis *in vitro*, polyamine, polyanion, daunomycin, neocarzinostatin, bleomycin

Unscheduled (repair) DNA synthesis in rapidly growing cells is extremely low compared with replicative DNA synthesis. And also replicative DNA synthesis in intact cells is relatively easily discriminated from unscheduled DNA synthesis. In *in vitro* DNA synthesis such as DNA synthesis in isolated nuclei, whereas, both replicative DNA synthesis and unscheduled DNA synthesis frequently occur simultaneously, because nuclear damage which is brought about during the process of nuclear preparation causes to decrease replicative DNA synthesis and to induce unscheduled DNA synthesis. To assess *in vitro* DNA synthesis, the discrimination of replicative DNA synthesis from unscheduled DNA synthesis is required. However, there are some advantages of *in vitro* DNA

synthesis systems. *In vitro* systems are simpler than *in vivo* systems, because no permeability barrier to low molecular weight substances is present and nucleotide-synthesizing systems are not necessary to consider. In *in vitro* systems, replicative DNA synthesis is mostly studied in isolated nuclei and in nucleotide-permeable cells. The impairment of replicative DNA synthesis and induction of unscheduled DNA synthesis are minimal in permeable cell systems (1-8). In the present communication, the effects of various compounds on DNA synthesis *in vitro* were studied by using both permeable cell and nuclear systems.

## MATERIALS AND METHODS

*Materials.* Reagents used in the present experiments were obtained from the following sources; deoxynucleoside triphosphates from P-L Biochemicals; [$^3$H] deoxythymidine triphosphate ([$^3$H] dTTP) from Radiochemical Centre, U.K.; spermidine, putrescine, cadaverine, heparin and 1-$\beta$-D-arabinofuranosyl cytosine 5'-triphosphate (araCTP) from Sigma Chemical Co.; aurintricarboxylic acid (formula weight, 473.44) from Wako Pure Chemicals Co. (Tokyo). Copper-free bleomycin $A_2$, daunorubicin hydrochloride (daunomycin) and neocarzinostatin were kindly provided by Nippon Kayaku Co. (Tokyo), Meiji Seika Co. (Tokyo) and Kayaku Antibiotics Research Co. (Tokyo), respectively. Mouse ascites sarcoma (SR-C3H/He) cells were obtained and maintained as reported previously (4). Ascites cells were collected from ascites fluid of mice 3-5 days (nearly exponentially growing phase) after a 0.05 ml ascites inoculation per mouse.

*Preparations of permeable cells and rat liver nuclei.* Permeable cells were prepared from SR-C3H/He cells by a hypotonic procedure (4) or by an isotonic detergent procedure (5). Nuclei were prepared from male Donryu rats weighing 250-300 g each by the procedure described previously (9).

*Assay of DNA synthetic activity.* Replicative DNA synthesis was measured as described previously (5). Permeable cells ($2\times10^6$ cells) suspended in 0.38 ml of permeabilizing buffer were mixed with 0.2 ml of a DNA replicase substrate mixture consisting of 0.1 M Tris-HCl buffer, 7 mM $MgCl_2$, 0.24 M NaCl, 7.5 mM ATP, 0.15 mM dATP, 0.15 mM dCTP, 0.15 mM dGTP and 7.5 $\mu$M [$^3$H] dTTP (0.5 Ci/mmol), adjusted to pH 8.0 at 25°C. Unscheduled DNA synthesis was measured by essentially the same method as that for replicative DNA synthesis except that isolated rat liver nuclei ($4\times10^6$ nuclei/assay) were usually used and that ATP was usually omitted from the assay mixture. Compounds to be examined were added at 0.01 or 0.02 ml per assay. The final reaction volume was adjusted to 0.6 ml per assay. Incubation was conducted at 37°C for 10 or 20 min. [$^3$H] dTMP incorporated into the acid-insoluble fraction was measured by the glass fiber disc method described previously (4).

## RESULTS

*Replicative DNA synthesis and unscheduled DNA synthesis.* The nature of DNA synthesis was determined by the deoxynucleoside triphosphate-require-

ment, ATP-dependency, N-ethylmaleimide sensitivity, araCTP sensitivity, and autoradiographical analysis of DNA synthesis, as described previously (9, 10). DNA synthesis measured in the presence of ATP and four deoxynucleoside triphosphates in permeable cells which were prepared from rapidly growing SR-C3H/He cells was mostly replicative. DNA synthesis in rat liver nuclei was mostly unscheduled.

*Effects of some polyamines on DNA synthesis.* Spermidine, putrescine and cadaverine all inhibited replicative DNA synthesis in permeable cells (Fig. 1, 2, 3). Unscheduled DNA synthesis in rat liver nuclei was inhibited by spermidine and cadaverine. Putrescine stimulated unscheduled DNA synthesis slightly at

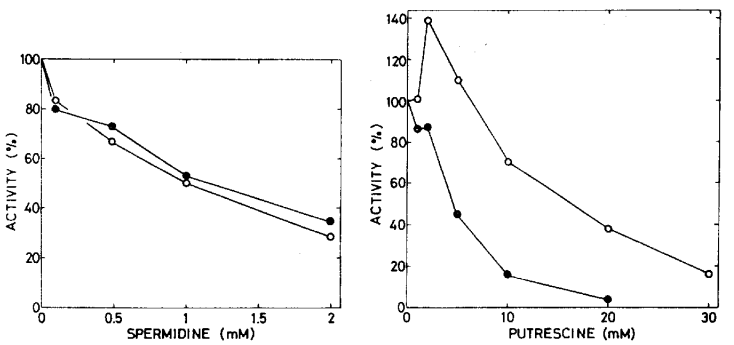


Fig. 1. Effect of spermidine on DNA synthesis. Permeable cells and rat liver nuclei were prepared as described in "Methods". Sperimidine was added at the concentrations indicated. DNA synthetic activity is expressed as per cent of spermidine-free control. ●, replicative DNA synthesis in permeable cells; ○, unscheduled DNA synthesis in rat liver nuclei.

Fig. 2. Effects of putrescine on DNA synthesis. Assay was conducted as described in "Methods". Putrescine was added at the concentrations indicated. DNA synthetic activity is expressed as per cent of putrescine-free control. ●, replicative DNA synthesis in permeable cells; ○, unscheduled DNA synthesis in rat liver nuclei.

low concentrations (around at 2 mM) and caused inhibition at higher concentrations (Fig. 2).

*Effect of some polyanions on DNA synthesis.* As shown in Fig. 4, aurintricarboxylic acid, a low molecular weight polyanion, inhibited both replicative DNA synthesis in permeable SR-C3H/He cells and unscheduled DNA synthesis in rat liver nuclei. Heparin, a high molecular weight polyanion, inhibited replicative DNA synthesis in permeable cells (Fig. 5). Unscheduled DNA synthesis was stimulated at low concentrations (2–20 $\mu$g/0.6 ml) of heparin and inhibited at higher concentrations (Fig. 5). Unscheduled DNA synthesis in nuclei isolated from other types of cells was also stimulated at low concentrations (9, 11).

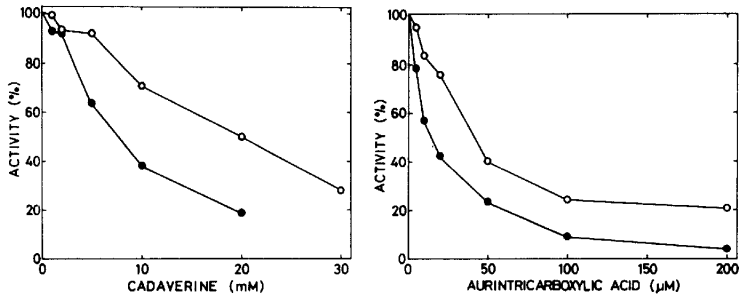


Fig. 3. Effect of cadaverine on DNA synthesis. Cadaverine was added in the assay mixture at the concentrations indicated. ●, replicative DNA synthesis in permeable cells; ○, unscheduled DNA synthesis in rat liver nuclei.

Fig. 4. Effect of aurintricarboxylic acid on DNA synthesis. Aurintricarboxylic acid was added at the concentrations indicated. ●, replicative DNA synthesis in permeable cells; ○, unscheduled DNA synthesis in rat liver nuclei.

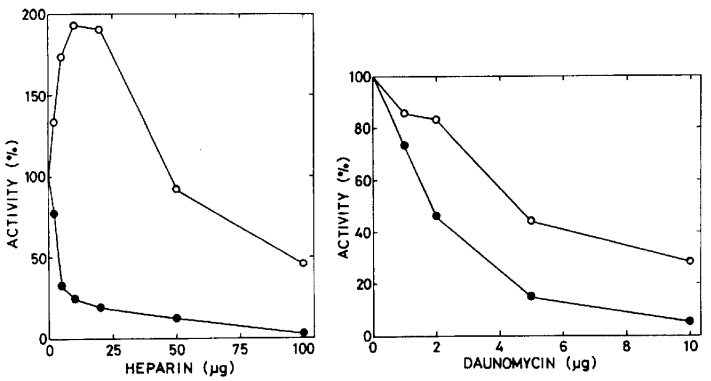


Fig. 5. Effects of heparin on DNA synthesis. Heparin was added in the assay mixture of 0.6 ml at the amounts indicated. ●, replicative DNA synthesis in permeable cells; ○, unscheduled DNA synthesis in rat liver nuclei.

Fig. 6. Effect of daunomycin on DNA synthesis. Daunomycin was added in the assay mixture of 0.6 ml at the amounts indicated. ●, replicative DNA synthesis in permeable cells; ○. unscheduled DNA synthesis in rat liver nuclei.

*Effects of some antitumor drugs on DNA synthesis.* Daunomycin inhibited both replicative DNA synthesis and unscheduled DNA synthesis (Fig. 6). Neocarzinostatin inhibited replicative DNA synthesis (Fig. 7A). Unscheduled DNA synthesis was not affected by neocarzinostatin without 2-mercaptoethanol, but stimulated in the presence of 2-mercaptoethanol (Fig. 7B). Bleomycin inhibited replicative DNA synthesis as shown in Fig. 8A. Unscheduled DNA synthesis was induced markedly not only in rat liver nuclei but also in permeable cells (Fig. 8A and 8B).

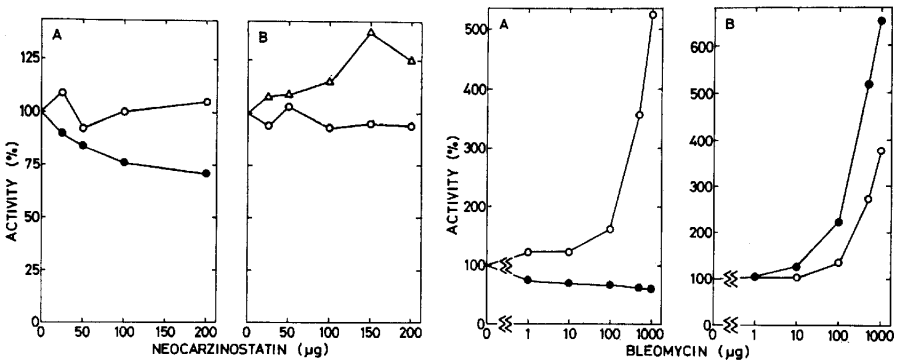


Fig. 7. Effects of neocarzinostatin on DNA synthesis. Neocarzinostatin was added in the assay mixture of 0.6 ml at the amounts indicated. A. ●, replicative DNA synthesis in permeable cells; ○, unscheduled DNA synthesis measured without ATP and 2-mercaptoethanol in permeable cells. B. △, unscheduled DNA synthesis in rat liver nuclei; ○, unscheduled DNA synthesis measured without 2-mercaptoethanol in liver nuclei.

Fig. 8. Effects of bleomycin on DNA synthesis. Bleomycin was added in the assay mixture of 0.6 ml at the amounts indicated. A. ●, replicative DNA synthesis in permeable cells; ○, unscheduled DNA synthesis measured without ATP in permeable cells. B. ○, unscheduled DNA synthesis in rat liver nuclei; ●, unscheduled DNA synthesis measured in rat liver nuclei with the addition of 2.5 mM ATP.

TABLE 1. EFFECTS OF VARIOUS COMPOUNDS ON REPLICATIVE DNA SYNTHESIS AND UNSCHEDULED DNA SYNTHESIS

| Compound | Replicative DNA synthesis | Unscheduled DNA synthesis |
|---|---|---|
| Spermidine | Inhibition | Inhibition |
| Putrescine | Inhibition | Stimulation & Inhibition[a] |
| Cadaverine | Inhibition | Inhibition |
| Aurintricarboxylic acid | Inhibition | Inhibition |
| heparin | Inhibition | Stimulation & Inhibition[a] |
| Daunomycin | Inhibition | Inhibition |
| Neocarzinostatin | Inhibition | No effect or Induction |
| Bleomycin | Inhibition | Induction |
| araCTP | Inhibition | No effect[b] |
| ddTTP | No effect[c] | Inhibition[d] |

*a* Stimulation at low concentrations and inhibition at high concentrations.
*b* No effect at the concentrations examined. *c* Data from ref. 13.
*d* An unpublished result.

## DISCUSSION

The separate effects of various compounds on replicative DNA synthesis and unscheduled DNA synthesis were studied in the present paper, and the results are summarized in Table 1. Compounds acting on DNA synthesis in-

hibited replicative DNA synthesis *in vitro*. None of the drugs tested stimulated replicative DNA synthesis. Unscheduled DNA synthesis was also inhibited by most of the compounds, but a few drugs stimulated or induced unscheduled DNA synthesis. As reported previously (10, 12), araCTP inhibited replicative DNA synthesis selectively at 0.2 mM. Selective inhibition of unscheduled DNA synthesis should occur with 2′, 3′-dideoxythymidine-5′-triphosphate (ddTTP) (13), although this has not been proven yet.

A close relationship was observed between high polyamine content in cells and rapid cell proliferation, suggesting a role for polyamines in DNA replication (14, 15). Yoshida *et al.* (16) have reported that low concentrations of polyamines stimulated the activity of calf thymus $\alpha$ and $\beta$ DNA polymerase. Slight stimulatory effects of polyamines on DNA synthesis have been observed in isolated rat liver nuclei (17) and in isolated HeLa nuclei (18). So far as we know, no comparative study of the effects of polyamine on replicative DNA synthesis and unscheduled DNA synthesis has been reported. The present experiment showed differential effects of putrescine on replicative DNA synthesis and unscheduled DNA synthesis. No stimulation of replicative DNA synthesis by polyamines was observed in permeable cells. Krokan and Eriksen (18) suggested that DNA synthesis-initiation, which might not occur in *in vitro* system such as the nuclear system, depends on polyamines.

The activities of purified DNA polymerase $\alpha$, $\beta$ and $\gamma$ were inhibited by heparin (19). Stimulation of *in vitro* nuclear DNA synthesis by high molecular weight polyanions including heparin was attributed to the release of the template restriction of the nuclear DNA (11). The difference in the effect of heparin on replicative DNA synthesis and unscheduled DNA synthesis is probably due to structural differences in chromatin sites of both types of DNA synthesis (9).

Daunomycin, an intercalating dye in double-stranded DNA, inhibited *in vitro* both replicative DNA synthesis and unscheduled DNA synthesis, probably by the same mechanism as it inhibits DNA synthesis *in vivo* (20). Neocarzinostatin induction of unscheduled DNA synthesis was attributed to the DNA damage and to repair of the damaged DNA, and has been shown in human lymphocytes (21), and in HeLa cells and isolated nuclei (22). A difference in the effects of neocarzinostatin on replicative DNA synthesis and unscheduled DNA synthesis, as reported in the present paper, has not been reported. Bleomycin which is also known to break DNA markedly induced unscheduled DNA synthesis *in vitro* (Fig. 8). Relatively low induction of unscheduled DNA synthesis with neocarzinostatin compared with the bleomycin-induction was probably due to the fact that neocarzinostatin produced DNA fragments with a 3′-phosphate end group (23), which has no primer activity on DNA polymerization. The reasons these drugs (neocarzinostatin and bleomycin) have different effects on

replicative DNA synthesis and unscheduled DNA synthesis are not known. Recently, Coetzee *et al.* (24) showed a selective response of DNA polymerase $\beta$ to bleomycin-induced breaks in DNA, and suggested that DNA polymerase $\beta$ is involved in DNA repair (unscheduled DNA synthesis).

The present comparative study system is thought to be useful for studying drug effects on DNA synthesis *in vitro* with a distinction between replicative DNA synthesis and unscheduled DNA synthesis.

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