Effect of Cisplatin Alone and in Combination with γ-Radiation on the Initiation of DNA Synthesis in Friend Leukemia Cells

Roumiana L. Ganeva and Lyuben M. Tzvetkov

Institute of Molecular Biology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

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The effect of the anticancer drug cisplatin (alone and in combination with γ-radiation) on the initiation of DNA synthesis in Friend leukemia cells was studied. A method for isolation of DNA fractions containing the origins of replication was used. It was found that cisplatin decreased the rate of the initiation of DNA synthesis. The mild γ-radiation has previously been observed to inhibit the initiation of DNA synthesis. In the present investigation the combination of cisplatin and γ-radiation showed additive effects without synergism on the initiation of DNA biosynthesis.

Cisplatin (*cis*-diamminedichloroplatinum(II), *cis*-DDP) is a square planar platinum complex. At present it is one of the most widely used agents for treatment of different malignancies. The cytotoxicity of the complex is believed to be due to the formation of DNA adducts (Lippert, 1992). The characteristic modes of binding of *cis*-DDP to DNA have been well defined (Fichtinger-Schepman *et al.,* 1985; Eastman, 1987). *Cis*-DDP – DNA intrastrand cross-links between two adjacent guanine residues seem to be a lesion responsible for the antitumor effect of the drug (Sherman and Lippard, 1987). *Cis*-DDP inhibits DNA synthesis (Howle and Gale, 1970). Gibbons *et al.* (1991) have used alkaline sucrose gradient sedimentation to determine the effect of some platinum compounds on the replicon initiation and DNA chain elongation in L1210 cell lines. They have shown that the initiation of DNA replication is inhibited by *cis*-DDP-DNA adducts to a greater extent then the chain elongation.

Reprint request to Dr. R. Ganeva.
Fax: 00359-2-723-507.

In the present investigation we studied the effect of *cis*-DDP alone and in combination with γ-radiation on the initiation of DNA synthesis in Friend leukemia cells. It has been previously shown that mild γ-radiation inhibits the initiation of DNA replication (Lallev *et al.,* 1993).

We studied the effect of different concentrations of cisplatin on the total biosynthesis of DNA in Friend leukemia cells. Two hours preincubation of the cells with 2 μM cisplatin inhibited the total DNA biosynthesis (about 30% – 0.031 pmol [^{3}H]thymidine per μg DNA was the incorporation in the control cells) and further increase of the concentration of the complex did not change its effect significantly. This rather small effect of cisplatin is due on the relatively short time of preincubation of the cells with cisplatin. Our purpose was to achieve such effect in order to may register the effect of the combined action of cisplatin and γ-radiation.

To investigate the effect of cisplatin and γ-radiation on the initiation of DNA biosynthesis we used the quantitative method for determination of the rate of the initiation of DNA synthesis developed in our institute (Russev and Vassilev, 1982). Briefly, after exposure to cisplatin and/or γ-radiation the cells were treated with 4,5',8-trimethylpsoralen (trioxsalen) and long wave ultraviolet light to introduce interstrand cross-links at a distance 3 kbp in DNA *in vivo* and were labelled with [^{3}H]thymidine for 45 min. In the course of this radiolabelling the precursor is incorporated into DNA as a result of three different pathways, each leading to the formation of different products. (a) Elongation of the already initiated DNA chains continues until the replication forks reach trioxsalen cross-links. This produces DNA stretches, which are covalently linked by their 5'-ends to the high molecular weight DNA and during subsequent procedures remain bound to it. (b) As a result of repair short stretches of labelled DNA are synthesised, which are covalently attached by both their ends to the high molecular weight DNA and during subsequent procedures remain bound to it. (c) Short DNA sequences initiated at origins of replication located between trioxsalen cross-links are syntesised. They are not ligated to the high

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molecular weight DNA and under denaturing conditions are released to form a fraction of newly synthesised DNA fragments. Upon electrophoresis under denaturing conditions cross-linked DNA forms a slow-moving peak, while the fragments synthesised at origins of replication form a fast-moving peak (Fig. 1). Provided equal amounts of DNA (measured as ^{14}C counts) were loaded onto the gel, the amount of ^{3}H counts incorporated in the low molecular mass peak containing the nascent DNA fragments is directly and quantitatively proportional to the number of initiation events.

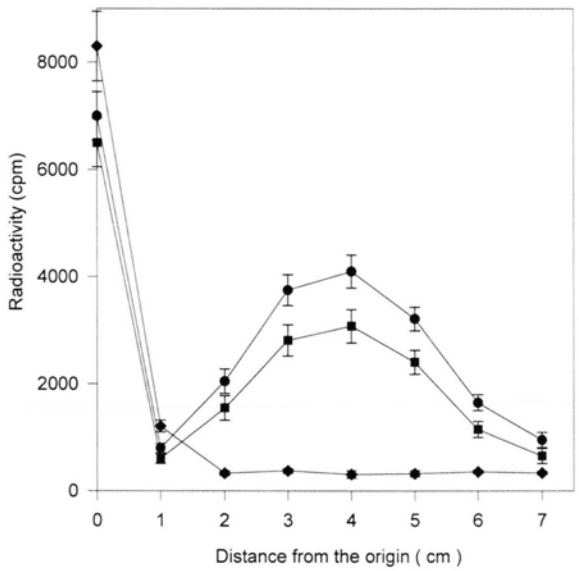

Fig. 1. Effect of cisplatin on the initiation rate of DNA synthesis in Friend leukemia cells. The cells were labelled uniformly with 0.004 MBq/ml [^{14}C]dThd for 24 h. Then they were preincubated with 2 μM cisplatin for two hours, cross-linked with trioxsalen to give 3 kb between the cross-links and labelled with 0.8 MBq/ml [^{3}H]dThd for 45 min. DNA was isolated, denatured and electrophoresed in a 1% agarose gel. Gels were cut into 1 cm slices and the radioactivity counted. Radioactivity was plotted against distance from the origin of gel. ● – control cells; ■ – cells preincubated with 2 μM cisplatin for two hours; ◆ – radioactivity from [^{14}C]thymidine, incorporated into DNA from control cells. The means of three experiments and the standard deviations are shown.

The electrophoretic profiles of cross-linked and denatured DNA samples, isolated from Friend cells after treatment with cisplatin, are shown in Fig. 1. The areas under the peaks are a direct marker for the initiation rates. Preincubation of the cells for two hours with 2 μM cisplatin decreased the rate of the initiation of DNA replication (about 25%). A further increase of cisplatin concentration did not significantly change the effect (results not shown).

The effect of the combination of cisplatin and γ-radiation on the initiation of the DNA biosynthesis is shown in Fig. 2. The cells were preincubated with 2 μM *cis*-DDP for two hours and exposed to γ-radiation (5 $J.cm^{-1}.min^{-1}$) before cross-linking with trioxsalen. The initiation rates were calculated from the areas of the respective fast-moving peaks in the gels and were expressed relative to the control. *Cis*-DDP and γ-radiation reduced the rate of initiation of DNA synthesis by about 25% and 40% respectively. The effect of the combined action of the two agents was additive without synergism – about 55% inhibition of the initiation of DNA biosynthesis. On the basis of this result it

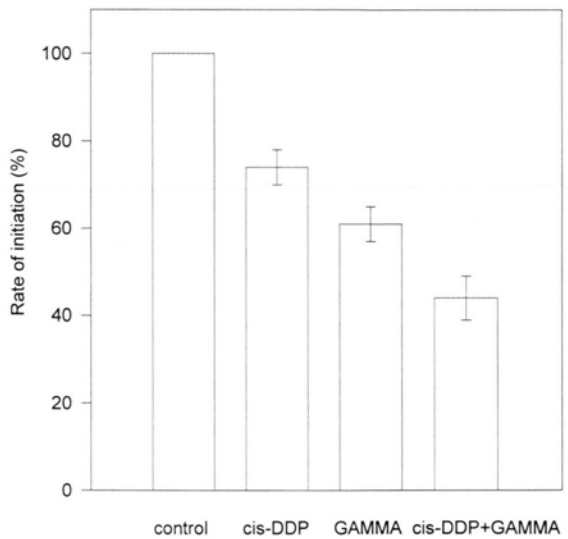

Fig. 2. Effect of the combined action of cisplatin and γ-radiation on the initiation rate of DNA in Friend leukemia cells. The cells were preincubated with 2 μM cis-DDP (cisplatin) for two hours and/or exposed to γ-radiation (5 $J.cm^{-1}.min^{-1}$) before cross-linking with trioxsalen. The DNA labelling and analysis were the same as in Fig. 1. Rates of replicon initiation were calculated from the areas of the respective fast-moving peaks in the gels and were expressed relative to the control. control – control cells; cis-DDP – cells preincubated with 2 μM cisplatin for two hours; GAMMA – cells exposed to γ-radiation (5 $J.cm^{-1}.min^{-1}$); cis-DDP+GAMMA – cells preincubated with 2 μM cisplatin for two hours and exposed to γ-radiation (5 $J.cm^{-1}.min^{-1}$). The means of three experiments and the standard deviations are shown.

may be assumed that both agents affect the initiation of DNA biosynthesis by different mechanisms.

Experimental

Cis-DDP was synthesised as described elsewhere (Spassovska *et al.*, 1981). Friend erythroleukemia cells, clone F_4N, were cultured in MEM-S, supplemented with 10% calf serum and antibiotics in 5% CO_2. To label uniformly cellular DNA cells were incubated with 0.004 MBq/ml [^{14}C]thymidine (dThd) for 24 h.

Cells (10^6 cells/ml) were treated with different concentrations of *cis*-DDP for 2 hours and/or irradiated with ^{60}Co-source at a rate of 5 J.cm^{-1}.min^{-1} in an ice bath. The cells were treated with 4,5',8-trimethylpsoralen (trioxsalen) and ultraviolet light (365 nm) to introduce interstrand trioxsalen cross-links between the two DNA chains as described in [7] (with distance between the cross-links of 3 kbp). The cross-linked cells were labelled with 0.8 MBq/ml [^{3}H]dThd for 45 min. To isolate high-molecular DNA cells were washed twice in NaCl/Pi (0.14 M NaCl, 0.01 M sodium phosphate) and were lysed in 1 M NaCl, 1% sodium dodecylsulfate, 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7, 10 mM EDTA at 37 °C. Proteins were digested with 200 μg/ml proteinase K and DNA was extracted with phenol/chloroform (1:1, by vol.) and precipitated with ethanol. DNA was dissolved in 10 mM Tris-HCl, 1 mM EDTA, pH 8 and the specific radioactivity was determined. DNA was denatured in glyoxal/dimethylsulfoxide/0.1 M sodium phosphate, pH 7 (2.2:5:1 v/v) at 55 °C for one hour (McMaster and Carmichael, 1977) and was electrophoresed in 1% agarose gel in 10 mM sodium phosphate, pH 7. The lanes were cut into 1 cm bands, dissolved by boiling in 0.5 ml 0.1 M HCl, and the radioactivity measured and plotted against the distance from the origin of the gel.

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