Note

Effects of cis-Platin on t-RNA of Saccharomyces cerevisiae

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Since the early 60's it has been known that platinum coordination complexes are significant in the treatment of human cancers. Among others, cis-diammine-dichloroplatinum(II) (Peyrone's chloride, cis-DDP) is widely used for platin-based chemotherapy. Cytotoxicity, anticancer activity and mutagenesis as well as carcinogenetic effects of this complex have been described. The anticancer activity is believed to result from chemical interaction with DNA leading to a DNA-synthesis inhibition as the major biochemical change in the cell. Since cis-DDP has a strong preference of coordination to purine nucleotides the most likely reaction is a direct attack of the platinum drug on the cellular DNA.1–18

It is necessary to make a distinction between cytotoxicity and anticancer activity. The former is nonselective, while the latter is very selective. Both possible diammine-dichloroplatinum-complexes, namely the cis- and the trans-configuration, are cytotoxic but only the cis-DDP is an anticancer agent, trans-DDP is not. Different reaction products of both complexes with DNA are described where the cis isomer forms a closed ring chelate complex with a guanine residue while the trans isomer acts via a monodentate bond.19

The effects of cis-DDP are often compared to alkylating agents but experiments with resistant and/or cross-resistant cells to cis-DDP and alkylating agents suggest that there may be additional mechanisms of action for cis-DDP.11,19–22 The mechanism common to all reactions of cis-DDP is thought to be substitution of nucleophilic groups for one or both of the coordinated chlorides. Probably cis-DDP is intracellularly converted into the more reactive aminated form in which the chloride groups have been exchanged by hydroxyl groups.23

cis-DDP also affects the control of several steroid hormones24 as well as the activity of some enzymes by reacting with essential SH-groups.25 As we generally investigate the effects of several drugs used for chemotherapy on t-RNA28 and no information is given about possible reactions of cis-DDP with RNA we studied the reactivity of t-RNA of Saccharomyces cerevisiae with this drug.

t-RNAs were prepared as described28,29 cis-DDP was purchased from Bristol-Myers, S. A. E. (Madrid, Spain). Cysteine, cysteine, homocysteine, and glutathione were purchased from Sigma (Munich, F.R.G). All other chemicals were of analytical grade. t-RNA (4 mg) was dissolved in different buffers and incubated with the cis-DDP compound for 10 h at room temperature. Fifty μl of this incubation solution was separated electrophoretically. PAGE was done by the method of Ebermann und Bodeneseher30 with a separation gel of 20% acrylamide, crosslinked 1:30 with N,N'-methylenbisacryl-amide. The buffer was a tris/glycine system, 0.2 M, pH 8.9. A constant voltage of 300 V and a starting current of 100 mA were used. After separation lasting for 3 h the gel was treated with 5% acetic acid for 1 h and incubated with 2% methylene blue for staining. Decoloring of the background was done by waterizing the gel for several hours.

Untreated t-RNA separated in one main fraction with the highest electrophoretic mobility, which can be subdivided in a lot of sharp bands. Two further clearly separated fractions with lower electrophoretic mobility were observed (Fig. 1, lane 1). Treatment with cis-DDP leads to changes in the electrophoretic characteristics of t-RNA, with means although the fractions migrate at the same position in the gel all the bands become blurred. The minimal concentration of cis-DDP causing this effect was found to be 10−4 M (Fig. 1, lane 2). The bands appear smeared after electrophoretic separation and no degradation products with lower molecular weight were observed. As with RNA, no interstrand crosslinks like those with DNA were possible. The effects of cis-DDP on t-RNA molecules seem to be based on decationation or the generation of RNA-monoadducts.9,16

Since the reaction of cis-DDP with thiols has been described, the effects of thiols on the alteration of t-RNA by cis-DDP was investigated. Several thiols, cysteine, cysteine, homocysteine, glutathione, and methionine, were tested and in all cases the alteration of t-RNA was inhibited depending on the concentration rate of cis-DDP and thiol. There were no differences in the effective concentrations of the different thiol except glutathione and methionine, which were just inhibiting t-RNA alteration at a tenfold concentration. In Fig. 1 the effects of different concentrations of cystine are shown. To investigate the dependence of the reaction on the incubation solution and pH we tested different buffers. The alterations in

Fig. 1. Electrophoretic Separation of t-RNA Treated with cis-DDP and Cysteine in Different Concentrations.

t-RNA (4 mg) was dissolved in 1 ml of phosphate buffer (0.01 M, pH 7.3) and incubated with cis-DDP (3.3 × 10−4 M) for 10 h at room temperature. t-RNA (lane 1); t-RNA + cis-DDP (lane 2). Inhibition experiments were done by addition of cysteine in different concentrations to the incubation solution containing t-RNA + cis-DDP. Cysteine concentration: 10−4 M (lane 3); 10−5 M (lane 4); 10−6 M (lane 5); 10−7 M (lane 6); 10−8 M (lane 7).
Fig. 2. Electrophoretic Separation of t-RNA Treated with cis-DDP in Different Solutions.

T-RNA (4 mg) was dissolved in 1 ml of different solutions and incubated with cis-DDP (3.3 x 10^{-3} M) for 10 h at room temperature. Control without cis-DDP (lane 1): phosphate buffer (0.01 M, pH 7.3) (lane 2); carbonate buffer (0.01 M, pH 7.3) (lane 3); Tris-citrate buffer (0.01 M, pH 7.3) (lane 4); phys. NaCl solution (lane 5).

t-RNA structure due to cis-DDP are stronger in the presence of inorganic phosphate. Less change of t-RNA can be detected after incubation in carbonate, Tris-citrate, and physiological NaCl-solution. Obviously the reaction of cis-DDP with t-RNA is greatly accelerated by inorganic phosphate (Fig. 2). No influence of pH is observed from pH 5.0 up to 8.0 (not shown). In the case of DNA it is assumed that the dichloro-diammino-Pt(II)-complex is converted by water to the more reactive dihydroxy-diammino-Pt(II)-complex in the first step. A possible explanation of the catalytic function of the phosphate ion in the case of t-RNA seems to be that the diphospho-complex is formed instead of the dihydroxy-complex, which might have a greater reactivity towards t-RNA. A theoretical study investigating the energetics of direct and through-water phosphate-ammine interactions makes the formation of a cis-diammine-diphosphato-Pt(II)-complex likely. Since phosphate is a substance involved in most biochemical processes understanding of phosphate-catalyzed reactions is therefore of fundamental interest.

References