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THE EFFECT OF CIS-DIAMMINEDICHLOOROPLATINUM (II) ON THE FORMATION OF DNA-Pt-DNA CROSS-LINKS

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The effect of cis-diamminedichloroplatium (II) (cis-DDP) on the formation of interstrand cross-links in DNA and in DNA and chromatin complex from leukocytes was studied. Following the use of cis-DDP the number of DNA-DNA interstrand cross-links was elevated with the increase of cis-DDP concentration and elongation of reaction time. It was also found that nucleic proteins reduce the quantity of the cis-DDP induced DNA-DNA interstrand cross-links in the DNA in nucleoprotein complex when compared with the links in the isolated DNA.

Cis-diamminedichloroplatium (cis-DDP) a drug used in tumour therapy, reacts in a cell preferentially with DNA nitrogen bases. A few possible models of the cis-DDP-DNA reaction are assumed: i, chelate binding to one base; ii, intrastrand cross-link; iii, interstrand cross-link; iii, DNA-protein cross-links.

DNA-DNA interstrand cross-links resulting from the coordination of two nitrogen bases from the opposing DNA chains are mainly found in the regions rich in G-C pairs thus suggesting the formation of difunctional links between guanine and cytosine or guanine and guanine. It was shown, however, that cis-DDP binds most efficiently to guanine N7, and cross-links are mostly made between guanine bases of adjacent DNA chains [1]. Our earlier assays on the in vitro model system demonstrated differences in the amount of DNA interstrand links depending on cis- and trans-platinum stereoisomer and temperature [2]. This work is an attempt to determine the kinetics of formation of the DNA interstrand cross-links so as to eventually determine the mechanism of action of the examined drug in a cell.

MATERIALS AND METHODS

DNA and chromatin isolated from pig peripheral blood were used in our experiments. DNA was isolated from blood by the phenol-detergent method according to Maniatis et al. [3]. Chromatin was isolated by Spelsberg

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& Hnilica’s method [4] from nuclei of leucocytes which were obtained according to Maniatis et al. [3]. The purity of isolated DNA chromatin was determined by UV-spectrophotometry using absorbance ratios at characteristic wavelengths. The DNA concentrations was measured by Burton’s method [5], RNA by the orcinol method [6] and protein according to Lowry et al. [7]. The isolated DNA showed high purity and was almost free from RNA and protein. The UV spectrum in 5 M urea was read at respective wavelengths and the absorbance ratios for isolated chromatin were found within accepted limits. DNA and chromatin samples were dissolved in borate buffer, pH 9.5 to a final concentration of 40 μg DNA/ml. DNA and chromatin samples were incubated with cis-DDP at the defined ri values: 0.025, 0.1, 0.5 (ri express μmols of incorporated platinum per μmol of DNA nucleotides) for 3.7 and 18 h at 37°C. The cis-DDP solution in 50 mM sodium perchlorate pH 6.2 was preincubated for 72 h at 37°C before adding to DNA samples to activate cis-DDP during hydrolysis.

The parallel control samples were incubated without cis-DDP. The cis-DDP solution in 50 mM sodium perchlorate pH 6.2 was preincubated for 72 h at 37°C before adding to DNA samples. The spectrofluorometry method [8, 9, 10] with etidium bromide (EB) was applied to demonstrate the presence of DNA-DNA cross-links. This procedure utilizes the difference in fluorescence after EB binding to double-strand and single-strand yielded by DNA thermal denaturation. The percentage of DNA-DNA interstrand cross-links (ci) was calculated according to:

\[ c_i = \left( \frac{f_i - f_n}{1 - f_n} \right) \times 100\% \]

where \( f_i \) is the ratio of fluorescence after thermal denaturation to fluorescence before denaturation of samples incubated with assayed compounds; \( f_n \) is the ratio of fluorescence after thermal denaturation to fluorescence before denaturation of samples incubated without the assayed compound. The formation of cross-links by the assayed compound protects DNA against complete thermal denaturation. The amount of double-stranded fragments yielded by the compound is proportional to the quantity of formed cross-links. The number of interstrand cross-links in DNA was calculated on the basis of the rate of fluorescence after thermal denaturation at alkaline pH.

RESULTS AND DISCUSSION

In this work we examine the formation of DNA-DNA cross-links after incubation of DNA and chromatin with cis-diaminedichloroplatinum (II) by the spectrofluorometry method. So far the experiments on cis-DDP interaction with cell structures suggested that antitumour activity of this drug could be dependent on the occurrence of DNA-Pt-DNA difunctional adducts
and nitrogen bases may be its binding site [11-13]. Using the spectrofluorometry method under the above described incubation of genetic material with \( \text{cis-DDP} \) DNA - Pt - DNA adducts were found (Table 1 and 2). The formation of interstrand cross-links by \( \text{cis-DDP} \) in DNA is in agreement with the results of other authors [14-15]. The occurrence of DNA - Pt - DNA adducts increases with elongation of incubation time for both DNA and chromatin at

**Table 1**

**The number of DNA-DNA interstrand cross-links formed after DNA incubation with cis-DDP**

<table>
<thead>
<tr>
<th>Time of incubation (h)</th>
<th>( C_i ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_i = 0.025 )</td>
<td>( r_i = 0.1 )</td>
</tr>
<tr>
<td>( r_i = 0.5 )</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 ± 6</td>
</tr>
<tr>
<td>7</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>18</td>
<td>28 ± 9</td>
</tr>
</tbody>
</table>

**Table 2**

**The number of DNA-DNA interstrand cross-links formed after chromatin incubation with cis-DDP**

<table>
<thead>
<tr>
<th>Time of incubation (h)</th>
<th>( C_i ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_i = 0.025 )</td>
<td>( r_i = 0.1 )</td>
</tr>
<tr>
<td>( r_i = 0.5 )</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12 ± 7</td>
</tr>
<tr>
<td>7</td>
<td>16 ± 8</td>
</tr>
<tr>
<td>18</td>
<td>30 ± 10</td>
</tr>
</tbody>
</table>

all \( \text{cis-DDP} \) concentrations examined (Figs. 1 and 2). A similar dependence was observed with respect to the percentage of produced cross-links \( (C_i) \) and \( \text{cis-DDP} \) concentration. At each incubation time the increase of \( \text{cis-DDP} \) concentration was accompanied by a higher percentage of the studied links (Figs. 1 and 2).

The highest rate of increase of the DNA-DNA interstrand cross-links formation for the samples studied can be noted at the \( \text{cis-DDP} \) concentration up to \( r_i = 0.1 \) and incubation time up to 7 h (Figs. 1 and 2). The maximum DNA saturation by \( \text{cis-DDP} \) in control samples is observed after 18 h incubation at the \( r_i \) concentration 0.5. Different plots of the dependence of the number of formed cross-links and incubation time and \( \text{cis-DDP} \) concentration for DNA and DNA in chromatin complex were spotted (Figs. 1 and 2).

The incubation of \( \text{cis-DDP} \) at the lowest \( r_i \) concentration 0.025 shows a slight increase of adducts in case of chromatin. The standard deviations and
accuracy of the method (Tables 1, 2) indicate, however, that the results for both samples are comparable. A significant drop of DNA-Pt-DNA adducts in DNA in a nucleoprotein complex in comparison with the isolated DNA was found for cis-DDP at the concentrations: \( r_1 = 0.1 \); \( r_1 = 0.5 \) and for all incubation times (Figs. 1, 2). The increase of adducts with time most probably originates directly from platination causing mainly the occurrence of monofunctional adducts, and initiating the formation of difunctional ones promoted by the elongation of incubation time. This is in agreement with Roberts et al. [15] who demonstrated using the alkaline elution method that the number both of cross-links and platinum atoms bound to DNA is elevated with the increase of incubation time. Our experiments are in agreement with the data obtained by Wilborn & Brendel in 1989 for DNA of Saccharomyces cerevisiae [16].
REFERENCES