Chromosomal aberration and micronucleus studies of two topoisomerase (II) targeting anthracyclines

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Abstract: Anthracycline antibiotics are widely used in cancer chemotherapy. Doxorubicin and Idarubicin, topoisomerase-targeting anthracyclines, were examined for their effect on chromosomal aberration and micronucleus induction in cultured human lymphocytes employing lymphocyte transformation test and cytokinesis-blocked micronucleus (CBMN) assay. A statistically significant dose-dependent increase in micronucleus frequency (p < 0.001) in binucleated cells was seen as well as a significant increase in chromosomal aberration frequency was also observed for both the drugs. A variety of aberrations were scored including terminal deletions, breaks, gaps, exchanges, fragment formation, ends rejoining, interstitial deletions etc. Nuclear division index was also calculated and showed a cell cycle delay towards higher doses. A number of necrotic and apoptotic cells were also observed at higher concentrations. This confirms the two drugs to be clastogenic and aneugenic.

Key words: Doxorubicin, Idarubicin, Chromosomal aberration assay, CBMN assay

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Introduction

Chemotherapeutic treatments have provided longer survival to the cancer patients with few types of malignancies despite their well known side effects. One of the delayed consequences of the treatment is probability of formation of secondary malignancy. Anticancer drugs have the ability to induce many types of DNA lesions. Some of these lesions could produce chromosomal aberrations representing a significant damage to the genome. The consequences of these effects could potentially lead to carcinogenesis (Albertini et al., 2000). It is, therefore, an essential task in chemotherapy to determine the damaging effects of these drugs on normally dividing cells (Gentile et al., 1998). Cell-based assays are used to identify molecules that are toxic to cells with a predetermined genetic background (Dunstan et al., 2002; Siddique and Afzal, 2005; Mathur and Bhatnagar, 2007).

Anthracycline antibiotics are extensively used in cancer chemotherapy. Doxorubicin, one of the primarily used anthracyclines, is regularly utilized for the treatment of breast cancer (Hiddemann et al., 2005; Henderson and Canelllos, 1980), whileidarubicin (4-demethoxy-daunorubicin) is second-generation anthracycline, a synthetic analog of daunorubicin, the first anthracycline antibiotic used. It is recommended primarily for the treatment of acute myelogenous leukemia, acute lymphoblastic leukemia in children, chronic myelogenous leukemia, myeloblastic syndrome and breast cancer (Cersosimo, 1992; Seiter, 2005). Anthracyclines consist of an aglycone ring coupled with an amino sugar and are able to produce a wide range of biological effects. The planar ring can intercalate between DNA base pairs and the sugar moiety (Zhang and Li, 2000), resulting in change in the shape of DNA helix, interfering with transcription and replication. The primary mechanism thought to mediate the antiproliferative and cytotoxic effects of anthracyclines is the inhibition of the regulation activity of the enzyme, topoisomerase II (Bridewell et al., 1997). Anthracyclines undergo one electron reduction and form free radicals. Blasiak et al. (2002) suggested that reactive oxygen species, including free radicals, might be involved in the formation of DNA lesions induced by idarubicin.

Present research work concentrates on genotoxic action of two anthracycline antibiotics routinely used in chemotherapy viz. doxorubicin and Idarubicin, employing two cytogenetic assays i.e. chromosomal aberration assay and cytokinesis-blocked micronucleus (CBMN) assay. Increased rate of chromosomal aberrations indicates an increased risk for secondary cancer (Hagmar et al., 1994; Bofetta et al., 2007). The CBMN assay provides a reliable index of both chromosomal breakage and loss, and thus provides an idea of any broken chromosome, which might have been lost but will show its presence as micronucleus (El Zein et al., 2006).

Materials and Methods

Chemicals: Phytohaemagglutinin (PHA), Medium RPMI-1640, foetal calf serum and antibiotic-antimycotic solutions were purchased from Gibco, BRL (Paisley, UK). Cytochalasin B was obtained from Sigma Chemical Co (Poole, UK). Doxorubicin was procured from Dabur Pharmaceuticals (Mumbai, India), while idarubicin from Pharmacia and Upjohn (Nerviano, Italy).

Chromosomal aberration assay: Lymphocyte transformation test was performed for the study of chromosomal aberrations employing the protocol by Watt and Stephen, 1986. Medium RPMI-1640, supplemented with 15% foetal calf serum and antibiotics was used for the culture, while PHA was used as mitotic inducer. Different concentrations were tested from 0.001 to 2.0 µg ml⁻¹ for three exposure
Table 1: Micronucleus frequency induced by idarubicin and doxorubicin. Data from two replicates (2000 cells in each) have been pooled and averaged.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (µg ml⁻¹)</th>
<th>Micronucleated cells (%)</th>
<th>Micronucleus per cell ± SE</th>
<th>NDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idarubicin</td>
<td>0.001</td>
<td>1.9*</td>
<td>0.019 ± 0.003</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>2.3**</td>
<td>0.023 ± 0.003</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3.9**</td>
<td>0.039 ± 0.004</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>6.1**</td>
<td>0.061 ± 0.005</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>6.7**</td>
<td>0.067 ± 0.006</td>
<td>1.15</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.001</td>
<td>1.3</td>
<td>0.013 ± 0.002</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>3.2**</td>
<td>0.032 ± 0.004</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>2.8**</td>
<td>0.028 ± 0.004</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5.1**</td>
<td>0.051 ± 0.005</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>5.6**</td>
<td>0.056 ± 0.005</td>
<td>1.29</td>
</tr>
<tr>
<td>Positive control (CP)</td>
<td>0.1</td>
<td>14.3**</td>
<td>0.143 ± 0.008</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td></td>
<td>0.008 ± 0.002</td>
<td>1.79</td>
</tr>
<tr>
<td>Negative control</td>
<td>Distilled water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CP = Cyclophosphamide, SE = Standard error * Significant at p < 0.01, ** Significant at p<0.001, NDI = Nuclear division index

Chromosomal aberrations observed were mainly gaps, breaks, dicentrics and fragments. Less frequently, rings were also observed.

Cytokinesis blocked micronucleus (CBMN) assay: Cytokinesis blocked micronucleus assay was performed as per the protocol of Fenech, 2000. Cultures were planted as mentioned earlier but for the micronucleus assay drug treatment was given at only 24 hr after initiation of culture. Testing concentrations for both the drugs remain the same as of chromosomal aberration assay and cultures were kept in duplicates. Cyclophosphamide was used as positive control and cultures without any drug were considered as negative control. After 44 hr of PHA stimulation, 4.5 µg of cytochalasin-B was added to per ml of culture. Twenty-eight hr after the addition of cytochalasin B, cultures were harvested and slides were prepared by air-drying method. Scoring of micronucleus was done as per the criteria accepted at Washington International Workshop on genotoxicity test procedures (Kirsch-Volders et al., 2000). About 2000 cells were scored per concentration. Student’s t-test was used for calculating the statistical significance. The level of significance was tested from standard statistical table of Fisher and Yates (1963). Nuclear division index (NDI) was also calculated employing the formula,

\[ \text{NDI} = \frac{(M_4 - 2M_2 + 3M_1)}{N} \]

Where \( M_i \) represent the number of cells with one to four nuclei and \( N \) is the total number of cells scored (Eastmond and Tucker, 1989).

Results and Discussion

The data of occurrence of chromosomal aberrations induced by idarubicin and doxorubicin in cultured peripheral blood lymphocytes is presented in Fig. 1 and 2 respectively. For each concentration and exposure duration, about 100 metaphases were observed. A significant dose dependent increase was found in the frequency of chromosomal aberrations induced by both the drugs. Chromosomal aberrations observed were mainly gaps, breaks, dicentrics and fragments. Less frequently, rings were also observed.

The results obtained from CBMN assay induced by two selected drugs are shown in Table 1. Approximately 2000 binucleated cells were observed for each concentration. A significant dose-dependent increase was observed at all concentrations. Cells with nucleoplasmic interactions were also observed. Nuclear division index for proliferating lymphocytes was also calculated (Fig. 3).

Anthracycline antibiotics have wide applications in chemotherapy. Idarubicin, one of the second-generation drugs, is recommended for better antineoplastic activity. The main aim of our study was to compare the genotoxic potential of selected anthracyclines towards normally dividing cells. The ability to induce chromosomal damage in non-cancerous cells may underlie one of the most serious side effects evoked by anticancer drugs i.e. induction of secondary malignancies.
The results obtained indicate that doxorubicin and idarubicin produce a significant increase in frequency of chromosomal aberrations and micronucleus formation. Chromosomal aberrations induced by idarubicin and doxorubicin in cultured peripheral blood lymphocytes were found to be both chromatid and chromosome types such as gaps, breaks and exchanges between sister-arms resulting in the formation of dicentric chromosomes. Increase in the aberrations was found to be significant in almost all the concentrations and exposure duration except few lower dose concentrations. A significant dose-dependent induction of abnormal cells has been previously observed for idarubicin and fludarabine. Similarly, other topoisomerase II inhibitors such as etoposide, aclacinomycin (Hajji et al., 2005), mitoxantrone (Suzuki and Nakane, 1994) and epirubicin (El Mahdy Sayed Othman, 2000) have been observed to induce different types of chromosomal aberrations in Chinese hamster cell lines.

Micronucleus frequency in cultured human lymphocytes was observed to be increased in dose dependent manner for both the drugs. Cultures treated with Doxorubicin displayed a significant (p < 0.001) increase in frequency of micronuclei going from 0.01 to 2.0 µg ml⁻¹ of drug concentration, while those treated with Idarubicin exhibit significant (p < 0.001) micronucleus induction at test concentrations. IDA has been previously reported to increase the frequency of micronuclei in human T lymphoblastoid CEM cell line (Stopper et al., 1999). An increase in micronucleus formation in human lymphocytes, over a similar dose range of anthracyclines, has also been stated earlier by Migliore et al. (1987).

In order to assess the effects of both the drugs on proliferative capacity of cultured human lymphocytes, the nuclear division index was also calculated. The results show cell cycle delay in lymphocytes exposed to higher exposure doses. Decrease in NDI in cells treated with Idarubicin was markedly prominent than cells treated with doxorubicin. Nucleoplasmic bridges were also observed in few binucleated cells. One previous study also corresponds to the findings of present analysis in which percentage of abnormal cells was found to increase in dose dependent manner with a decrease in mitotic index at higher concentration (Gonzalez-Cid et al., 2002).

Both the drugs were found to induce significant genotoxic damage in cultured peripheral blood lymphocytes but damages induced by idarubicin were more prominent than doxorubicin at similar concentration. Anthracycline antibiotics, target DNA topoisomerase II, leading to DNA fragmentation, especially in dividing cells (Szlafowska and Czyz, 2006; Hande, 1998). Idarubicin is a synthetic analog of daunorubicin, a sister compound of doxorubicin. Deletion of methoxy group increases lipophilicity of idarubicin, compared with that of doxorubicin, enhances its uptake into cells and may increase its binding to DNA, resulting in added damage (Platel et al., 1999). Induction of myocardial and hepatic lipid peroxidation may also be responsible for enhanced DNA damage (Bagchi et al., 1995). There is an apparent relationship among chromosomal abnormalities, mutagenesis and carcinogenesis (Kantidze and Razin, 2007; Boffetta et al., 2007). Although these drugs have not yet been
proved to be carcinogenic in man, their long term in vivo and in vitro effects need to be monitored.

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References


