Ciprofloxacin Increased Cytotoxicity of Normal Bone Marrow Stem Cells in Mice Treated With Cyclophosphamide and Etoposide

Muthana I. Maleek¹, Safaa A. Faraj², Suhool Kareem³*

1. Department of Biology, College of Science, University of Wasit, Kut, Wasit, Iraq.
2. Department of pediatrics, College of medicine, University of Wasit, Kut, Wasit, Iraq.

*Corresponding Authors: Suhool kareem

Abstract

The effects of ciprofloxacin (Cipx) on the cytotoxicity of cyclophosphamide (CP), etoposide (VP-16), and combinations thereof on normal bone marrow stem cells in mice were studied. Cipx was administered prior to or after treatment with CP and VP-16. Chromosomal aberration (CA), micronucleus (MN) and mitotic index (MI) were used as markers. CP, VP-16 and Cipx and their combinations were injected intraperitoneally at concentrations of 2, 20, 9.5 mg/kg, respectively. Control groups were injected with normal saline and DMSO solution. Cytogenetic analyses were carried out after 24 hrs of treatment. CP and VP-16 reduced MI and increased CA and MN; while Cipx did not produce significant cytotoxic effects when used alone. Additional cytotoxic effects were seen when Cipx was injected before CP and VP-16 combination. Highest effect was observed when Cipx was injected after injecting combination of CP and VP-16 (p≤0.05). In conclusion; Cipx increased the cytotoxic effects of CP and VP-16 when used before and after treatments with combinations of CP and VP-16.

Keywords: Cyclophosphamide; Etoposide; Ciprofloxacin; bone marrow; chromosomal aberration; micronucleus; Mitotic index; cytotoxicity.

Introduction

For the last 50 years, chemotherapy has been a general therapeutic treatment for a wide range of tumors [1]. Chemotherapy includes the management of drugs that destroy cancer cells. Unfortunately, this cytotoxicity does not target tumor cells only but destroy numerous normal cells as well [2]. Chemotherapy drugs such as procarbazine cyclophosphamide and etoposide create chromosomal abnormalities leading to mutagenesis and chromosomal instability [3-5].

Cyclophosphamide (CP) is a common anticancer chemotherapeutic agent, also known as Cytoxan or Endoxan. It is nitrogen mustard alkylating agent (C7H15Cl2N2P.H2O), which adds an alkyl group (CnH2n+1) to DNA and attaches it to guanine base of DNA [6, 7]. It’s a pharmaceutical harvest used as an antineoplastic in the therapy of a wide variety of cancers such as many types of multiple myeloma, leukemia, neuroblastomas, and certain malignant neoplasm of the lung and adenocarcinomas of the ovary [8]. It is also used as an immunosuppressant drugs for scleroderma, arthritis, glomerulonephritis, multiple sclerosis, chronic hepatitis, and organ transplantation, used alone or in combination with other chemotherapeutic agents [9].

Etoposide (VP-16) is one of the common chemotherapeutic drugs used in medical oncological practice despite the fact that it also affects rapidly dividing cells including haemopoietic cells, gastrointestinal mucosal cells and bone marrow [10].

VP-16, a semi synthetic podophyllotoxin spin-off, is an inhibitor of DNA topoisomerase II (topo II) [11]. Cancerous cells use more topo II than healthy cells, which explains why cancer cells need this enzyme to regulate the proliferation. VP-16 makes a ternary complex with topo II and DNA causing DNA breakages and thus apoptosis [12]. Ciprofloxacin (Cipx) is a second-generation fluoroquinolone. It is an artificial broad spectrum antimicrobial (bactericidal) drug used for the treatment of various diseases,
mainly urinary tract infections and infections associated with chemotherapy [13]. Cipx inhibits DNA replication by producing tremendous amount of reactive oxygen species [14, 15, 16], which creates excessive amounts of free radicals that break lipids, proteins and nucleic acid structures [17].

It also inhibits bacterial DNA gyrase and topo IV, which are required for DNA replication [18].

Materials and Methods

Mice

Albino Swiss male mice were obtained from National Center for Drug Control and Research/ Ministry of Health/ Baghdad. Their age was 8 to 12 weeks and average weights was 25 ±2 g. Mice were kept at room temperature (23-25ºC) and fed standard pellets and fresh water to avoid stressful conditions. A permission to conduct these experiments using lab animals was obtained from the College of Science ethics committee.

Cyclophosphamide

Cyclophosphamide (Hella /Germany) was used after dilution with normal saline. It was injected at a concentration of 2 mg/kg [19].

Etoposide

Etoposide solution (Ebewe/Austria) was dissolved in 5% Dimethyl sulfoxide (DMSO) in normal saline, mixed on a magnetic stirrer for 30 min prior to administration of 20 mg/kg [20].

Ciprofloxacin

Ciprofloxacin (500 mg) film-coated tablets (Aiwa /Germany) was dissolved in normal saline to prepare the required concentration. Mice were injected with 9.5mg/kg of Cipx [21].

Laboratory Animal’s Management

Mice were divided to groups according to types of treatments and each group consisted of 10 mice. Mice were sacrificed after 24 hours of last treatment.

Group 1: control mice injected with 0.25 ml of normal saline.

Group 2: mice injected with 0.2 ml of DMSO

Group 3: mice injected with 2 mg/kg of CP

Group 4: mice injected with 20 mg/kg of VP-16.

Group 5: mice injected with 9.5 mg/kg of Cipx.

Group 6: mice injected with CP and VP-16

Group 7: mice were injected with CP and VP-16 and after 24 hours they were injected with Cipx 8 hours for 5 days and sacrificed after 24 hours from the last injection of Cipx.

Group 8: mice were injected with Cipx every 8 hours for 5 days and after 24 hours from the last injection mice were injected with combination of CP and VP-16 and sacrificed after 24 hours.

Cytogenetic Analysis

Preparation of Mouse Bone Marrow Stem Cells for Examination

Mice bone marrow samples were collected and prepared for cytotoxic analysis experiments according to methods reported previously [22, 23].

Mitotic Index (MI) Assay

Mitotic Index assay was performed as previously reported [24, 25].

Chromosomal Aberration (CA) Assay

Chromosomal aberrations (Acentric Fragment, Gap, Polyploidy, Ring, Fragment and Breaks) were checked and calculated in metaphase cells as previously reported [23]. Photographs of the cytotoxic changes are shown in Fig. 1.
Micronucleus (MN) Assay

Micronucleus assay was done according to the method reported by Schmid, 1975 [26]. A sample photograph showing MN is shown in Fig. 2. The micronucleus index was calculated using the following equation [27]:

\[
\text{Micronucleus Index} = \frac{\text{Number of micronuclei}}{\text{Total count cells (1000)}} \times 100
\]
Statistical Analysis
Data were tabulated and processed using SPSS (Statistical package for the social sciences) V.20 for mac. Quantitative data are expressed as frequency and percentage. Chi-square test was used to identify the association between groups and studied independent variables. P-values equal or less than 0.05 were considered significant [28].

Results and Discussion
The two drugs, CP and VP-16, are used for the treatment of tumors. They induce different cytotoxic changes causing cell death. Cipx is an antimicrobial drug usually used in association with chemotherapy to prevent possible infection due to immunity suppression. In the present investigation this drug was tested in combination with CP and VP-16 and the cytotoxic data (MI, CA and MN) are presented in Tables 1, 2 and 3. No changes in MI were seen in mice injected with DMSO solution giving a value of 6.42%. Cipx slightly reduced MI when used alone giving a value of 5.08%. However, when CP and VP-16 were used individually, significant decreases in MI amounting 4.26% and 3.88%, respectively, was observed. More reduction in MI (3.66%) was seen when CP and VP-16 were used together. A comparable value of 3.34% was obtained when a combination of CP and VP-16 were administered 24 hours prior to the injection of Cipx. However, when Cipx was given before the administration of CP and VP-16, a value of 3.7% was obtained.

Table 1: Percentages of mitotic index in mice bone marrow stem cells for control and treatment groups. In each treatment 5 animals were used and 5000 cells were examined

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mitotic index</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Control (normal saline) for 24h</td>
<td>349</td>
<td>6.98</td>
</tr>
<tr>
<td>Group 2: DMSO for 24h</td>
<td>321</td>
<td>6.42</td>
</tr>
<tr>
<td>Group 3: CP for 24h</td>
<td>213</td>
<td>4.26</td>
</tr>
<tr>
<td>Group 4: VP-16 for 24h</td>
<td>194</td>
<td>3.88</td>
</tr>
<tr>
<td>Group 5: Cipx for 24h</td>
<td>254</td>
<td>5.08</td>
</tr>
<tr>
<td>Group 6: CP and VP-16 injected together for 24h</td>
<td>183</td>
<td>3.66</td>
</tr>
<tr>
<td>Group 7: CP and VP-16 and after 24h Cipx was injected (3 doses/day for 5 days)</td>
<td>167</td>
<td>3.34</td>
</tr>
<tr>
<td>Group 8: Cipx was injected (3 doses/day for 5 days) and after 24h CP and VP-16 was injected</td>
<td>185</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Similarly, frequency of CA was also affected 39.2% and 32.6%, respectively. Much higher dramatically when same treatment was tested. value (42.8%) was obtained when mice were Mice received saline alone gave CA value of injected with combination of CP and VP-16. The 13.2% and DMSO solution alone was 14.4%, highest values of CA of 54.2% and 49.4% were while Cipx alone gave a slightly higher value produced when combinations of CP and VP-16 (17.4%), as shown in Table 2. The value of CA were injected after and before Cipx injection, increased significantly in mice injected respectively. separately with CP and VP-16 giving values of

Table 2: Percentages of different types of chromosomal aberrations (CA) in mice bone marrow stem cells for different treatments groups in each treatment 5 animals were used and a total of 500 cells were examined

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acentric Fragment</th>
<th>Gap</th>
<th>Break</th>
<th>Fragment</th>
<th>Ring</th>
<th>Polyploid</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>Group 1: Control (normal saline) for 24h</td>
<td>41</td>
<td>8.2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0.6</td>
<td>21</td>
</tr>
<tr>
<td>Group 2: DMSO for 24h</td>
<td>44</td>
<td>8.8</td>
<td>1</td>
<td>0.2</td>
<td>3</td>
<td>0.6</td>
<td>23</td>
</tr>
<tr>
<td>Group 3: CP for 24h</td>
<td>86</td>
<td>17.2</td>
<td>7</td>
<td>1.4</td>
<td>29</td>
<td>5.8</td>
<td>61</td>
</tr>
<tr>
<td>Group 4: VP-16 for 24h</td>
<td>63</td>
<td>12.6</td>
<td>10</td>
<td>2.0</td>
<td>27</td>
<td>5.4</td>
<td>44</td>
</tr>
<tr>
<td>Group 5: Cipx for 24h</td>
<td>29</td>
<td>5.8</td>
<td>2</td>
<td>0.4</td>
<td>13</td>
<td>2.6</td>
<td>35</td>
</tr>
<tr>
<td>Group 6: CP and VP-16 injected together for 24h</td>
<td>56</td>
<td>11.2</td>
<td>17</td>
<td>3.4</td>
<td>35</td>
<td>7.0</td>
<td>50</td>
</tr>
<tr>
<td>Group 7: CP and VP-16 and after 24h Cipx was injected (3 doses/day for 5 days)</td>
<td>27</td>
<td>14.4</td>
<td>7</td>
<td>1.4</td>
<td>62</td>
<td>12.4</td>
<td>74</td>
</tr>
<tr>
<td>Group 8: Cipx was injected</td>
<td>58</td>
<td>11.6</td>
<td>8</td>
<td>1.6</td>
<td>57</td>
<td>11.4</td>
<td>75</td>
</tr>
</tbody>
</table>
In Table 3, Micronuclei (MN) percentages were reported. DMSO and ciprofloxacin did not produce any significant change giving values of MN of 2.54% and 2.80%, respectively. However, MN increased significantly in mice treated with CP and VP-16 separately to give values of 4.22% and 3.88%, respectively. When combinations of CP and VP-16 were injected, MN increased to 4.54%. Values of MN went up furthermore to 5.72% and 5.50% when mice were treated with Cipx after and prior to CP and VP-16 injection.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Micronucleus</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Control (normal saline) for 24h</td>
<td>113</td>
<td>2.66</td>
</tr>
<tr>
<td>Group 2: DMSO for 24h</td>
<td>127</td>
<td>2.54</td>
</tr>
<tr>
<td>Group 3: CP for 24h</td>
<td>211</td>
<td>4.22</td>
</tr>
<tr>
<td>Group 4: VP-16 for 24h</td>
<td>194</td>
<td>3.88</td>
</tr>
<tr>
<td>Group 5: Cipx for 24h</td>
<td>140</td>
<td>2.8</td>
</tr>
<tr>
<td>Group 6: CP and VP-16 injected together for 24h</td>
<td>227</td>
<td>4.54</td>
</tr>
<tr>
<td>Group 7: CP and VP-16 and after 24h Cipx was injected (3 doses/day for 5 days)</td>
<td>286</td>
<td>5.72</td>
</tr>
<tr>
<td>Group 8: Cipx was injected (3 doses/day for 5 days) and after 24h CP and VP-16 was injected</td>
<td>280</td>
<td>5.6</td>
</tr>
</tbody>
</table>

It should be pointed out that DMSO did not produce a noticeable change in any of the treatments because the concentration employed was a safe dose [29]. Mice injected with CP and VP-16 has shown a decline in percentage of MI and increases in CA and MN. This was due to the fact that these drugs work on rapidly growing cells, particularly on stem cells and possibly, where DNA replication and chromosome building and configuration are at higher rates.

Accordingly these cells become the main targets of these drugs and therefore these drugs are used for the treatment of cancer. CP is a cytogenetic alkylating nitrogen mustards agent, which exerts its mechanism by forming covalent bonds between alkyl groups and different nucleophilic molecules in cell [30].

This characteristic has enabled CP to bind with DNA and produce DNA cross-linking, which occurs when alkyl group attaches to the guanine base of DNA at the number 7 nitrogen atom of the imidazole ring. This intervenes with DNA replication leading to apoptosis and inhibition of DNA replication by forming intrastrand and interstrand DNA cross-linking [31]. Therefore the main action of CP occurs during the replication because some parts of DNA are not paired and become more susceptible to alkylating specially guanine base of DNA. These effects manifest themselves, however, during the S phase, causing a blockage in G2 and the subsequent cellular death [32]. That is why CP can produce large amount of CA and MN in stem cells. The information that has become recognized as basics and constants, the free radicals or Reactive Oxygen Species (ROS) can induce damages of cells and specially effected on DNA molecule because their high chemical reactivity. Therefore CP induces DNA damage, mononuclear, predominant lethal mutation because (CP) can produce free radicals [33].

For all reasons above (CP) reduce MI and increased CA and MN as shown in Tables 1, 2 and 3, respectively. Similarly, VP-16 reduces MI and increases CA and MN because it induces both single and double breaks in DNA [34] and affects S and G2 phases of cell cycle by creating errors in DNA synthesis in the pre mitotic phase and can causes death of cells [35].

As VP-16 affects DNA topoisomerases (Topo) which are essential enzymes that regulate the topological state of the genetic material by introducing transient breaks in the DNA molecule. They are involved in DNA replication, transcription, DNA repair and chromatin remodeling [36]. For DNA
stability and correct multiplication, the unwinding and rewinding of the double helix, the protein movement along DNA and the coiling of DNA in higher-order structures leading to topological entanglements that are resolved by topoisomerases through enabling topological transformation through two trans esterification reactions [37]. PV-16 poisons the Topo II cleavage complexes (TopoIIcc) and inhibits the second step of the reaction (i.e. DNA re-ligation).

The recent high-resolution of the ternary complex between TopoII, DNA and PV-16 has revealed the elements that are crucial to the stabilization of the cleavable complex [38]. However etoposide has also a high-affinity for chromatin and histones, in particular H1, suggesting that beside Topo II, chromatin can be a target of the drug [39]. Therefore, the effects of VP-16 on Topo has led to an increase in the number of CA and MN and decreasing in MI. Another side effects of Topoisomerase II inhibition by VP-16 frequently cause rearrangements involving the mixed lineage leukemia (MLL) gene on chromosome 11q23, which is associated with secondary leukemia [40-42]. Cipx targets DNA gyrase (is an enzyme within the class of topoisomerase II) and topoisomerase IV, trapping these enzymes at the DNA cleavage stage and inhibits DNA repair and bacterial growth, which leads to bacteriostasis and finally to cell death [43, 44]. Cipx also reduces mammalian tendon cell proliferation and causes cell cycle arrest at the G2/M phase and mitotic arrest, misaligned chromosomes, and poor bipolar spindle formation were observed in Cipx-treated tendon cells [45]. In another study, Cipx serves as anti-proliferative and apoptosis inducing activity on prostate cancer cells.

These effects were mediated by cell cycle arrest at S-G2/M phase of the cell cycle, Bax translocation to mitochondrial membrane and by increasing the Bax/Bel-2 ratio in PC3 prostate cancer cells [46]. The mode of action of Cipx is different from that of CP and VP-16. Nonetheless, it did not produce significant changes in percentage of AC, MN and MI of treated mice. Our result partially agrees with Forsgren, et al. [47] where they reported that cell cycle of lymphocyte slightly inhibited by Cipx after 2 days but was increased on days 3 to 6. This little discrepancy can be attributed to that the analysis of the present investigation was carried out 24 hours after injection only.

Conclusion

The use of Cipx with combinations of CP and CV-16 clearly increased their effect in producing chromosomal aberrations, slightly increased micronucleus formation and decreased mitotic index. These data strongly recommend that Cipx should not be used to treat infections associated with cancer treatment by CP and CV-16.

References


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