Relationship of Glutathione Concentrations with Cytotoxicity of Cisplatin in Different Cell Lines after Confront Vitamin C and E.

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Abstract: One of the well-known cellular defenses after exposure to cytotoxic agents is the glutathione (GSH) related mechanisms. Resistant to cisplatin (DP) chemotherapy has been strongly correlated to GSH-mediated mechanisms in many articles. In this study, we have evaluated the effects of cisplatin on the cellular total GSH level in different tumor and normal cell lines. Five different cell lines of human hepatic carcinoma (HePG2), human lung adenocarcinoma (A549), human ovarian carcinoma (SKOV3), dog kidney (LLCPK1), Chinese Hamster Ovary (CHO) and Human gingival fibroblast (GHFI) cell lines were exposed to their respected IC50 concentrations of cisplatin for two hours. Cisplatin cytotoxicity was measured using clonogenic assay and the total cellular GSH level was analyzed using a photometrical assay. The results showed that cisplatin had different degrees of cytotoxicities on different cell lines as shown by IC50 values: 0.87±0.07 for HepG2, 3.27±0.35 for A549, 0.99±0.08 for SKOV3, 5.50±0.35 for LLCPK1, 5.50±0.21 for CHO and 1.60±0.21 for GHFI1 cell lines. GSH level after exposure to cisplatin (GSH-DP) were also different for different cell lines compare to their controls (GSH-C): 85.33±8 for HepG2, 637.00±81 for A549, 2691.00±416 for SKOV3, 1388.30±261 for LLCPK1, 412.60±32 for CHO and 783.24±30 for GHFI1 cell line. It is shown that compare to the matched controls, the cellular GSH level increased in LLCPK1, A549, SKOV3 and GHFI1 cell lines, but decreased in CHO and HepG2 cell lines. The highest significant variation of GSH in cancer cell line was belonging to SKOV3 and in normal cell lines to LLCPK1, after treated with cisplatin. It is concluded that the total GSH variation after exposure to cisplatin is different for different cell lines. We were not able to correlate between the level of resistance to cisplatin (based on the IC50 levels) and GSH level or variations in this study. It might indicate that in spite of many publications so far, the GSH is neither the unique, nor the most important mechanism of resistance to cisplatin in these cell lines. Internal and Eternal GSH level in Studied cell lines will be changed in several ways when contaminated with different concentration of vitamins (for example, Vit C, Vit E and Vit C+E) and observed that variation was more prominent in cancer cell line.

Key words: Cisplatin, glutathione, cell lines, Vit C, Vit E

INTRODUCTION

The discovery and development of platinum compounds has been one of the greatest achievements of cancer chemotherapy in the past three decades. Cisplatin was accidentally discovered by Rosenberg in 1968 as an antibiotic and very rapidly as a very powerful antiproliferative agent against tumor cells. It was rapidly introduced in clinical use, principally for the treatment of germ cell tumors of testis and ovary (Rosenberg, 1985). Cisplatin (cis-dichlorodiammineplatinum-II) gained a widespread use against various malignant tumors in different experimental animals (Cummings and Schnellmann, 2002; Prasad and Giri, 1994) and in a variety

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of human malignancies (Fokkema et al., 2002). Many of the biological properties and effects of cisplatin have been well documented (Rosenberg, 1985; Blasiak et al., 1985) with numerous reports indicating that the cellular DNA could be the primary target in its antinecancer activity (Zamble and Lippard, 1995). However, the therapeutic efficacy of cisplatin is limited due to the development of drug resistance (Boschma and Mulder, 1992) and major side effects, nephrotoxicity (Krakoff, 1979). An increased carcinogenic risk with the development of secondary malignancies in animals/patients treated with cisplatin has also been reported (Kempf and Ivankovic, 1986; Greene, 1992). In an attempt to overcome these impediments, the development of many new cisplatin analogues (Christian, 1992) or the use of cisplatin in combination with other agents have been tried with different degrees of success (Treskes and Vigh, 1993).

Cisplatin is known to generate oxygen free radicals, (Masuda et al., 1994). Though the role of free radicals in cisplatin induced nephrotoxicity remains a matter of debate, (Vermeulen and Baldew, 1992), free radical scavengers have been shown to ameliorate the toxicities of cytotoxic agents (Torri et al., 1993).

Thiol (sulfhydryl) groups, such as those on glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH) and metallothionein (MT), defend the cell against cisplatin, (Kraker et al., 1985; Masahiko et al., 2000; Zhang et al., 2001; Tsunyu et al., 2003). Since the thiolate anion has a high affinity for Pt, Pt ions entering the cell may preferentially bind to sulfur atoms rather than the bases of DNA, (Dedon and Boroch, 1987; Lai et al., 1989; Ishikawa et al., 1994). Although it is easy to overwhelm this protective mechanism in first-time patients receiving cisplatin, continuous exposure to the drug ultimately produces resistance due to increased sulfhydryl levels (e.g., GSH and MT) (Takehiko et al., 2001; Godwin et al., 1992a). Plant and animal cells eliminate a broad range of hydrophobic toxins from the cytosol to the extra cellular space after their conjugation with GSH, (Ishikawa, 1992). This transport is mediated by a novel class of organic anion transporters belonging to the family of ATP-binding cassette carriers, the GS-X pumps, (Ishikawa et al., 1997). GS-X pump activity was found in canalicular and basolateral rat hepatocyte plasma membranes, heart sarcomema vesicles, human erythrocytes and tumor cells (Ishikawa et al., 1997; Muller et al., 1994). Many publications have claimed that Glutathione is the key component of a ubiquitous antioxidant system that defends the cell against the toxic effects of cisplatin (Ishikawa et al., 1997).

α-Tocopherol protects against lipid peroxidation. This protection is achieved via the scavenging of lipid of lipid peroxyl radicals by α-Tocopherol. The α-tocopherol radicals thus formed can be regenerated in the membrane by cytosolic antioxidants such as vitamin C or GSH. Nevertheless, some of the α-tocopherol radicals will not be captured by this recycling process and will be oxidized further to α-tocopherol quinone, which has no antioxidant properties (Kashif and Banu, 2004).

Vitamin C (ascorbic acid), an essential nutrient and active reducing agent, is involved in numerous biological effects. The chemopreventive/therapeutic roles of vitamin C against cancers have been widely reported. However, the definite role of vitamin C in cancer treatment still remains controversial (Giri et al., 1998).

In addition to the importance of the glutathione status of cells for resistance to oxynradical generating drugs, the ability of the cell to maintain the natural antioxidant vitamins, α-tocopherol and L-ascorbic acid in their reduced forms is essential. Both vitamins play vital roles in scavenging toxic oxygen free radicals and they are closely integrated with one another and the glutathione redox system. Previous investigations of human tumor cells, in vitro, for relative sensitivity to cisplatin have not considered the importance of ascorbic acid in intracellular compartments, because the vitamin was omitted from the culture medium. The half-life of ascorbic acid in tissue culture media is estimated to be 0.9 h and the potential proxidant action of ascorbic acid in the presence of transition metal ions, especially iron, promotes lipid membrane per oxidation. Therefore, ascorbic acid is often omitted from media for human cells (William et al., 1995; Meijer et al., 1994).

Whether GSH is the principle parameter in the cisplatin cytotoxicity is the main question of this study. If it is so, the intracellular amount of initial GSH, should be able to present a measurable scale of cellular resistance in different cell lines. On the other hand, it should be possible that variations in cellular GSH content and consumption after the exposure to cisplatin represent the degree of sensitivity and resistant to this drug in different cell lines. In this manuscript, we are presenting the cellular toxicity of cisplatin in different cell lines, as well as the cellular GSH levels before and after the exposure to cisplatin and vitamins (C, E, C+E) to examine the accuracy of above hypothesis.

This study was accomplished Faculty of Pharmacy, Sheheed Beheshtis University of Medical Science, Tehran, Iran, in the year 2005.

MATERIALS AND METHODS

Chemicals: All chemicals that used in this study as following: (cisplatin, media serum DMEM F12 (Gibco BRL), antibiotic (Streptomycin, Penicillin Gibco BRL), FBS
Cell lines and clonogenic assay: Human hepatoma cell (HepG2) was purchased from the Pasteur institute in Tehran (Iran). Human lung carcinoma (A549), Human ovary carcinoma (SKOV3), Dog renal normal cell (LLCPK1), Chinese Hamster Ovary (CHO) and Human gingival fibroblast (GHFI1) were as generous gift from Dr. Rakesh Goel, Ottawa Regional Cancer Center, Ottawa, Canada. All cells were grown in DMEM/F12 media (Gibco BRL, USA) supplied with 10% fetal bovine serum and 1% antibiotic. Cells were maintained in a humidified 37°C incubator with 5% CO₂ for three passages before the start of experiments. Cells were exposed to the different concentrations of cisplatin in a range of 0.5 to 10 μM for 1 h. Clonogenic assay was carried out after the cell exposure to cisplatin based on Von Hoff method, (Von Hoff et al., 1985; Conney, 2003).

Sample preparation and analysis of vitamins: For preparation of different concentrations of Vitamin C, 0.031 g Vit C powder sample was weighted and then dissolved in 10 mL DDW. From this solution AC (17.6 mm), another solution prepared with using of sterile PBS 2 mL of solution AC plus 2 mL PBS used for preparation of solution BC (8.8 mm) and then 2 mL of solution BC plus 2 mL PBS used for making of solution CC (4.4 mm) and further 2 mL of solution CC plus 2 mL PBS mixed for preparation of solution DC (2.2 mm), on the other hand for Preparation of different concentrations of vitamin E (act fast as this is sensitive to light and air), 1 mL of vit E powder sample added to 4 mL of DMSO in a tube under the hood for making the solution called, solution AE (465.11) and then 2 mL of solution AE mixed with 2 mL PBS to making solution BE (232.5 mM) afterward 2 mL of solution BE combined to 2 mL PBS for preparation the solution of CE (116.2 mM) and at last 2 mL of solution CE mixed to 2 mL PBS to make solution DE (85.12 mM).

Final procedure was about Preparation of different concentrations of complex Vitamin C and Vitamin E at first 40 μL each of these solutions added to each three Petri-dishes with specific codes as observe (col), control vit C (SS, SC), different concentration (AC, BC, CC and DC), control vit E (SE1, SE2, SE3) different concentration vit E (AE, BE, CE and DE) and max vit E+C (AEC, BEC, CEC and DEC).

GSH assay: GSH analysis was performed spectrophotometrically according to the enzymatic method of Tietze and Florenani, (Neuschwander and Roll, 1989; Blum and Fridovich, 1985). In brief, different cell lines (HepG2, A549, SKOV3, LLCPK1, CHO and GHFI1) were grown to 80% confluences as was described above, in 25 mm plates. Experiment and control cells were harvested and collected in 1 mL of PBS, Commented and subsequently stored at -20°C. Determination of GSH was performed by a modification of the method of Cohn and Lyle. To 0.5 mL of the 100,000g supernatant, 4.5 mL of the phosphate-EDTA buffer, pH 8.0 was added. The final assay mixture (2.0 mL) contained 100 μL of the diluted cell lines supernatant, 1.8 mL of phosphate-EDTA buffer and 100 μL of the OPT solution (Fluorescence material which compound in the GSH).

After thorough mixing and incubation at room temperature for 15 min, the solution was transferred to quartz cuvette. Fluorescence at 420 nm was determined with the excitation at 330 nm (Hissin and Hilf, 1976; Griffith, 1980).

Figure 1 mark the total amount of glutathione in the samples was determined as GSH (μmol) Per million cells using a standard curve obtained by plotting the known amounts of GSH (100, 80, 50, 40, 25 and 12.5 μm), incubated under the same experimental conditions, versus the rate of change of absorbance at 420 nm [r = 0.989].

Animal study: The animals (male mice 10-12 week old, weighing 25-30 g) were divided into nine groups of 8 animals each:

Experimental protocol
Group 1: Normal control, received only the normal saline (5 mg kg⁻¹, i.p.) for 5 days.
Group 2: Positive control, received only single doses of cisplatin (8 mg kg\(^{-1}\), i.p.) only at 6th day.

Group 3: Control group with carrier of Vit E, received only soybean oil (10 mg kg\(^{-1}\), i.p.) for 5 days.

Group 4: Control group with Vit C, received only Vit C (100 mg kg\(^{-1}\), i.p.) for 5 days.

Group 5: Control group with Vit C, received only Vit E (500 mg kg\(^{-1}\), i.p.) for 5 days.

Group 6: Control group with Vit C and Vit E, received does of Vit C (100 mg kg\(^{-1}\), i.p.) and Vit E (500 mg kg\(^{-1}\), i.p.) for 5 days.

Group 7: Treatment group: received Vit C (100 mg kg\(^{-1}\), i.p.) for 5 days and single does of cisplatin (8 mg kg\(^{-1}\), i.p.) only at 6th day.

Group 8: Treatment group: received Vit E (500 mg kg\(^{-1}\), i.p.) for 5 days and single does of cisplatin (8 mg kg\(^{-1}\), i.p.) only at 6th day.

Group 9: Treatment group: received Vit C (100 mg kg\(^{-1}\), i.p.) with Vit E (500 mg kg\(^{-1}\), i.p.) for 5 days and single does of cisplatin (8 mg kg\(^{-1}\), i.p.) at 6th day.

Twenty four and 96 h after 6th day, the animal was killed and taken 2CC blood sample for biochemical analysis and extracted the whole liver and kidney and then fixed in formalin (9%) solution for later pathological studies.

**Statistical analysis:** The results are expressed as mean±SEM. Differences between means were elaborated by one way analysis of variance or the Turkey-kramer method for multiple comparisons. Differences at p<0.05 were considered to be significant. IC\(_{50}\) was calculated using Graph pad prism software.

**RESULTS**

Table 1 represents the IC\(_{50}\) amounts of cisplatin in different under-investigation cell lines (HEPG2, A549, SKOV3, LLCPK1, CHO and GHEF1). The IC\(_{50}\) of cisplatin in these cell lines are HEPG2: 0.87±0.07, A549: 3.27±0.35, SKOV3: 0.99±0.08, LLCPK1: 5.5±0.35, CHO: 5.5±0.21 and GHEF1: 1.6±0.21.

As is shown in Fig. 2, the rank order of IC\(_{50}\) are A549> SKVO3> HEPG2 for cancer cell lines and CHO> LLCPK1> GHEF1 in normal cells. There is a statistically significant difference (p<0.05) between the IC\(_{50}\) of cisplatin among normal and cancer cell lines. Based on the information presented in Fig. 2, among normal cell lines (between LLCPK1 and CHO with GHEF1 and then cancer cell lines between HEPG2 and SKOV3 with A549, a significant relation was seen (p<0.05).

**Fig. 2:** Pathologic kidney tissue sample after contamination with cisplatin including fatty changes of renal tubular

**Table 1: Comparison of cisplatin IC\(_{50}\) (μmol) on different cell lines**

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>HEPG2</th>
<th>A549</th>
<th>SKOV3</th>
<th>LLCPK1</th>
<th>CHO</th>
<th>GHEF1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC(_{50}) (μg mL(^{-1}))</td>
<td>0.87±0.07</td>
<td>3.27±0.35</td>
<td>0.99±0.08</td>
<td>5.5±0.35</td>
<td>5.5±0.21</td>
<td>1.6±0.21</td>
</tr>
</tbody>
</table>

Table 1-4 represents the concentrations of GSH in these cell lines in mmol/million cells, before and after the 1h exposure to the IC\(_{50}\) concentration of cisplatin for each cell line. Based on this figure, the rank order of total GSH concentration for under investigation cell lines is as LLCPK1> GHEF1> CHO in normal cell lines and SKOV3> A549> HEPG2 in tumor cell lines. The rank order of cellular GSH level among these cell lines remains the same before and after the exposure to cisplatin.

Correlation between HEPG2 (cancer cell line) with cisplatin in Internal GSH levels with main blank (col: without any treatment) was seen as SS (with IC\(_{50}\) of cisplatin), SC (with IC\(_{50}\) of cisplatin plus normal saline control group) and SE2 (with IC\(_{50}\) cisplatin + soybean oil) that Correlation between main blank (col) with BC, CC, DC, AE and DE group, was significant (p<0.0001) and then Correlation between SS and SC with AC, BE, ABC, BCC and CEC was significant at p<0.001 and finally Correlation between SE2 with DE, AEC, BCC and DCC was significant at p<0.001. This results with external GSH level showed that correlation between main blank (col) with BC, CC, DC, AE, DE, AEC, BCC and CEC was significant at p<0.001 and relation between SS and SC with AC, BE, CE, ABC, BCC, CBC and DEC was significant (p<0.001) and at last correlation between SE2 with DE, AEC, BCC, CEC and DEC was significant at 0.001 (Table 3 and 4).

Above relationship with SKOV3 (cancer cell line) about Internal and external GSH level showed that
Table 2: GSH levels in cancer and normal cell lines before and after the exposure to IC50 concentration cisplatin

<table>
<thead>
<tr>
<th>Cell line</th>
<th>HEPG2</th>
<th>A549</th>
<th>SKOV3</th>
<th>LLC-PK1</th>
<th>CHO</th>
<th>HGF1</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH+C6</td>
<td>177±19</td>
<td>355±53</td>
<td>957±108</td>
<td>1035±81</td>
<td>480±25</td>
<td>635±23</td>
</tr>
<tr>
<td>GSH+Cu</td>
<td>858±44</td>
<td>637±48</td>
<td>819±46</td>
<td>1288±261</td>
<td>412±38</td>
<td>728±30</td>
</tr>
</tbody>
</table>

Table 3: Comparison mean and mean standard error GSH levels in the cancer cell lines after confront cisplatin and Vitamin concentrations

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>In</th>
<th>Ex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPEG2</td>
<td>In</td>
<td>Ex</td>
<td>Total</td>
</tr>
<tr>
<td>COL</td>
<td>51±5</td>
<td>126±10</td>
<td>177±19</td>
</tr>
<tr>
<td>Control</td>
<td>SS</td>
<td>24±2</td>
<td>61±8</td>
</tr>
<tr>
<td>Vit C</td>
<td>19±1</td>
<td>47±2</td>
<td>66±6</td>
</tr>
<tr>
<td>BC</td>
<td>31±67</td>
<td>21±10</td>
<td>21±18</td>
</tr>
<tr>
<td>DC</td>
<td>20±086</td>
<td>50±3</td>
<td>70±6</td>
</tr>
<tr>
<td>CC</td>
<td>16±05</td>
<td>41±2</td>
<td>57±8</td>
</tr>
<tr>
<td>Control</td>
<td>SE1</td>
<td>26±1</td>
<td>64±2</td>
</tr>
<tr>
<td>SE2</td>
<td>42±15</td>
<td>104±3</td>
<td>146±13</td>
</tr>
<tr>
<td>SE3</td>
<td>31±15</td>
<td>84±25</td>
<td>117±12</td>
</tr>
<tr>
<td>Vit E</td>
<td>AE</td>
<td>33±2</td>
<td>83±4</td>
</tr>
<tr>
<td>BE</td>
<td>45±22</td>
<td>112±3</td>
<td>157±11</td>
</tr>
<tr>
<td>CE</td>
<td>43±3</td>
<td>109±2</td>
<td>152±10</td>
</tr>
<tr>
<td>DE</td>
<td>25±1</td>
<td>63±2</td>
<td>88±6</td>
</tr>
<tr>
<td>Vit C+E</td>
<td>AEC</td>
<td>14±6</td>
<td>36±5</td>
</tr>
<tr>
<td>BCC</td>
<td>79±2</td>
<td>186±7</td>
<td>265±11</td>
</tr>
<tr>
<td>CEC</td>
<td>66±25</td>
<td>167±7</td>
<td>234±12</td>
</tr>
<tr>
<td>DEC</td>
<td>58±2</td>
<td>144±6</td>
<td>202±14</td>
</tr>
</tbody>
</table>

Table 4: Comparison mean and mean standard error GSH levels in the normal cell lines after confront cisplatin and Vitamin concentrations

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>In</th>
<th>Ex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLC-PK1</td>
<td>In</td>
<td>Ex</td>
<td>Total</td>
</tr>
<tr>
<td>COL</td>
<td>370±24</td>
<td>695±32</td>
<td>1065±81</td>
</tr>
<tr>
<td>Control</td>
<td>SS</td>
<td>42±51</td>
<td>86±6</td>
</tr>
<tr>
<td>SC</td>
<td>397±62</td>
<td>79±59</td>
<td>119±49</td>
</tr>
<tr>
<td>AC</td>
<td>433±67</td>
<td>86±104</td>
<td>129±215</td>
</tr>
<tr>
<td>BC</td>
<td>440±64</td>
<td>88±49</td>
<td>132±240</td>
</tr>
<tr>
<td>CC</td>
<td>438±27</td>
<td>878±68</td>
<td>131±363</td>
</tr>
<tr>
<td>DC</td>
<td>406±58</td>
<td>81±51</td>
<td>121±95</td>
</tr>
<tr>
<td>Control</td>
<td>SE1</td>
<td>362±42</td>
<td>72±49</td>
</tr>
<tr>
<td>SE2</td>
<td>431±30</td>
<td>82±86</td>
<td>124±162</td>
</tr>
<tr>
<td>SE3</td>
<td>428±25</td>
<td>85±75</td>
<td>128±146</td>
</tr>
<tr>
<td>Vit E</td>
<td>AE</td>
<td>461±28</td>
<td>934±62</td>
</tr>
<tr>
<td>BE</td>
<td>594±34</td>
<td>918±89</td>
<td>1376±122</td>
</tr>
<tr>
<td>CE</td>
<td>449±41</td>
<td>899±39</td>
<td>1348±134</td>
</tr>
<tr>
<td>DE</td>
<td>439±29</td>
<td>879±57</td>
<td>1318±108</td>
</tr>
<tr>
<td>Vit C+E</td>
<td>AEC</td>
<td>470±31</td>
<td>941±78</td>
</tr>
<tr>
<td>BCC</td>
<td>463±26</td>
<td>925±79</td>
<td>1288±209</td>
</tr>
<tr>
<td>CEC</td>
<td>447±19</td>
<td>895±31</td>
<td>1342±127</td>
</tr>
<tr>
<td>DCC</td>
<td>440±51</td>
<td>881±52</td>
<td>1321±98</td>
</tr>
</tbody>
</table>

The correlation between main blank (col) with all studied groups was significant (p<0.001) and between SS, SC and SE2 with all studied groups was non-significant. A549 (Cancer cell line) with cisplatin at internal GSH level represented that main blank (col) with AC, AE, BE, DE, AEC, BCC, CE and DEC, groups was significant at p<0.05 and control group SS was significant at p<0.001 with BC, AEC and at p<0.01 with DC and finally control group SC was significant at p<0.01 with BC and p<0.001 with AEC. Results with external GSH level showed that correlation of main blank (col) with all studied groups was significant at p<0.001 and control group SS with all SS with all studied groups have not significant. Control group SC with all AEC at p<0.01 was significant and finally control group SE2 with AEC group was significant at p<0.05. Treatment with LLCPK1 (normal cell line) with
cisplatin in internal and external GSH level showed no relationship between control groups with different vitamin groups. Correlation between CHO (normal cell line) with cisplatin about internal GSH level presented relation between main blank (col) and SE2 with all studied groups was not significant and between SS, SC and SE2 that was significant only with BEC at p<0.05. This relation about external GSH level showed as non-significant correlation between main blank with all studied groups and significant correlation between SS and SC only with DEB group at p<0.01 and finally correlation between SE2 was significant with BEC at p<0.001 and with ABC at p<0.01. Correlation between HGF1 (Normal cell line) with cisplatin in internal GSH Level illustrated significant correlation between main blank (col) with AE, BE, BEC, CEC at p<0.05 and with ACE at p<0.01 and non-significant correlation between SS, SC and SE2 with all studied groups external GSH Level in this study showed significant correlation between main blank (col) with all studied groups was at p<0.05 and between SC, SS control only with ABC at p<0.05 and finally correlation between SE2 control was not significant with all studied groups.

According to results in Table 3, reduction in total GSH level in HEPG2 cell line remarkably seen in SC and SS Control group versus blank (col), but in cell line contaminated with Vit C and complex Vit C and E, showed increase in GSH level, versus to blank and control groups. All samples that contaminated with different concentration of vit E, showed remarkably decrease in GSH level versus to control and blank. In the other hand, A549 cell line showed increase in GSH level in SS and SC control group versus blank (col), while vit E, vit C and complex vit C and E groups, showed increase level in GSH that was highest concentration of GSH were seen in AC, AE and BEC groups. SKOV3 cell line showed remarkably increase in total GSH level in SS and SC control versus blank (col) and in Vit C, Vit E and complex, increase in total GSH level was seen that highest level was found in AC, AE and BEC.

According to results in Table 4, LLCPK1 cell line has caused increase in total GSH level in SS and SC control group versus blank and this increase seen in Vit C, Vit E and complex, versus blank and control and highest level was seen in BC, AE and AEC. CHO cell line showed remarkably reduction of total GSH level in SC and SS control group versus blank and this condition seen in all Vit C and Vit E Concentration, but in complex increase in total GSH level seen versus control group that BEC concentration was very obvious. In HGF1 cell line, increase in total GSH level was seen in SS and SC control group versus blank and this condition was seen in Vit C, Vit E and complex versus control and blank AC, AE and finally ABC concentration was very obvious.

According to animal study protocol, BUN and creatinin were measured in time of 24 and 96 h after contamination with cisplatin and vitamins (C, E, Complex E + C) and no significant different was seen between nine test groups.

Pathological assessment from mice liver and kidney Tissue samples by fluorescent microscopy showed that Considered alteration in tissue samples remarkably seen in group that contaminated directly with cisplatin (Group 1).

Main alteration in kidney tissue sample after contamination with cisplatin including fatty changes of renal tubular epithelium, Tubular epithelium is necrotic in areas and preserved epithelial lining of the tubules (Fig. 2 and 3).

Basic alteration in hepatic tissue sample after contamination with cisplatin including Apoptotic cell, Necrotic hepatic parenchyma cells, central vein dilatation and kupper cell hyperplasic (Fig. 4 and 5).

Fig. 3: Pathologic kidney tissue sample after contamination with cisplatin including tubular epithelium is necrotic in areas

Fig. 4: Pathologic hepatic tissue sample after contamination with cisplatin including focal cellular necrosis, kupper cell hyperplasic and apoptotic cell
DISCUSSION

Cell lines from many different tumors may develop resistance to cisplatin. In most cases, the level of resistance is less than 50-fold, although there are reports of up to 1000-fold resistance (Neuschwander and Roll, 1989). Nevertheless, even a small increase in resistance of a tumor to cisplatin can be clinically important.

Biochemical studies have not succeeded in identifying conclusively the basis of resistance in any type of cell selected with cDDP, but they have defined several mechanisms that can contribute to resistance. The effectiveness of cell killing is a function of how much drug gets into the cell, how much of this actually reacts with DNA, how tolerant the cell is of lesions in its DNA and how effectively it removes these adducts from DNA (Rooseboom and Schaaf, 2002).

Thiol (sulphhydryl) groups, such as those on glutathione (GSH) and metallothionein (MT), defend the cell against cisplatin (Kraker et al., 1985; Masahiko et al., 2000; Zhang et al., 2001; Tsuruya et al., 2003). Since the thiolate anion has a high affinity for platinum atom, Pt ions entering the cell may preferentially bind to sulfur atoms rather than the bases of DNA (Dedon and Borch, 1987; Lai et al., 1989; Ishikawa et al., 1994).

Glutathione (GSH) is a tripeptide thiol, y-glutamylesteimyglycine. Concentrations of 0.5-10 mM make it the most abundant thiol in the cell. Glutathione is synthesized in a two-step pathway involving the ATP-dependent enzymes glutamylcysteine synthetase and glutathione synthetase (Meister, 1991). The first step is rate-limiting and inhibited by glutathione itself and by buthionine sulfoximine (BSO). As a potent nucleophile, GSH reacts with alkylating agents as well as cisplatin. The reaction of GSH and cisplatin in a 2:1 molar ratio forms a GSH-platinum complex that is then eliminated from the cell by an ATP-dependent glutathione S-conjugate export pump (Meister, 1991; Rooseboom and Schaaf, 2002). GSH may protect cells by intercepting reactive platinum complexes before they can react with DNA. GSH also protects cells by supporting DNA repair, possibly by stabilizing repair enzymes such as DNA polymerase (Rooseboom and Schaaf, 2002). Increased glutathione levels have been found in some cisplatin resistant cells but not in all cell lines. On the other hand, continued exposure to the drug in patients receiving cisplatin, ultimately produces resistance due to increased sulphhydryl levels (e.g., GSH and MT) (Meister, 1991; Godwin et al., 1992b).

It has been suggested that the capacity of the cell to synthesize GSH in response to stress may be more important than the steady state GSH level (Schneider et al., 2002). We have therefore examined the hypotheses that different cell lines have variable potency in adapting a higher level of GSH level as protective mechanism in long-term or short-term exposure to platinum compounds, which might reflect in their level of sensitivity to cisplatin.

To investigate on this postulation, we have examined the effects of cisplatin on the cellular total GSH level of three tumor (HePG2, A549, SKOV3) and normal (LLCPK1, CHO, GHI1) cell lines. Present results showed that different cells have different levels of GSH. A comparison of normal GSH level with the IC_{50} of cisplatin for each cell line did not reveal any kind of correlation, to conclude that intracellular amount of GSH might be the critical index for the cell line resistance to this drug. As an example, although LLCPK1 cell line has the highest IC_{50} value and also GSH level among these cell lines (1035.7±81 nM/10^6 cells), but the CHO cell line with the same concentration of IC_{50} contain a median level of GSH (480.1±25 nM/10^6 cells) within this group of cells. Our results were therefore failed to show any correlations between the level of intracellular GSH and resistance to cisplatin.

To examine the importance of GSH increase in response to stress, cells were exposed to cisplatin. IC_{50} concentrations in the same exposure time were selected for the cisplatin to be exposed for each cell line to produce the same level of potency for the drug and stress on the cell line. As is shown in this study, compare to the matched controls, the cellular GSH level following exposure to cisplatin were increased in LLCPK1, A549, SKOV3 and GHI1 cell lines, but decreased in CHO and HepG2 cell lines. The cellular total level of GSH in various cell lines after exposure to the corresponding IC_{50} concentrations of cisplatin for each cell line was hence different and not as a good indicator of sensitivity to this drug (Cummings and Schnellmann, 2002; Tsuruya et al., 2003).
On the other hand, intracellular GSH level was evaluated in some but decreased in other cell lines after exposure to cisplatin. We have therefore tried to find any correlation between the decrease and/or increase in GSH and cellular resistance to cisplatin, but was not successful to find any pattern. As examples, SKOV3 (IC_{50} = 0.99±0.08) and HepG2 (IC_{50} = 0.87±0.07) have shown almost the same level of sensitivity, however, after the exposure to cisplatin the GSH level was increased dramatically (about 2.5 times), but decreased significantly (about 2 times) in these cell lines, respectively. LLCPK1 and CHO cell lines with the same level of IC_{50} showed the same pattern, as GSH level increased in LLCPK1, but decreased in CHO cell line after exposure to the same toxic level of cisplatin for the same duration of time (Paolicchi et al., 2003; Hanigan et al., 2001).

There was also not significant difference between normal and tumor cells in their pattern of GSH level or variation for cisplatin. The highest significant GSH variation in cancer cell line was belong to SKOV3 and between normal cell lines to LLCPK1 after treated with cisplatin (Schilder et al., 2002; Hanigan et al., 2001).

Answer to questions of cellular recognition of stress after exposure to cisplatin, level and speed of cellular reaction to express GSH related genes and the two-step process of GSH production in different cells and specially possible differences in the cellular uptake and hence the actual exposure of cellular GSH molecule exposure to cisplatin, are remained to be answered in further more detail studies. However, in spite of many publications on the GSH critical role, but the fact that our study was not able to show any kind of correlation between GSH level and/or variations with cisplatin sensitivity, suggests a more important role for other cellular thiol pools (e.g., MT and thiol groups on the chromatin) and resistance mechanisms (e.g., cellular entrance and DNA) repair in cisplatin clinical response (Masahito et al., 2000; Tsuruya et al., 2003; Schilder et al., 2002; Chu, 1994).


REFERENCES


