Antioxidative Potential of Vitamin C Against Chemotherapeutic Agent Mitomycin C Induced Genotoxicity in Somatic and Germ Cells in Mouse Test Model

Mehnaz Mazumdar*, Sarbani Giri*, Supriya Singh*, Afisa Kausar*, Anirudha Giri* and Gauri Dutt Sharma*

Molecular Cytogenetics Laboratory*, Toxicology Laboratory*, Laboratory of Microbial Ecology*
Department of Life Sciences and Bioinformatics, Assam University, Silchar-788011, Assam, India

*Corresponding author email: girisarbani@yahoo.com

Abstract

The objective of the present study is to investigate the effects of chemotherapeutic agent Mitomycin C and the potential antioxidative properties of Vitamin C when acting in combination with Mitomycin C in the mammalian somatic and germ cell in vivo. Mitomycin C is a chemotherapeutic agent. However, application of such a drug may lead to severe cytogenetical consequences like damage to the normal, healthy proliferative cells and tissues of the body. Such effects may lead to development of secondary neoplasms, faulty genome and various other physical and physiological side-effects. In our study we have observed that administration of Mitomycin C (2 mg/kg/bw) to Sprague-dawley's mice induced significant level (P < 0.001) of chromosomal aberrations (CA), micronucleus formation (MN) and sperm head abnormalities (SHA) when compared to untreated control. However with prior administration of Vitamin C in various doses (125, 250 and 500 mg/kg/bw) significantly reduced (P < 0.001) the frequency of CA, MN and SHA. This indicates the antioxidative potential of Vitamin C and its protective intervention against Mitomycin C induced cytogenetical damages.

Keywords: Mitomycin C, Vitamin C, Chromosomal aberration assay, Micronucleus assay, Sperm head abnormality assay.

Introduction

The most common and easily available natural antioxidant is the water soluble glucose derivative Vitamin C (\(C_6H_8O_6\)). It is a well known antioxidant and a known free radical scavenger, present abundantly in tropical diet rich in citrus fruits and vegetables. Vitamin C has been reported to be effective as a protectant against a variety of toxic chemical agents including heavy metals (Holloway and Peterson, 1984). Vitamin C has also been useful in treatment of various cancers and neuro-degenerative diseases (Coulter et al., 2006).

Mitomycin C \([C_{20}H_{19}N_{10}O_9]\) has been efficiently used in the treatment of various cancers (Verweij and Pinedo, 1990). It was found to have potential to directly damage the DNA by formation of free radicals or by forming DNA crosslinks (Korkina et al., 2000). So also it is capable of damaging other biomolecules of cells in addition to DNA. Considering the striking level of damage delivered by such drugs it is very essential to rescue the normal cells and tissues by alternative means and so the concept of concurrent application of antioxidants with drugs to protect cells against intermediate metabolites, reactive oxygen species such as hydrogen peroxide (\(H_2O_2\)), superoxide radical and singlet oxygen radical generated by chemotherapeutic drugs. This work is aimed to study the role of the antioxidant Vitamin C when used prior to chemotherapeutic drug Mitomycin.
Antioxidative Potential of Vitamin C Against

C and to find out whether the antioxidant exhibit any positive role to play along with Mitomycin C or whether it potentiate the effects of the drug or reversibly negates the side effects of the drug.

Materials and Methods

Test chemicals:

Mitomycin C (MMC) was obtained from Cadila Pharmaceuticals, India. Vitamin C and Colchicine were purchased from Sigma chemicals Co. [St Louis, MO]. Giemsa stain, Glacial acetic acid, Methanol, Eosin stain etc, were purchased from HiMedia Laboratories Pvt. Ltd, Mumbai, India.

Test animal:

Swiss albino mice of both sexes were used for chromosomal aberration, micronucleus assay and sperm head abnormality assay. They were purchased from Pasteur Institute, Shillong, Meghalaya, India. 6-10 week old mice, weighing 22-30g were selected. Animals were maintained at room temperature at 25.0 ± 5.0 °C and in 12 h dark and 12 h light cycles. Standard food pellets and water ad libitum was provided to the animals.

Dose and treatment:

MMC (2 mg per kg / b.w) was used to study the genotoxic effect in mice bone marrow and germ cells. It was administered intraperitoneally (i.p) . Vitamin C of 3 different doses 120 mg/kg (V1), 250 mg/kg (V2) and 500 mg/kg (V3) bw were selected and administered orally (p.o) for 5 consecutive days prior to MMC treatment. The dose were selected and standerdized on the basis of available literature.

Chromosomal aberration assay (CA):

CA is defined as a modification of the number or structure in chromosomes. It is an important parameter for genotoxicity studies because it was considered to be an indicator of cancer risk (Bonassi et al., 2004). The CA assay was conducted as per Krishna and Hayashi (2000) protocol with minor modifications. 100 metaphase chromosomes were studied for various type of abnormalities like chromatid breaks and gaps, isochromatid breaks and gaps, exchanges, Robertsonian translocations, sister chromatid unions (Figure 2A-D) etc. Suppression percent was calculated as 100 - [Total aberrant cells studied induced by combination treatment with Vitamin C and MMC / Total aberrant cells induced by MMC alone]*100.

Micronucleus assay (MN)

MN study is another very important and useful bio-marker for genotoxicity studies (Heddele et al., 1983). It is defined as an acentric fragment of a chromosome or the whole chromosome itself which lags behind in the cytoplasm during anaphase of cell division and does not get incorporated in the daughter nuclei. MN is induced basically due to certain clastogenic or aneugenic effects of chemicals or other mutagens. The MN study is conducted in the early (Poly chromatic Erythrocytes, PCE) (Figure 2 E-G) and late maturing stages (Normochromatic Erythrocyte : NCE) (Figure 2 H-J) of RBC. MN slides were prepared as per method of Schmid (1976). 2000 PCE were studied and their corresponding NCE was scored.

Sperm head Abnormality assay (SHA)

1000 sperms from per animal were scored and the abnormalities categorized based on Wyrobek and Bruce (1978) categorization of various types of abnormal sperms such as hookless, hooked, giant, dwarf, altered head, triangular, banana, needle, pin-head, amorphous, beaked (Figure 2 K-Q).

Statistical analysis:

ANOVA was used to determine the significance of data. Pair wise comparison of significance between the different groups was determined using Tukey’s test. ANOVA values were calculated using GraphPad Prism Version 4.03 (Graph pad Inc., San Diego, CA, USA).

Results

In CA study, total aberrations, % aberrant cells and Suppression percent was calculated out. It was seen that, MMC induced CAs which was significantly high (P < 0.001) compared to the untreated control after 24 and 48 hour study (Table 1 and Table 2). However in Vitamin C treated
groups it was seen that all the three doses had no effect on the frequency of CAs and % aberrant cells too. In the combination treatment of Vitamin C and MMC, it was observed (Table 1 and Table 2) that with the increase in the Vitamin C doses, there was a significant decline in the incidence of CAs and % aberrant cells when compared to only MMC treated groups after 24 and 48 hour exposure. The lowest dose of Vitamin C (125 mg/kg/bw) did not show significant decline in MMC induced CAs. However, the middle (250 mg/kg/bw) and the highest dose (500 mg/kg/bw) of Vitamin C delivered significant decline (P < 0.001) in CA values (Table 1). It was also observed that suppression of MMC induced clastogenicity by Vitamin C doses were found to increase in a dose dependent manner with the lowest dose showing 6.08%, middle dose showing 26.95%, and the highest dose showing 53.98% (Table 1) after 24 hour and 15.84%, 31.72%, and 59.97% after 48 hour study respectively (Table 2). It was also clearly observed that there was a dose dependent and time dependent decrease in % aberrant cells and CAs.

MMC (2 mg/kg/bw) induced significant level (P < 0.001) of MN in PCEs at both 24 and 48 hour study (Figure 1). It was also observed that there was a decline in the PCE/NCE ratio which was found to be statistically significant when compared to the untreated control value. In all the Vitamin C treated groups it was observed that there was no significant incidence of MN in PCEs. The PCE/NCE ratio was also found similar to that of control values. In combination treatment with Vitamin C and MMC, there was significant decline (P < 0.001) in the incidence of MN with increase in Vitamin C doses in both 24 and 48 hour study. Cytotoxicity was also found to decrease with the administration of Vitamin C.

In SHA assay (Table 3), MMC treated groups show significant rise (P < 0.001) in SHAs when compared to control. On the other hand Vitamin C treated groups did not exhibit any significant rise in SHAs when compared to control. In the combination treatment of Vitamin C with MMC it was observed that the lowest dose of Vitamin C (125 mg/kg/bw) did not show any significant protection in SHAs. However, the middle (250 mg/kg/bw) and the highest dose (500 mg/kg/bw) of Vitamin C significantly (P < 0.001) reduced the frequency of SHAs from 11.66 ± 0.61 to 9.00 ± 0.40 and 4.74 ± 0.24 respectively in the middle and higher dose (Table 3).

**Discussion:**

The drug MMC used in the experiments has properties of known clastogenicity. In the present work, it was observed that the drug induced significant level of genotoxicity (P< 0.001) in CA (Table 1 and Table 2), MN (Figure 1) and SHA assay (Table 3).

MMC possess a quinone chemical structure which through a cascade of bio-reductive process generates OH radical which potentially can damage the DNA (Korkina et al., 2000) and other biomolecules of the cell. Since, free radicals (ROS) are highly reactive they can undergo reduction by oxidation of surrounding molecules (DNA, lipids, proteins). Such damages to biological molecules by redox reactions may lead to numerous pathological disorders including aging and cancer (Ames et al., 1983, Feig et al., 1994). In addition, MMC can damage DNA by cross-linking bases in the same or adjacent strands of DNA principally at the N2 position of the guanine (G) (Warren et al., 2001) which may eventually lead to apoptotic cell death (Fritsche et al., 1993). Since in the present study, MMC induced significant level of SHA after 35 days of exposure, it could be due to the induction of point mutations in the early spermatocytes and spermatogonia at the premeiotic stages of spermatogenesis (Hugenholtz and Bruce, 1983) by MMC. Since, genes which are responsible for controlling the sperm head shapes are contained in the autosomes, there is a possibility of the drug used in the experiment might have caused certain alterations in the testicular DNA which had resulted into sperms with abnormal heads. There may also be the possibility that the drug like other chemicals interfere and cause hindrance in the differentiation process of sperm development (Rai and Vijayalaxmi, 2001), bringing small alterations in the testis DNA (Topham, 1980).
On the other hand antioxidant, Vitamin C is a known free radical scavenger and its application prior to MMC administration reduced the genotoxicity level significantly. This clearly indicates that Vitamin C has the capacity to modulate the oxidative damage induced by MMC in bone marrow and germ cells of mice.

There are reports supporting our the present findings. In one report, Vitamin C decreased the frequency of sister-chromatid exchange induced by MMC and Cyclophosphamide (Krishna et al., 1986). So also Vitamin C or olive oil showed protection against antitumoral drug Doxorubicin in mice (Antunes and Takahashi, 1998). Vitamin C has also shown protection against the genotoxicity of the drug Cisplatin in in vivo (Giri et al., 1998) as well as in vitro (Nefic, 2001). Genotoxicity induced by drug like norfloxacin could also be minimized by application of Vitamin C (Alba et al., 2008). Vitamin C was reported to protect sperm DNA from the damage induced by exogenous oxidative stress in vitro (Song et al., 2006). It was found that concurrent administration of Vitamin C to pesticide fed animals ameliorates the induced sperm morphology and significantly improves the sperm count (Khan and Sinha, 1996).

It was also reported that Vitamin C pretreatment prevented hydrogen peroxide induced sperm DNA damage (Donnelly et al., 1999). Most recently, it was shown that there is a direct correlation between Vitamin C and fertility in man (Colagar and Marzony, 2009). Such behavior of Vitamin C against wide range of chemicals and oxidation can be attributed to its free radical scavenging property, activation of other endogenous antioxidants (Kutsky, 1973) and also regenerating other antioxidants (Chan, 1993).

It can be concluded that Vitamin C plays a very important role in protecting the gene pool from the severe effects of MMC. Vitamin C in almost all the applied doses has been found to be highly effective against MMC induced genotoxicity. The capacity of donating electrons to free radicals, activating and regenerating other antioxidants and increasing resistance of toxin-susceptible cells may be confounding factors behind the antioxidative

Table 1 Frequency of chromosomal aberrations in the bone marrow cells of mice induced by anticancer agent Mitomycin C (MMC) and protective intervention by different doses of antioxidant Vitamin C (V) at 24 hour of treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Total Cells (n)</th>
<th>Aberrant cells (Total)</th>
<th>% Aberrant cells ± S.D</th>
<th>Chromatid Breaks/Gaps</th>
<th>Isochromatid Breaks/Gaps</th>
<th>Exchanges</th>
<th>SCU</th>
<th>R.T.</th>
<th>Total Aberrations mean ± SD (excluding gaps)</th>
<th>Total Aberrations mean ± SD (including gaps)</th>
<th>Suppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>p.o.</td>
<td>303/3</td>
<td>8</td>
<td>2.61 ± 0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.30 ± 0.54</td>
<td>2.62 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>p.o.</td>
<td>307/3</td>
<td>4</td>
<td>1.33 ± 0.51</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td></td>
<td></td>
<td>3.33 ± 1.36</td>
<td>3.33 ± 1.36</td>
<td></td>
</tr>
<tr>
<td>V2</td>
<td>p.o.</td>
<td>301/3</td>
<td>7</td>
<td>2.33 ± 0.51</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
<td>3.65 ± 0.51</td>
<td>3.65 ± 0.51</td>
<td></td>
</tr>
<tr>
<td>V3</td>
<td>p.o.</td>
<td>300/3</td>
<td>8</td>
<td>2.66 ± 1.86</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td></td>
<td>4.33 ± 1.36</td>
<td>4.33 ± 1.36</td>
<td></td>
</tr>
<tr>
<td>MMC</td>
<td>i.p.</td>
<td>306/3</td>
<td>88</td>
<td>28.75 ± 2.05</td>
<td>65</td>
<td>3</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>51.05 ± 1.56**</td>
<td>37.57 ± 2.01**</td>
<td>60%</td>
</tr>
<tr>
<td>V1+MMC</td>
<td>i.p.</td>
<td>300/3</td>
<td>81</td>
<td>27.00 ± 1.78</td>
<td>65</td>
<td>3</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>28.66 ± 0.51**</td>
<td>29.66 ± 1.86**</td>
<td>6.08</td>
</tr>
<tr>
<td>V2+MMC</td>
<td>i.p.</td>
<td>300/3</td>
<td>63</td>
<td>21.00 ± 1.76</td>
<td>50</td>
<td>0</td>
<td>6</td>
<td>7</td>
<td>4</td>
<td>23.33 ± 0.51**</td>
<td>23.33 ± 0.51**</td>
<td>26.95</td>
</tr>
<tr>
<td>V3+MMC</td>
<td>i.p.</td>
<td>302/3</td>
<td>40</td>
<td>13.23 ± 1.27**</td>
<td>41</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>15.58 ± 3.46**</td>
<td>16.58 ± 4.31**</td>
<td>53.98</td>
</tr>
</tbody>
</table>

Con: received only vehicle; MMC: Mitomycin C (2 mg/kg/bw); Vitamin C: V1: 125 mg/kg/bw; V2: 250 mg/kg/bw; V3: 500 mg/kg/bw; p.o.: Per oral; i.p: Intraperitoneal; SCU: Sister chromatid union; R.T: Robertsonian translocation. n: Total number of animals; Groups bearing the same superscript are significantly different from each other [b, c, g = P < 0.001***; a = P < 0.05*]; Groups bearing the any of the following symbol is significantly different from the Control groups, **P < 0.01; *P < 0.05; **P < 0.05; ***P < 0.001; **P < 0.01; *P < 0.05.
Table 2 Frequency of chromosomal aberrations in the bone marrow cells of mice induced by anticancer agent Mitomycin C (MMC) and protective intervention by different doses of antioxidant Vitamin C (V) at 48 hour of treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Total Cells (n)</th>
<th>Aberrant cells</th>
<th>% Aberrant cells ± S.D</th>
<th>Chromatid Break/Gaps</th>
<th>Isochromatid Breaks/Gaps</th>
<th>Exchanges</th>
<th>SCU</th>
<th>R.T</th>
<th>Total Aberrations mean ± SD(excluding gaps)</th>
<th>Total Aberrations mean ± SD (including gaps)</th>
<th>Suppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>-</td>
<td>305/3</td>
<td>8</td>
<td>2.61 ± 0.48</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00±0.54</td>
<td>2.62 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>p.o</td>
<td>300/3</td>
<td>5</td>
<td>1.66 ± 0.51</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.00 ± 0.89</td>
<td>2.00 ± 0.89</td>
<td></td>
</tr>
<tr>
<td>V2</td>
<td>p.o</td>
<td>300/3</td>
<td>6</td>
<td>2.00 ± 0.89</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2.66 ± 0.51</td>
<td>2.66 ± 0.51</td>
<td></td>
</tr>
<tr>
<td>V3</td>
<td>p.o</td>
<td>300/3</td>
<td>8</td>
<td>2.66 ± 1.36</td>
<td>11</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3.33 ± 2.58</td>
<td>4.66 ± 0.51</td>
<td></td>
</tr>
<tr>
<td>MMC</td>
<td>i.p</td>
<td>300/3</td>
<td>82</td>
<td>27.33 ± 1.86</td>
<td>65</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>3.33 ± 0.51</td>
<td>33.33 ± 1.03</td>
<td></td>
</tr>
<tr>
<td>V1+MMC</td>
<td>p.o+i.p</td>
<td>300/3</td>
<td>69</td>
<td>23.00 ± 0.89</td>
<td>43</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>7</td>
<td>21.66 ± 0.51</td>
<td>23.66 ± 1.86</td>
<td>15.84</td>
</tr>
<tr>
<td>V2+MMC</td>
<td>p.o+i.p</td>
<td>300/3</td>
<td>56</td>
<td>18.66 ± 2.25</td>
<td>46</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>19.33 ± 0.51</td>
<td>21.33 ± 0.51</td>
<td>31.72</td>
</tr>
<tr>
<td>V3+MMC</td>
<td>p.o+i.p</td>
<td>300/3</td>
<td>33</td>
<td>10.94 ± 1.86</td>
<td>21</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>10.94 ± 2.421</td>
<td>12.27 ± 2.81</td>
<td>59.97</td>
</tr>
</tbody>
</table>

Con: received only vehicle; MMC: Mitomycin C (2 mg/kg/bw); Vitamin C: V1: 125 mg/kg/bw; V2: 250 mg/kg/bw; V3: 500 mg/kg/bw; p.o.: Per oral; i.p: Intraperitoneal; SCU: Sister chromatid union; R.T: Robertsonian translocation. n : Total number of animals; Groups bearing the same superscript are significantly different from each other [a, b, c, d = P<0.001***; f = P<0.01**; e = P<0.05*]; Groups bearing the any of the following symbol is significantly different from the Control groups, ***P<0.001; **P<0.01; *P<0.05.

Table 3 Frequency of Sperm Head Abnormality (SHA) in mice induced by Mitomycin C (MMC) and intervention with various doses of Vitamin C (V).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total sperm cells studied(n)</th>
<th>Total aberrant sperm cells studied</th>
<th>Types of abnormalities studied</th>
<th>% Aberration mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>3005/3</td>
<td>101</td>
<td>Abnormalities (Mean ± SD)</td>
<td>3.36 ± 0.64</td>
</tr>
<tr>
<td>V1</td>
<td>3002/3</td>
<td>141</td>
<td>Abnormalities (Mean ± SD)</td>
<td>4.69 ± 0.17</td>
</tr>
<tr>
<td>V2</td>
<td>3002/3</td>
<td>129</td>
<td>Abnormalities (Mean ± SD)</td>
<td>4.29 ± 0.09</td>
</tr>
<tr>
<td>V3</td>
<td>3000/3</td>
<td>142</td>
<td>Abnormalities (Mean ± SD)</td>
<td>4.73 ± 0.22</td>
</tr>
<tr>
<td>MMC</td>
<td>3019/3</td>
<td>352</td>
<td>Abnormalities (Mean ± SD)</td>
<td>11.66 ± 0.61*</td>
</tr>
<tr>
<td>V1+MMC</td>
<td>3001/3</td>
<td>338</td>
<td>Abnormalities (Mean ± SD)</td>
<td>11.26 ± 0.45*</td>
</tr>
<tr>
<td>V2+MMC</td>
<td>3000/3</td>
<td>270</td>
<td>Abnormalities (Mean ± SD)</td>
<td>9.00 ± 0.40**</td>
</tr>
<tr>
<td>V3+MMC</td>
<td>3013/3</td>
<td>143</td>
<td>Abnormalities (Mean ± SD)</td>
<td>4.74 ± 0.24**</td>
</tr>
</tbody>
</table>

Con: received only vehicle; MMC: Mitomycin C (2 mg/kg/bw); Vitamin C: V1: 125 mg/kg/bw; V2: 250 mg/kg/bw; V3: 500 mg/kg/bw; p.o.: Per oral; i.p: Intraperitoneal; SCU: Sister chromatid union; R.T: Robertsonian translocation. n : Total number of animals; Groups bearing the same superscript are significantly different from each other [a, b, c, d = P<0.001***; f = P<0.01**; e = P<0.05*]; Groups bearing the any of the following symbol is significantly different from the Control groups, ***P<0.001; **P<0.01; *P<0.05.
Figure 1 Histograms showing the frequency of micronucleated polychromatic erythrocytes (PCE) after 24 h and 48 h exposure in the bone marrow cells following Vitamin C and Mitomycin C treatment. Con = Control, MMC = Mitomycin C (2 mg/kg/bw); V1, V2, V3 = Vitamin C (125, 250 and 500 mg/kg/bw respectively). *** = P< 0.001.

Antioxidative Potential of Vitamin C Against......

essence of Vitamin C. Hence, its judicious application in right doses and for proper duration can minimize and mitigate the dreaded side-effects of MMC on the normal cells and tissues of the body.

References


Acknowledgement

The authors are thankful to Assam University for providing laboratory support, DST, GOI for financial support to SG and University Grant Commission for granting SRF to MM.


