Effect of an anticancer drug-Methotrexate in the serum and seminal vesicles of adult male albino rats

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Citation
EFFECT OF AN ANTICANCER DRUG-METHOTREXATE IN THE SERUM AND SEMINAL VESICLES OF ADULT MALE ALBINO RATS

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ABSTRACT: Methotrexate (MTX) an anti-folate is widely used for the treatment for both neoplastic and non-neoplastic diseases. Though effective drug, methotrexate toxicity to normal proliferating tissue such as bone marrow, intestinal mucosa and gonads is often encountered. These toxic side effects have been largely prevented by the use of Leucovorin, afolate derivative. Vitamin A and related retinoids have anticancer properties. Hence for this study in mature adult male Wistar albino rats, the effect of Methotrexate in combination with or without leucovorin and vitamin A supplementation were taken .The body weight, testicular weight, accessory sex organs, few serum and tissue biochemical parameters were studied in the seminal vesicles of Albino rats.It was found that Leucovorin supplementation alone was ineffective in the study, further supplementation with vitamin A brought the majority of the biochemical profile taken for the study to near normalcy.

INTRODUCTION

The efficacy and toxicity of cancer chemotherapy is an important, Still controversial and under research area. In general, drugs used in the treatment of cancer are well known to produce a number of reversible toxic effects on normal tissue. (Schilsy et.al., 1980). All chemotherapeutic drugs including antimetabolites, the therapeutic and toxic effects often depend heavily on duration of exposure to the drugs. The drugs used to treat cancer today are a confusing array of compounds with differing organs, mechanisms of action, anti-tumor spectra and toxicity(Black& Livingston, 1990). The antimetabolite consist of methotrexate, pyrimidine and purine analoges and pentostatin, an adenosine deaminase inhibitor and relative new comer to the class (Pinedo, 1978). All the above cancer chemotherpeutic drugs act on the folate metabolism.

Later development and studies on the antimetabolite drugs provided a great impetus to evaluate the single and combined dose drug toxicity in every organ. Among them, infertility must be considered an unavoidable and unexpected side effects of many patients (Van scott and Renerton
Co-administration of Methotrexate and the reversing agents leucovorin is referred to as protection while delayed use of latter has been termed methotrexate rescue interest in antifolate has increased greatly with the introduction of high dose regiments with rescue of host toxicity by leucovorin (Stover and Schirch, 1993). Leucovorin is capable replenishing intracellular pools of reduced folate affected by methotrexated inhibition of DHFR. Theoretically, leucovorin in low concentration rescue normal cells which retain an active transport mechanism for reduced folate. But it was noticed that leucovorin produced a potentiating effect of Methotrexate toxicity instead of nullifying the toxic effects of methotrexate (Sampathraj, 1994). Chemotherapy can injure cancer cells by creating oxidative damage. As a result, some oncologists recommend that patients avoid supplementing antioxidants if they are undergoing chemotherapy. Limited test tube research occasionally does support the idea that an antioxidant can interfere with oxidative damage to cancer cells (Witenbery et al., 1999). However, most scientific research does not support this supposition. A modified form of vitamin A has been reported to work synergistically with chemotherapy in test tube research (Sacks et al., 1995). Vitamin C appears to increase the effectiveness of chemotherapy of in animals (Tapper et al., 1987) and with human breast cancer cells in test tube research (Kurbacher et al., 1996).

Hence in the present investigation an attempt is made to supplement Vitamin A along with Leucovorin to assess the reproductive toxicity in the male albino rat.

**MATERIALS AND METHODS**

**Experimental Animal:**

Mature adult male Wister strain albino rats weighing about 180-200gm body weight were selected for this work. They were maintained in a well-ventilated animal house with constant 12 hours of darkness and 12 hours of light schedule. Clean water and standard pellet diet (Hindustan Lever Ltd., India) were available to them 'adlibitum'.

**Experimental design:**

The animals were divided into four groups, each consisting of 5 animals.

- **GROUP I**: Control rats-received 0.9% saline Intramuscularly (IM)
- **GROUP II**: Rats received Methotrexate (MTX) at the dose of 8.5 mg/kg body weight (IM) for 7 consecutive days.
- **GROUP III**: Rats received MTX at the dose of 8.5 mg/kg body weight (IM) + Leucovorin (LCN) at the dose of 1.5 mg/kg body weight (IM) as supplement after 4 hours of MTX administration for 7 consecutive days.
GROUP IV: Rats received MTX at the dose of 8.5 mg/kg body weight (IM) + LCN at the dose of 1.5 mg/kg body weight (IM) as supplement after 4 hours of MTX administration for 7 days consecutively + vitamin A at the dose of 5 mg/kg body weight before MTX and LCN administration orally.

All the treatment was given between 9.30 and 10.00 hour in the morning. After 24 hours of last injection, the final body weight was recorded and the animals were sacrificed by decapitation. Blood was collected, sera separated by centrifugation at 3000 x g for 10 minutes and stored at -20C until used for various biochemical assays. Then testis, epididymis, vasdeferens, seminal vesicle and ventral prostate were dissected out, trimmed off extraneous tissues and weighed accurately on a torsion balance.

Assessment of sperm motility:

The motility of the spermatozoa was observed under microscopic at 100x magnification. The distance traversed by the sperm was determined using an occulometer.

Sperm count determination:

Collection of epididymal fluid

Sperm count was carried out by using Neubauer haemocytometer as described by Zaneveld and Polakoski (1977).

Estimation of blood glucose

The blood glucose was estimated by the method of Folin and Wu (1920).

Estimation of serum protein

The protein concentration of the serum was estimated by the method of Lowry et al., (1951)

Estimation of serum albumin

Estimation of albumin was done by Folin's method.

Estimation of serum globulin

serum globin was estimated by substracting the concentration of albumin from the total protein and expressed as gm/dl.

Estimation of urea

The serum urea was estimated by modified DAM-TSC method (Marsh et al.,1965).

Estimation of creatinine

The serum creatinine was determined using the Jaffe's reaction by the method of Owen et al., (1954).

Toxicity studies:
Liver and kidney function tests

The liver and kidney function tests were carried out with the estimation of glutamate - Oxalo - acetic transaminase (GOT) and glutamate pyruvic transaminase (GPT) in serum samples of control, MTX, MTX + LCN and MTX + LCN + Vit A treatment groups following the method of Reitman and Frankel (1957).

Statistical Analysis:

The data were statistically analysed and expressed as mean ± standard error of mean (S.E.M). The S.E.M were calculated as follows (Ostle, 1966)

\[
S.E.M = \frac{\sum X^2 - (\sum X)^2}{n(n-1)}
\]

and Student’s ‘T’ test was used to compare the mean values of two groups. The number of values of probability was obtained from the degree of freedom by using the standard table given by Fisher and Yates, 1963.

RESULTS

Fig: Decreased secretion in the seminal vesicles of MTX treated groups

Fig: Necrotic condition of vas deferens in the MTX treated groups

Fig: Macroscopic changes in MTX treated groups in the testis and epididymis
### Table 1: Effect of Vitamin A and Leucovorin supplementation on the body and reproductive organ weight in MTX treated adult Albino rats.

<table>
<thead>
<tr>
<th>S.N o</th>
<th>Groups</th>
<th>Body Weight (gm)</th>
<th>Testis (gm)</th>
<th>Epididymis (mg)</th>
<th>Vas deferens (mg)</th>
<th>Seminal Vesicle (mg)</th>
<th>Prostate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caput</td>
<td>Cauda</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>211±10.3</td>
<td>1.894±0.094</td>
<td>258.14±10.1</td>
<td>193.16±11.3</td>
<td>88.4±6.1</td>
<td>369.40±21.6</td>
</tr>
<tr>
<td>2</td>
<td>MTX</td>
<td>181±9.8</td>
<td>1.31±0.07</td>
<td>184.13±9.5</td>
<td>141.17±9.3</td>
<td>69.3±5.4</td>
<td>343.16±20.4</td>
</tr>
<tr>
<td>3</td>
<td>MTX + LCN</td>
<td>192±10.4</td>
<td>1.436±0.09</td>
<td>192.31±7.8</td>
<td>156.57±8.8</td>
<td>74.5±4.8</td>
<td>326.17±21.5</td>
</tr>
<tr>
<td>4</td>
<td>MTX + LCN + Vit. A</td>
<td>204±9.8</td>
<td>1.786±0.08</td>
<td>213.46±8.1</td>
<td>181.64±8.8</td>
<td>81.5±7.1</td>
<td>351.73±18.9</td>
</tr>
</tbody>
</table>

### Table 2: Effect of Vitamin A and Leucovorin supplementation on the sperm concentration and motility in the Epididymis in MTX treated adult Albino rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>MTX</th>
<th>MTX + LCN</th>
<th>MTX + LCN + Vit. A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration</td>
<td>494.00±53.00</td>
<td>556.00±48.00</td>
<td>404.40±33.00</td>
<td>492.00±29.00</td>
</tr>
<tr>
<td>Sperm Motility (FMI)</td>
<td>106±1.40</td>
<td>-</td>
<td>81.00±1.50</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 3: Effect of Vitamin A and Leucovorin supplementation on few Serum Biochemical profile in MTX treated adult Albino rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>MTX</th>
<th>MTX + LCN</th>
<th>MTX + LCN + Vit. A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein(g/dl)</td>
<td>6.18±0.56</td>
<td>4.94±0.71</td>
<td>5.06±0.81</td>
<td>6.88±0.91</td>
</tr>
<tr>
<td>Albumin(g/dl)</td>
<td>3.38±0.46</td>
<td>3.13±0.04</td>
<td>2.75±0.73</td>
<td>3.78±0.31</td>
</tr>
<tr>
<td>Globulin(mg/dl)</td>
<td>3.60±0.20</td>
<td>1.81±0.02</td>
<td>2.51±0.63</td>
<td>3.10±0.29</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>68.41±5.10</td>
<td>48.31±1.60</td>
<td>52.62±3.87</td>
<td>59.43±5.90</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>12.56±1.2</td>
<td>24.31±1.30</td>
<td>15.34±1.40</td>
<td>13.44±1.20</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.66±0.04</td>
<td>0.78±0.08</td>
<td>0.74±0.04</td>
<td>0.73±0.07</td>
</tr>
</tbody>
</table>

### Table 4: Effect of Vitamin A and Leucovorin supplementation on the Protein, Fructose concentration and a few enzymes of the Seminal vesicle in MTX treated adult Albino rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>MTX</th>
<th>MTX+ LCN</th>
<th>MTX+ LCN + Vit.A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (mg/ml)</td>
<td>11.8±2.1</td>
<td>7.4±1.2</td>
<td>8.1±1.8</td>
<td>10.9±1.3</td>
</tr>
<tr>
<td>Fructose (mg/ml)</td>
<td>17.4±3.4</td>
<td>26.4±2.6</td>
<td>20.6±2.1</td>
<td>18.1±2.8</td>
</tr>
<tr>
<td>ALP(moles of P-nitrophenol formed / hr / mg protein)</td>
<td>68.6±8.5</td>
<td>86.3±7.4</td>
<td>82.4±3.2</td>
<td>72.1±6.4</td>
</tr>
<tr>
<td>SGPT(Unit / min / mg / proteins)</td>
<td>14.6±1.2</td>
<td>38.6±2.4</td>
<td>30.2±2.1</td>
<td>21.6±3.1</td>
</tr>
<tr>
<td>SGOT( Unit / min / mg / proteins)</td>
<td>19.2±1.6</td>
<td>29.6±3.1</td>
<td>22.4±1.8</td>
<td>20.1±2.6</td>
</tr>
</tbody>
</table>

Each value is * SEM of 5 animals
**DISCUSSION:**

**Body weight**

The reduced body weight observed in 7 days in Methotrexate treated groups suggest that MTX has an adverse effect in general body metabolism. This observation is consistent with the early report of Aarsaether et al., 1988. MTX drug treatment was known to cause acute side effects including nausea, vomiting, stomatitis, alopecia, bone marrow depression and injury to the gastrointestinal epithelium (Morgan et al., 1990). Hepatotoxicity, abnormal function test and renal dysfunction are more marked during low dose MTX therapy in human. The sudden traumatic alteration in the normal health conditions of the animal, the less food intake and gastrointestinal intolerance may be the cause for reduced body weight in drug treated groups. The toxic effect of low dose (7.5 - 15 mg/week) often mimics folate deficiency (Blakely, 1969) which resulted in alteration in the protein synthesis. Cytoplasmic growth is determined by this factor. Hence, could markedly expect a decrease in organ weight.

Testis is an organ, in which cells undergoing divisions constantly are most susceptible to damage leading to cell death. Therefore the reduced weight of testis can be correlated to spermatogenic arrest and depletion of germ cells in the seminiferous tubules, which form 80% of the cell population in the testis. Thespermatogenic arrest is clearly demonstrated in the present study elsewhere, by the reduced concentration of caput and caudal epididymal spermatozoa.

**Accessory sex organs:**

The decrease in caput epididymal and seminal vesicle weight observed in MTX treatment and other supplemented groups may partly be due to the decreased sperm concentration and seminal vesicular secreting activity. A similar reduction in the epididymal segment and seminal vesicle weight was also observed in low dose MTX treatment (Sampathraj, 1994) and other DHFR inhibits pyramethamine (Awoniyi et al., 1993). The double supplementation groups showed no significant reduction evidenced well in nullifying effect of MTX toxicity. This warranted for further studies on the mechanism.
Sperm count and motility:

In the present study, the observed decreased testicular weight revealed a high degree of spermatogenic disruption, leading to the inhibition of spermatogenesis. Hence, the decreased number of sperm count is due to the inhibition of sperm production itself. Accumulation of carnitine in the rat epididymis may be directly associated with acquisition of flagella movement. Since spermatozoa are dependent on fatty acid metabolism, carnitine may play a crucial role in preserving sperm viability and motility in the epididymis (Casillas., 1971; Voglmayr., 1975). Further, fatty metamorphosis of liver is a side effect of long –term treatment of patients as well as rats with antifolatedrug, Methotrexate in low dose treatment (Custer et al., 1977; Nyfors and Poulson., 1997). In the present investigation, the probable effect may be the inhibition of fatty acid metabolism and decreased concentration of seminal fructose which leads to impairment of sperm motility and viability.

Serum biochemical profile:

Methotrexate is a well known DNA inhibitory drug. The decrease in protein concentration in MTX, MTX + LCN treated groups may be due to the inhibition of protein synthesis though DNA synthesis machinery (Yamato et al., 1988). Since MTX enters both tumor and normal cell to alter the protein biochemical sequences and its interaction within the cell further elucidated a depletion in the serum protein concentration in the present study.

The reduction in both the in the serum globulin concentration in both the treated groups, except vitamin A supplementation shows that MTX has an adverse effect on the immunoglobulin level, since it is an immunosuppressive drug. The increased levels of urea, creatinine in the drug treated groups may be due to the altered rate of anabolic and catabolic activity in the liver and kidney organs. Vitamin A supplementation is effective in retrieving all the serum biochemical profile except the total urea concentration. Further study on liver and kidney marker enzymes may elucidate the exact cause on MTX drug toxicity.

Tissue biochemistry:

A specific maker enzymes like ALP, in the seminal vesicle was found to be depleted on MTX treatment and MTX+LCN treatment and the same effect was not seen in the vitamin A supplemented groups. Vitamin A supplementation is effective in bringing back the normalcy in tissue marker enzymes like SGOT, SGPT and ALP in the seminal vesicle because of its antioxidant
property. Further study is required to find out the influx and efflux of enzyme activity in the seminal activity in the seminal vesicle is needed.

CONCLUSION

Methotrexate exerted an adverse effect on both the serum and seminal vesicle biochemical parameters. Leucovorin supplementation alone was ineffective in the study. While further supplementation with vitamin A brought the majority of the above mentioned biochemical profile to near normalcy in the present study. Further investigations on this line may give more insight in to the role of vitamin A on the seminal vesicular secretion after MTX administration.

REFERENCES:


