Measuring Tolerance Dose of the Methotrexate for Induction of Oral Mucositis in Dark a Gourt\(\text{r}\) (Pilot Study)

Dr. Bahaa A. A. Hadi\(^1\), Dr. Jamal N. Ahmed\(^2\)

\(^1\)B.D.S. Msc. Ph.D Student Oral Medicine, Oral Diagnosis Department, College of Dentistry, University of Baghdad, Baghdad, Republic of Iraq
\(^2\)Professor, B.D.S. Msc. Ph.D Oral Medicine, Oral Diagnosis Department, College of Dentistry, University of Baghdad, Baghdad, Republic of Iraq

**Abstract:** Oral mucositis severe sideeffect, caused by treatment with radiotherapy and chemotherapy (CT) for cancer. Major progress has been made in recent years in understanding the mechanisms of oral and enteral mucositis. This progress is largely due to the development of representative laboratory animal models of mucositis. A total of sixteen rats were included in this study and divided into three groups: group (A) included five rats, group (B) included six rats and group (C) included five rats. A were injected with a dose of 80mg/kg methotrexate (MTX) cytotoxic drug with a single intraperitoneal (I.P) injection at base line time (day zero). Group (B) were injected with a dose of 60mg/kg MTX (I.P) at day zero, group (C) the rats were injected with a dose of 40mg/kg MTX at day zero. There was a statistical significant negative correlation that correlate the doses of MTX with the mortality duration of rats among the pilot experimental study groups. We found all rats induced with oral mucositis at day seven with the main scores range (0-2), the rats had died 21 days after treatment.

**Keywords:** Oral mucositis, methotrexate, tolerance dose& dark agoutri rat

1. **Introduction**

Oral mucositis (OM) is considered an acute sideeffect reported in patients undergoing mucotoxic chemotherapy (CT) [10]. When severe and/or widespread, it is associated with intense pain and bleeding, increasing the risk of systemic infection; need for fluids and nutritional support; and significant additional hospitalization costs [5, 3]. Although the pathogenesis of OM has not yet been completely elucidated, it has become accepted that proinflammatory cytokines are released in response to reactive oxygen species produced in the cells, resulting from the effect of CT [2,6]. One of the cytokines most involved in the pathogenesis of OM is the tumor necrosis factor (TNF-\(\alpha\)) related damage to the epithelium, accelerating the formation of OM [6, 9]. Clinically, OM is characterized by pain and erythema, and when severe, it may be characterized by confluent ulcerative lesions that interfere with normal oral functions [7]. Although many palliative interventions have been used in OM management, recent studies have indicated the use of phototherapies as an effective and promising treatment [5, 3]. The drugs used in chemotherapy like Methotrexate (MTX) destroy rapidly dividing cells in the body like the gastrointestinal epithelium, including the buccal epithelium [10,5]. This condition affects the quality of life and nutritional state of the patient whomay require hospitalization thus increasing health care costs [3]. Adjusting the optimal toxic dosage of MTX that can be used in a standard research protocols is an important demand. Reduce the mortality rate of the experimental animals for reproducibility of this research in multi scientific topics.

2. **Materials and Methods**

A total of ten rats were included in this study and divided into three groups: group (A) included five rats, group (B) included six rats and group (C) included five rats. Group A were injected with a dose of 80mg/kg methotrexate (MTX) cytotoxic drug with a single intraperitoneal (I.P) injection at base line time (day zero). Group (B) were injected with a dose of 60mg/kg MTX (I.P) at day zero, group (C) the rats were injected with a dose of 40mg/kg MTX at day zero. The animals were kept in the cage under standard condition (room temperature, standard rat chow and water drinking). The animals were also kept under strict observation every day to watch their health condition.

**Table 1:** Scale of the WHO modified for animal[9].

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Pouch completely healthy, No erythema or vasodilation</td>
</tr>
<tr>
<td>1</td>
<td>Light to severe erythema AND vasodilation. No erosion of mucosa.</td>
</tr>
<tr>
<td>2</td>
<td>Sever erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased slipping of mucosa.</td>
</tr>
<tr>
<td>3</td>
<td>Formation of off white ulcers in one or more places. Ulcers may have a yellow/gray due to pseudomembrane.</td>
</tr>
<tr>
<td>4</td>
<td>Cumulative seize of ulcers should equal about1/2 of the pouch. Loss of pliability. Sever erythema and vasodilation.</td>
</tr>
<tr>
<td>5</td>
<td>Virtually all of pouch is ulcerated. Loss of pliability (pouch can only partially be extracted from mouth)</td>
</tr>
</tbody>
</table>
3. Results

Tolerance of the rats to the chemotherapy methotrexate (MTX) and induction of oral mucositis:

A total of sixteen rats were included in this study and divided into three groups: group (A) included five rats, group (B) included six rats and group (C) included five rats. Group A were injected with a dose of 80 mg/kg methotrexate (MTX) cytotoxic drug with a single intraperitoneal (IP) injection at base line time day zero. The rats had died four days after injection due to severe diarrhea. Mucosal changes were noticed redness in two rats, in other words score (1) Group (B) were injected with a dose of 60 mg/kg MTX (IP) at day zero, we found all rats induced with oral mucositis at day

Table 2: Experimental doses of methotrexate with different life span

<table>
<thead>
<tr>
<th>Study group/(N)</th>
<th>Variables</th>
<th>Life span</th>
<th>MTX DOSE</th>
<th>Oral mucositis Induction score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>10.40 ± 5.562</td>
<td>64 ±15.776</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>1.759</td>
<td>4.989</td>
<td>0.133</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>14</td>
<td>60</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>17</td>
<td>40</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>4</td>
<td>40</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>21</td>
<td>80</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>(4,21) days</td>
<td>(80,60,40) mg/kg</td>
<td>(0,2)</td>
<td></td>
</tr>
</tbody>
</table>

Group A (5) *P value (60-80) mg/kg 0.025
Group B (6) *P value (60-40) mg/kg 0.025
Group C (5) *P value (80,40) mg/kg 0.011

4. Discussion

Mucositis is a major oncological problem. The entire gastrointestinal and genitourinary tract and also other mucosal surfaces can be affected in recipients of radiotherapy, and/or chemotherapy. Major progress has been made in recent years in understanding the mechanisms of oral and small intestinal mucositis, which appears to be more prominent than colonic damage. This progress is largely due to the development of representative laboratory animal models of mucositis[10]. Methotrexate (MTX) is one of the antimitotic drugs. These drugs act by blocking deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis, inducing apoptosis and slowing down or stopping cell proliferation in rapidly proliferating cells such as tumors, bone marrow and gut mucosa cells[11]. MTX, a folic acid antagonist, is widely preferred as a cytotoxic/chemotherapeutic agent but its efficacy is limited due to its side effects[13]. Severe OM can negatively influence the patient’s prognosis and have important economic impact, resulting from costs associated with management of the symptoms [17]. The raised mortality rate that has emerged in this study could be due to chronic diarrhea, renal and hepatic toxicity. The present work coincided with the results that obtained from Lotfy & Zayed, 2009 [1] had a photomicrograph of buccal mucosa of rat treated by 80 mg/kg MTX showed a significant decrease in the thickness of the epithelium. The direct inhibitory effects of chemotherapy on DNA

Figure 1: Intra peritoneal injection of oral mucositis

Figure 2: Photograph showing clinical scoring of experimental oral mucositis (rats), 0,1,2

Volume 6 Issue 6, June 2017
www.ijsr.net
Licensed Under Creative Commons Attribution CC BY
Paper ID: ART20174877 DOI: 10.21275/ART20174877 2018
replication and mucosal cellular proliferation result in reduction in the renewal capacity of the basal epithelium [1].

The present pilot study revealed mild degree of oral mucositis at 40 mg/kg of MTX dosage, while at 60 mg/kg of MTX the rats were developed oral mucositis with a range of severity (0-2) with sufficient span of life that permit us to monitor the path of development in amanar that mimic oral mucositis in human model. Lotfy & Zayed, 2009 showed the animals that received 10 mg/kg, 20 mg/kg, and 40 mg/kg of MTX, there were increase in the thickness of the epithelium and the number of sub epithelial inflammatory cellsand congested blood vessels. In ischemic inflammatory/vascularphase of mucositis, the epithelial, endothelial, and connective tissues in the buccal mucosa release free radicals, modified proteins, and proinflammatory cytokines, including interleukin-1β, prostaglandins, and Tumor Necrosis Factor (TNF). These inflammatory mediators cause further damage either directly or indirectly by increasing vascular permeability and angiogenesis, thereby enhancing cytotoxic drug uptake into the oral mucosa [1]. In addition to that, there was increase in the number of connective tissue congested blood vessels. In ischemic phase, the chemotherapeutic causes the release of cytokines from the epithelium and the connective tissue which initiate an inflammatory response that may result in increased sub epithelial vascularity. This phase is considered to be relatively acute [13]. The result also showed that, when there was increase the dose of MTX the cytotoxic effect increased. Munaretto et al. [13] showed immune suppressed the mice with subcutaneous injections of 2.5 mg/kg MTX for three consecutive days, the epithelial thickness of the ventral surface of the tongue was increased significantly in the second day of the experiment, but it decreased gradually later on. The number of blood vessels and inflammatory cells per field in the connective tissue was similar in control and experimental samples. The differences between this study and other results in the procedure used avoid direct assessment between them. Conclusions: MTX at 60 mg/kg can be used in experimental induction of oral mucositis in dark agouti rat with minimal mortality rate and representative clinical grading of oral lesions that can be synchronous human oral mucositis.

Abbreviations

- CT: chemotherapy
- MTX: methotrexate
- OM: oral mucositis
- LP: intraperitoneal
- RNA: ribonucleic acid
- DNA: deoxyribonucleic acid
- Mg: milligram
- Kg: kilogram

References


