International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Measuring Tolerance Dose of the Methotrexate for Induction of Oral Mucositis in Dark a Gourti Rat. (Pilot Study)

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Abstract: ***Oral mucositis severe side effect, 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. 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 60 mg/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 correlates the doss of MTX with the mortality duration of rats among the plot 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 side effect reported in patients undergoing mucotoxic chemotherapy (CT) [10]. When severe and / or wide spread, it is associated with intense pain and bleeding, increasing the risk of systematic infection; need for fluids and nutritional support; and significant additional hospitalization costs [5, 3]. Although the pathogenesis of OM has not yetbeen 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 pathogeneses of OM is the tumor necrosis factor (TNF-α) related to 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 who may 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 60 mg/kg MTX (I.P) at day zero. Group (C) the rats were injected with a dose of 40mg/kg MTX at day zero. The animal's were kept in the cage under standard condition (room temperature, standard rat chow and water drinking). The animal's were also kept under strict observation every day to watch their health condition.

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

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

Figure 1: Intra peritoneal injuction of oral mucositis

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 80mg/kg methotrexate (MTX) cytotoxic drug with a single intraperitoneal (I.P) 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 (I.P) at day zero, we found all rats induced with oral mucositis at day seven with the main scores range (1-2), the rats had died 14 days after injection. Group (C) the rat's were injected with a dose of 40mg/kg MTX at day 0, they haven't developed oral mucosal changes and died 21 days after injection. We did these pilots experimental study for adjusting the optimal toxic dosage of MTX that promote adequate lifespan as long as possible to study oral mucosal changes (histologically and clinically) along the course of experimental oral mucositis.

Table 2: Experimental doses of methotrexate with different life span

Study group/ (N)	Variables	Life span	MTX DOSE	Oral mucositis Induction score
	Mean ± SD	10.40 ± 5.562	64 ±15.776	0.80 ±0.422
	Std. Error of Mean	1.759	4.989	0.133
	Median	14	60	1
	Range	17	40	1
	Minimum	4	40	0
	Maximum	21	80	1
	Frequency	(4, 21) days	(80, 60, 40)mg/kg	(0, 2)
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

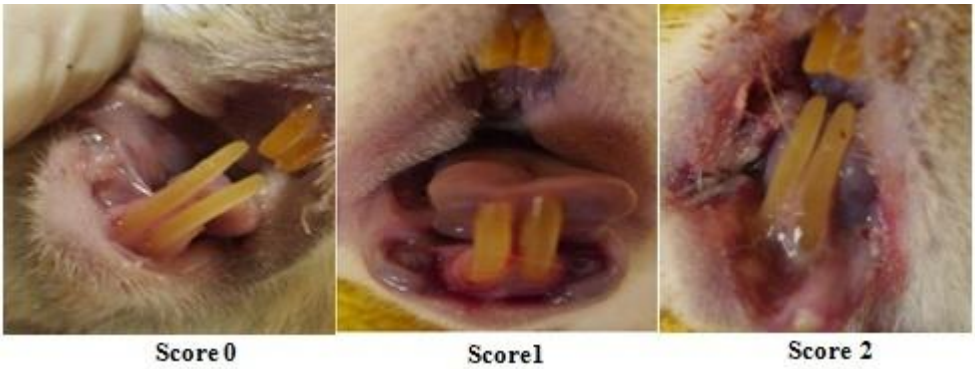

Figure 2: Photograph showing clinical scoring of experimental oral mucositis (rats), 0, 1, 2

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 cytotoxicchemotherapeutic agent but the 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 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 60mg/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 cells and congested blood vessels. In the initial inflammatory / vascular phase of mucositis, the epithelial, endothelial, and connective tissue cells in the buccal mucosa release free radicals, modified proteins, and proinflammatory cytokines, including interleukin-1B, 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 up take into the oral mucosa [1]. In addition to that, there was increase in the number of connective tissue congested blood vessels. In the initial phase, the chemotherapy causes the release of cytokines from the epithelium and the connective tissues which incite 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 in the dose of MTX the cytotoxic effect increased. Munaretto et al. [13] showed immune suppressed the mice with the 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 cellsper 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 a 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 synonymous human oral mucositis.

Abbreviations
CT chemotherapy
MTX methotrexate
OM oral mucositis
I.P intraperitoneal
RNA ribonucleic acid
DNA deoxyribonucleic acid
Mg milligram
Kg kilogram

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