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EVALUATION OF TOXICITIES INDUCED BY SHORT INTERVAL, HIGH DOSE METHOTREXATE

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Folic acid plays an important role in DNA synthesis, during cell division. Folic acid antagonists, therefore, interfere in cell growth, and are in use in cancer chemotherapy since long. Methotrexate, a folic acid antagonist, has shown good results in the treatment of a number of malignancies. Most of the clinical protocols, applied in cancer treatment, are not in use in their standard form to achieve maximum benefit and they are often leading to considerable toxicities. Evaluation of toxicities, induced by high doses of Methotrexate, repeated after short periods of time, was carried out in rabbits. Each animal received 40 mg/kg of body weight/72 hr. upto four doses, of Methotrexate, without citrovorum factor rescue through intraperitoneal route. Nine different gross toxicities, which are usually developed during Methotrexate therapy, were observed. Hepatotoxicities and nephrotoxicities were determined by biochemical and histopathological changes. No considerable or statistically significant general, biochemical or histopathological changes were found.

Key words. Methotrexate, Toxicity, Chemotherapy.

Introduction

Antimetabolites are in use in cancer chemotherapy since long. After the isolation and synthesis of folic acid [1-3] it was found very soon, that folic acid antagonists would interfere cell growth [4]. The beneficial role of these antagonists was reported when aminopterin [5] was used in the treatment of acute lymphoblastic leukemia [6]. Since then the Methotrexate has become the most commonly used folic acid antagonist, in the treatment of several malignant diseases.

Methotrexate has shown dramatic results both in combination and alone in anticancer therapy. It is important in the treatment of acute lymphoblastic leukemia [7,8], lymphomas [9], osteogenic sarcoma [10], squamous cell carcinoma of head and neck [11] and breast cancer [12]. It is considered to produce objective responses in some patients with lung cancer, epidermoid carcinoma of the cervix and some other solid tumors [13].

The treatment schedules, based on Methotrexate have become more numerous, varied and sometimes more complicated over the last twenty to thirty years, with increase in its use by clinicians and laboratory workers. Now, it is not possible to offer a simple, clear cut guideline that any clinician may follow and be certain of achieving a particular result in a malignancy. As the treatment schedules are not consistent with strategies and can not be used in their efficient forms in developing countries, so it is necessary to work out a new plan. In the present study, we have tried to evolve such a plan for the cancer chemotherapy by Methotrexate.

Materials and Methods

This study was conducted on healthy male rabbits with an average weight of 1327 gm divided in two groups of seven rabbits each (totally 14 rabbits). The apparent healthy conditions of the rabbits were observed for several days during the conditioning period. Special attention was given to hair loss, ulceration, lack of activity, lack of appetite, etc. Both groups, A and B, were also tested for their liver functions, renal functions etc., before giving any drug or placebo. Group 'A' was given distilled water intraperitoneally (I.P.) and was used as a control group. Group 'B' received Methotrexate 40 mg/kg body weight intraperitoneally. The doses were repeated every 72 hr. up to four doses. In this way, each rabbit in group 'B' received a total of 200 mg Methotrexate, approximately.

The animals of both groups were maintained on a mixed free weight diet. The diet schedule was such that, before experimentation the animals were fed morning and evening while during the experimentation they were given a diet in the afternoon also. Blood samples were obtained by cardiac puncture technique from both groups (one animal of each group expired by this invasive technique due to cardiac temponade) and were collected in 5ml rubber stoppered tubes without using any anticoagulant. Samples were stored at 2° and analyzed within 12 hr. The alkaline phosphatase, SGOT, SGPT, bilirubin, urea, creatinine and cholesterol levels in these samples were determined by specific reagents kits Merck by autoanalyzer (Hitachi 750 system). The first, second and third samples were taken after one, five and ten days of last dose respectively. The general toxicities were also noted at each collection of sample.

After the tenth day, the animals were sacrificed. Autopsies were performed. All organs were examined for gross changes if any. The samples of liver and kidneys were collected for histopathological examination. Student 't' test was applied for the statistical analysis of data.

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Results Discussion

Table 1 shows general toxicities noted during the experiment. No. animal of any group developed vomiting, diarrhoea, haematuria, loss of hair, skin ulceration, loss of physical activities, oedema of dependent parts or loss of interest in food. However, weight loss after five and ten days of drug administration was noted in animals of group 'B'.

Table 2 shows growth inhibition in animals, evaluated by inability to gain weight after increasing the number of diets per day. Animals of both groups gained weight after day one, but animals of group 'A' kept on gaining, whereas animals of group 'B' lost weight after the fifth and tenth day of drug administration. The weight gain and loss were observed in all animals of both groups. The loss of weight indicates a general inhibition of body building components after Methotrexate administration.

Table 3 presents important liver parameters observed at various stages. No significant difference (P>0.05) was found in the alkaline phosphatase of both the groups. However, 40% of the animals of group 'B' showed higher values of the enzyme after the tenth day. Similarly, no significant difference (P>0.05) was found in SGOT and SGPT of both the groups. However, a definite pattern was noted in SGOT, which was found to be raised on the first day, but decreased after the fifth day and came back to the control values after the tenth day.

Table 4 shows important renal parameters. Urea did not change significantly (P>0.05) after the first and fifth day of total drug administration but increased significantly (P<0.05) after the tenth day in the animals of group 'B'. Creatinine rise was insignificant (P>0.05) after the first day, but there was a significant increase (P<0.05) after the fifth day; however, after

TABLE 1. METHOTREXATE INDUCED GENERAL TOXICITIES.

Toxicities	Interval	Group 'A'	Group 'B'
Vomitting	1st*	None	None
	2nd**	None	None
	3rd***	None	None
Diarrhoea	1st	None	None
	2nd	None	None
	3rd	None	None
Gross Hemituria	1st	None	None
	2nd	None	None
	3rd	None	None
Loss of hair	1st	None	None
	2nd	None	None
	3rd	None	None
Skin ulceration	1st	None	None
	2nd	None	None
	3rd	None	None
Weight loss	1st	None	None
	2nd	Present	Present
	3rd	Present	Present
Loss of activity	1st	None	None
	2nd	None	None
	3rd	None	None
Oedema	1st	None	None
	2nd	Non	None
	3rd	None	None
Loss of interest in food	1st	None	None
	2nd	None	None
	3rd	None	None

*After one day of total drug administration.; **After five days of total drug administration.; ***After ten days of total drug administration.

TABLE 2. METHOTREXATE INDUCED GROWTH INHIBITION.

Interval	Group 'A'	Group 'B'
1st	+125 gm (6)*	+102.5 gm (6)
2nd	+32 gm (6)	-37 gm (6)**
3rd	+10 gm (6)	-75 gm (6)

*Average weight gain (No. of animals).; **Average weight loss (No. of animals).

TABLE 3. METHOTREXATE INDUCED HEPATOTOXICITIES.

Parameter	Interval	Group 'A'	Group 'B'	Significance of difference
Alkaline phosphatase (U/L)	Ist	54.6± 13.8(6)*	73.6± 15.8(6)	P>0.05
	2nd	57.6 ±15.2(6)	76.1± 20.0(6)	P>0.05
	3rd	54.6± 15.1(6)	95.2± 28.4(6)	P>0.05
SGOT (U/L)	Ist	47.6± 06.6(6)	65.3± 26.6(6)	P>0.05
	2nd	41.6± 17.0(6)	44.0± 10.8(6)	P>0.05
	3rd	41.8± 05.7(6)	50.8± 10.4(6)	P>0.05
SGPT (U/L)	Ist	55.1± 06.6(6)	58.0± 04.1(6)	P>0.05
	2nd	63.0± 07.3(6)	48.8± 08.6(6)	P>0.05
	3rd	66.8± 06.2(6)	55.3± 06.8(6)	P>0.05
Bilirubin (mg%)	Ist	0.01±00.0(6)	0.21± 0.1(6)	P>0.05
	2nd	0.01± 0.15(5)	0.21± 0.04(5)	P>0.05
	3rd	0.01± 0.01(5)	0.18± 0.04(5)	P>0.05

*Average value ± S.E. (No. of animals).

TABLE 4. METHOTREXATE INDUCED NEPHROTOXICITIES.

Parameter	Interval	Group 'A'	Group 'B'	Significance of difference
Urea (mg%)	Ist	52.8± 1.7(7)	54.5± 2.9(6)	p>0.05
	2nd	52.8 ± 1.7(7)	54.3± 3.2(6)	P>0.05
	3rd	55.5± 4.7(6)	79.0± 6.9(5)	P<0.05
Creatinine (mg%)	Ist	0.98± 0.00(6)	1.02± 0.07(6)	P>0.05
	2nd	0.97± 0.01(6)	1.2± 0.07(6)	P<0.05
	3rd	0.96± 0.02(6)	1.05± 0.05(6)	P>0.05

the tenth day of drug administration it fell significantly ($P<0.05$), as compared to group 'A' animals. This pattern shows nephrotoxicity induced by Methotrexate, which was however reversible.

Table 5 presents effects of Methotrexate on cholesterol levels, which remained significantly low ($P<0.05$) in the animals of group 'A' as compared to the animals of group 'B', throughout the study.

TABLE 5. EFFECT OF METHOTREXATE ON CHOLESTEROL LEVEL (mg%).

Interval	Group 'A'	Group 'B'	Significance of difference
1st	47.16± 6.16(6)	28.5± 4.17(6)	P<0.05
2nd	41.33± 6.18(6)	28.0±22.70(6)	P<0.05
3rd	45.37± 4.64(6)	27.33±4.50(6	P<0.05

Histopathological examination of liver and kidneys in both groups of animals revealed no significant difference. Mild mononuclear infiltration was seen in one of the livers and focal interstitial infiltration in one of the kidneys in the experimental group, only.

Multidrug cancer chemotherapy can not be highly appreciated in developing countries like Pakistan, where cancer treatment by protocols using a number of drugs is a difficult task to achieve in its standard form, due to the non-availability of all the component drugs all the time throughout the treatment, high prices of one or more of the drugs, busy out door patient settings in hospitals, lack of training of medical and paramedical staff for the administration of different drugs in actual dosages and timingly schedules. Single drug cancer chemotherapy has, therefore, considerable advantages in the treatment of malignant diseases, when and where possible. The role of Methotrexate which is being used in a number of centres in such countries as a single agent in the treatment of different cancers especially head and neck malignancies, should not be neglected. However, the drug has been found to be associated with considerable toxicities [14].

In spite of the problems, encountered in connection with combination therapy, the good results of combination of drugs can not be ignored i.e. one leukemic cell may proliferate to a number that proves lethal to an animal [15], an observation that has been confirmed many times [16]. However, a particular dose of a chemotherapeutic agent will kill a certain percentage of leukemic cells, regardless of the number present [17]. So, a certain agent reduces the number of diseased cells within an organism and further doses of the same agent will kill diseased cells in the same percentage, but fewer in number. Therefore, at cessation of the therapy, if only one leukemic cell is viable, it may proliferate to a population which, if left unchecked, will become overwhelming and fatal. So, at the time of relapse, in case of leukemia if the patient is given two different drugs, each capable of killing half of the leukemic cells, then theoretically, the patient would be cured. But if only one agent, that may kill 90% of the leukemic cells, is given, and the doses is repeated several times, the patient will recover, but more slowly. It leads to the idea that, if the diseased cells are permeable and accessible for a certain drug, the larger the dose administrated, the greater its potential for accomplishing total cell kill. This is enough justification for the use of higher dose of a drug. Clincically a dose more than 1 g/m^2 or 20 mg/kg of Methotrexate is regarded as a high dose [18].

Previous studies have shown that Methotrexate infusions, when continued for more than 30 hr., were usually associated with significant toxicities [19,20], whereas short duration infusions were well tolerated. However, it has also been found that a low dose schedule for shorter periods has led to high toxicities [21]. Both these limitations can be observed if high doses are given by a 'push in' technique with 'short duration' as adopted in the present study. Although some changes which were not so apparent on first or fifth but become considerable on tenth day e.g. more growth inhibition, relatively high alkaline phosphatase and urea levels on tenth day are noted in our study, but they look transit or self limiting, as they do not occur with or supported by other biochemical parameters or histopathological examination of vital organs so they are not a reflection of entire organ toxicity.

The study of the biochemical mechanism of the acquired resistance to Methotrexate has been clearly demonstrated.

(a) Impaired transfer of drug into cells. (b) Production of altered forms of dihydrofolate reductase. (c) Increased concentration of intracellular dihydrofolate reductase [22].

These mechanisms are supported by the evidences that, (i) Blood elements with marked increase in the activity of dihydrofolate reductase appeared within day after the treatment of patients with single doses of Methotrexate (23). (ii) Excess of dihydrofolate reductase was found in Methotrexate resistant cell lines 24-26]. (iii) Replacement of Methotrexate by other active therapeutic agents, which have different mechanisms, were found to response the Methotrexate resistant tumours. (iv) Decreased formation of polyglutamates was found in Methotrexate non-responsive cells [27-30].

Hence, resistance is a consequence of inadequate and prolonged exposure of the malignant cells to the drug, it provides another justification for a high dose short gaped Methotrexate protocol.

Our study shows no considerable toxicities in animals after administration of high doses of Methotrexate spaced by short gaps. Now it requires further evaluation in humans before development of a clinical protocol.

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