A BLIND STUDY TO EVALUATE THE ROLE OF ALPHA-TOCOPHEROL TO REDUCE METHOTREXATE INDUCED TOXICITIES IN CANCER MANAGEMENT

MOHAMMAD T. AFTAB*
S.H.M. ZAIDI*
ZAFAR S. SAIFY*
ASIF B. REHMAN*
SHAHID RASHID*

SUMMARY: Methotrexate is the most commonly used antimetabolite, frequently producing dramatic results in a number of malignant diseases. However, the efficacy of the drug becomes limited due to unexpected and apparently quite sudden severe toxicity, which even persists despite the administration of citrovorum factor. Since alpha-tocopherol has been found to play a significant role against the development of anticancer therapy induced toxicities, a double blind study is conducted on twenty six patients, who were on prolonged Methotrexate therapy, for residue or recurrence of their head and neck malignancies. A significant decrease in renal toxicity is noted in patients who received alpha-tocopherol. Antioxidant action of alpha tocopherol probably plays an important role in this regard.

Key Words: Alpha-tocopherol, methotrexate toxicity, cancer.

INTRODUCTION

Antimetabolites have a definite role in systemic chemotherapy of malignant diseases (3,4,15,29,31). Methotrexate is the most commonly used antimetabolite, which competes with folic acid for the enzyme dihydrofolate reductase (5), blocks the formation of tetrahydrofolic acid compounds and cell division (11,30). This drug has shown dramatic results in the treatment of acute lymphoblastic leukemia (18,27), lymphomas (14), osteogenic sarcoma (20), squamous cell carcinoma of head and neck (23) and breast cancer (8). However, the dose of the drug has frequently to be reduced or the drug had to be totally stopped, due to supervening toxicity, resulting directly from the drug use (24), which even persisted with citrovorum factor (21). This means that strategy to get maximum therapeutic response with minimum toxicities still requires exploration. Alpha-tocopherol has received considerable attention, because of its role against carcinogenesis (2,6,7,10,17,22,25,33,35). It is found to be low in patients developing anticancer drug toxicities (1,12) and has proved to act against the development of these toxicities (32,36).

PATIENTS AND METHODS

Twenty six patients were chosen for this study. They were randomly selected, and were on prolong Methotrexate therapy. All had established diagnosis of head and neck cancers and were in stage 3 or 4. They had received radiation and were on Methotrexate therapy due to residue or recurrence. They were receiving 50 mg Methotrexate intravenously fortnightly.

Karnofsky scale was applied to grade their physical status. WHO criteria of drug toxicities was used to note their apparent toxicities. The important informations about their treatment were obtained from their hospital medical records. Before randomization, about 7 cc of blood was collected from each patient, from a prominent cubital vein, by disposable syringe. The collection was

*From Department of Pharmacology and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan.

fourteen days after the last Methotrexate intravenous therapy. Each sample was divided into two glass bottles, 2 cc in one, having an equal amount of heparin and 5 cc in other, without any anticoagulant and were used to determine important hematological and other parameters respectively. Renal parameters viz. urea and creatinine and hepatic parameters viz. bilirubin, SGPT and alkaline phosphatase were assessed by specific Merck Kits on autoanalyzer, within twenty four hours of collections.

After baseline data, the patients were randomized into two groups A and B and were given idintical capsules, having alpha-tocopherol acetate or placebo (200 mg in each). These patients were prescribed capsules thrice daily upto fourteen days. During this period the patients continuously received their Methotrexate therapy according to the protocol. After completion of the last day the blood samples of these patients, were collected again and treated in the previous manner to measure their hematological, hepatic and renal parameters again.

Table 1: General characteristic of the patients.

No. of Patients entered in the study	26
Mean age	57,33 years
Sex	Male-15, Female-11
Site of lesion (Residue or Recurrence)	Head and Neck
Karnophsky score	Between 60-90
Average duration of methotrexate treatment	6.37 months
Average total dose given	412.50 mg
Minimum drug free interval	15 days

RESULTS

Table 1 shows characteristics of the patients.

Table 2 presents gross toxicities noted according to

Table 2: Drug related Gross Toxicities.

No. Toxicity	Group A (Receiving alpha-tocopherol)		Group B (Receiving placebo)	
	Before treatment	After treatment	Before treatment	After treatment
1 Loss of hair	None	None	None	None
2 Consciousness	Alert	Alert	Alert	Alert
3 Fever	None	None	None	None
4 Oral ulceration	None	None	None	None
5 Nausea and vomitting	None	None	None	None
6 Allergy	None	None	None	None
7 Cutaneous	None	None	None	None
8 Hemorhage	None	None	None	None
9 Pain (treatment related)	None	None	None	None
10 Peripheral Neurotoxicity	None	None	None	None
11 Hematuria	None	None	None	None
12 Diarrhoea	None	None	None	None
13 Constipation	None	None	None	None
14 Infection	None	None	None	None

Table 3: Comparison of toxicities between groups A and B patients after 15 days of drug treatment.

Toxicity		Group A (Receiving alpha-tocopherol)				Group B (Receiving placebo)			
		Improvement		Impairment		Improvement		Impairment	
Hematological	Hb	NIL	(00/8)*	62.50	(05/8)	42.80	(03/07)	42.80	(03/07)
	RBC	NIL	(0/8)	50.00	(04/08)	50.00	(04/08)	25.00	(02/08)
	WBC	37.50	(03/08)	37.50	(03/08)	25.00	(02/08)	50.00	(04/08)
	Platelets	37.50	(03/08)	37.50	(03/08)	37.50	(03/08)	50.00	(04/08)
Renal	Urea	11.11	(01/09)	22.22	(02/09)	NIL	(00/07)	42.85	(03/07)
	Creatinine	44.44	(04/09)	11.11	(01/09)	14.28	(01/07)	57.14	(04/07)
	Bilirubin	NIL	(00/09)	33.30	(03/09)	NIL	(00/08)	12.50	(01/08)
Hepatic	SGPT	11.11	(01/09)	33.30	(03/09)	12.50	(01/08)	25.00	(02/08)
	Alkaline phosphatase	33.33	(03/09)	22.22	(02/09)	12.50	(01/08)	NIL	(00/08)

*Percentage of patients (No. of patients showing response/total no. of patients).

Table 4: Comparison of toxicity levels between groups A and B patients.

Toxicity	Profile	Group A (Receiving alpha-tocopherol)		Group B (Receiving placebo)	
		Before therapy	After therapy	Before therapy	After therapy
Hematological	Hb (mg%)	12.18±1.88 (08)*	11.10±2.241 (08)	10.81±2.08 (07)	10.62±1.72 (07)
	RBC ($x10^6/cm^3$)	5.55±0.67 (08)	5.04±0.93 (08)	4.56±1.09 (08)	4.57±1.01 (08)
	WBC ($x10^3/cm^3$)	9.21±2.61 (08)	9.33±2.76 (08)	8.37±3.38 (08)	8.26±4.17 (08)
	Platelets($x10^3/cm^3$)	469.62±177.18 (08)	415.00±163.74 (08)	421.25±167.08 (08)	440.62±159.85 (08)
Renal	Urea (mg%)	24.33±12.80 (09)	24.44±11.20 (09)	25.00±10.14 (07)	34.42±17.12 (07)
	Cneatinine (mg%)	0.90±0.259 (09)	0.85±0.267 (09)	1.06±0.23 (07)	1.16±1980.39 (07)
Hepatic	Bilirubin (mg%)	0.53±0.12 (09)	0.60±0.11 (09)	0.50±0.08 (08)	0.53±0.11 (08)
	SGPT (μ/L)	21.77±9.24 (09)	24.77±13.92 (09)	19.22±8.08 (08)	18.72±8.39 (08)
	Alkaline phosphatase (μ/L)	277.66±78.71 (09)	252.55±65.51 (09)	175.75±52.72 (08)	165.37±39.34 (08)

* Mean±SD (No. of patients).

WHO criteria. No significant toxicities were present, before and after the therapy in the patients of both the groups.

Table 3 shows comparison of toxicity distributions between groups A and B patients. An improvement in renal toxicity, presented by decreased urea and creatinine levels, was noted in group A patients.

Table 4 presents comparison of toxicity levels in group A and B patients. No statistically significant difference ($p>0.05$) was found in hematological and hepatic parameters of groups A and B patients, before and after the therapy. A statistically significant raise ($p<0.05$) was noted in urea and creatinine levels in group B but not in group A patients, after the therapy, showing a protective role of alpha-tocopherol.

DISCUSSION

An evaluation of results of this study leads to point a significant role of alpha-tocopherol in reducing renal toxicity, induced by Methotrexate. It is an important finding, because other toxic effects of Methotrexate are also, found to be related to its delayed renal excretion (34).

The nephrotoxicity of Methotrexate is a result of its excretion through the kidneys and its interaction with the renal tissue. Animal studies have shown, that up to 90 percent of the drug may be cleared from the kidneys, nine hours after short term intra-arterial or intravenous infusions. This clearance is delayed when the drug causes functional and morphological changes in the epithelium of the convulated tubules (13). Its precipitation in the distal tubules may result in tubular dilatation, oliguria, and anurea (28). These complications lead to slow clearance of the drug and so accentuate the toxic reactions effecting the other body systems. Therefore the other toxicities of the drug can be reduced by reducing its renal toxicities. Although we are unable to draw a definite result for hematological and hepatic toxicities, from our data, but a convincing support to this fact is provided by less impairment but more improvement as is seen in case of leucocytes and platelets counts and alkaline phosphatase levels.

In Rhesus monkeys, crystalline deposits of Methotrexate and Methotrexate derived material have been demonstrated, after therapy (19). Most of the nonprotein bound drug remaining in the monkey kidney after 24 hours (77 percent) represents the 7-hydroxymethotrexate metabolite (19). According to our current understanding, most of the nephrotoxicity would appear to be due to deposition of these crystals in the renal tubules (19). Antioxidant action of alpha-tocopherol either inhibits formation of these crystals or prevents cellular damage due to free radicals (16) in urinary tubules, a fact, which has already been proved in case of cardiotoxicity induced by adriamycin (26).

REFERENCES

1. Atukorala TM, Dickerson JW, Basu TK, McElwan TJ : Longitudinal studies of nutritional status in patients having chemotherapy for testicular teratomas. Clin Oncol 9:3-10, 1983.

2. Bennet JD : Ulcerative Collitis, the result of an altered bacterial metabolism of bile acid and cholesterol. Med Hypothesis 20:125-132, 1986.

3. Bertino JR (ed) : Folate Antagonists as chemotherapeutics agents. Ann NY Acad Sci 186:1, 1971.

4. Bertino JR : Folate Antagonists in Antineoplastic and Immuno suppressive Agents. II. ed by Sartorelli AC, Johns DG. Berlin. Spring-Verlag, pp 468-483, 1975.

5. Bertino JR, Booth BA, Cashmore A, Bieber AL, Sartorelli AC : Studies of the inhibition of dihydrofolate reductase by folate antagonists. J Biol Chem 239:479, 1964.

6. Beth M, Berger MR, Aksoy M, Schmachl D : Effect of vitamin A and E supplementation to diets containing two different fat levels on methyl nitrosourea induced mammary Carcinogenesis in female SD-rats. Br J Cancer, 56:445-449, 1987.

7. Birt DF : Update on the effects of Vitamins A, C and E and selenium on carcinogenesis. Proc Soc Exp Biol Med, 183:311-20, 1986.

8. Bonadonna G, Brusomolino E, Valagussa P, Rossi A, Bugnatelli L, Brambilla C, DeLena M, Tacini G, Bajetta E, Musumeci R, Veronesi U : Combination chemotherapy as an adjuvant treatment in operable breast cancer. New Eng J Med, 294:405, 1976.

9. Borsa, Whitamore : Studies relating to the mode of action of Methotrexate. III. Inhibition of thymidylate synthetase in tissue culture cells and in cell free systems. Mol Pharmacol 5:318, 1969.

10. Calhoun KH, Stanley D, Stieruberg CM, Ahmed AE : Vitamins A and E do protect against oral Carcinoma. Arch Otolaryngol Head, Neck Surg, 115:484-488, 1989.

11. Chabner BA, Allegra CJ, Curt GA, Clendeninn NJ, Baram J, Koizumi S, Drake JC, Jolivet J : Polyglutamation of Methotrexate. Is Methotrexate a pro-drug? J Clin Invest, 76:907-912, 1958.

12. Clemens MR, Ladner C, Ehninger G, Einsele H, Renn W, Buchler E, Waller WD, Gey KF : Plasma vit and beta carotene concentration during radiochemotherapy preceding bone marrow transplantation. Am J Clin Nutr, 51:216-219, 1990.

13. Condit PT, Chanes RE, Joel W : Renal toxicity of Methotrexate. Cancer 23:126-131, 1969.

14. Djerassi I, Royer G, Treat C, Carim H : Management of childhood lymphosarcoma and reticulum cell Sarcoma with high dose intermittent methotrexate and citrovorum factor. Proc Am Assoc Cancer Res, 9:18, 1968.

15. Faber S, et al. : Action of Peteryl glutamic conjugates on man. Science 106:619-621, 1947.

16. Fritsma GA : Vitamin E and Autoxidation. Ann J Med Tech, 49:453-456, 1983.

17. Genster HL, Magdaleno M : Topical vitamin E inhibition of Immunosuppression and Tumorigenesis induced by ultraviolet irradiation. Nutr Cancer, 15:97-106, 1991.

18. Henderson ES : Acute lymphoblastic leukemia in Cancer Medicine. Ed by Holland JF, E Frei III. Philadelphia: Lea and Febiger, pp 1173-1199, 1973.

19. Jacobs SA, Stoller RG, Chabner BA, John DG : 7-Hydroxymethotrexate as a urinary metabolite in human subjects and Rhesus monkeys, receiving high dose Methotrexate. J Clin Invest, 57:534, 1976.

20. Jaffe N, Frei III E, Traggis D, Bishop Y : Adjuvant methotrexate and citrovorum factor treatment of Osteogenic sarcoma. New Eng J Med, 291:994, 1974.

21. Jaffe N, Traggis D : Toxicity of high dose Methotrexate and citrovorum factor in osteogenic sarcoma. Cancer Chemother Rep, 6:31-36, 1975.

22. LeGardeur BY, Lopez SA, Johnson WD : A care-control study of serum vitamins A, E and C in lung cancer patients. Nutr Cancer, 14:133-140, 1990.

23. Levit M, Mosher MB, DeConti RC, Farber LR, Skeel RT, Marsh JC, Mitchell MS, Papac RJ, Thomas ED, Bertino JR : Improved therapeutic index of methetroxate with "Leocovorin rescue". Cancer Res, 33:1729, 1974.

24. Livingstone RB, Carter SK : Single agents in Cancer Chemotherapy NY, IFL Plenum, 1970.

25. Longnecker MP, Moreno JMM, Knekt P, Nomura AMY, Schober SE, Stahelin HB, Wald NJ, Gey KF, Willett WC : Serum alpha-tocopherol concentration in relation to subsequent colorectal cancer: Pooled data from five Cohorts J Natl Cancer Inst, 84:430-435, 1992.

26. Mielei J, Boveris A, Llesuy S, Molina HA, Storino R, Ortega D, Milei SE : Amelioration of Adriamycin- induced Cardiotoxicity in Rabbits by Phenylamine and vitamins A and E. Am Heart J, 111:95-102, 1986.

27. Nagamoto A, Sullivan MP : Methotrexate therapy for meningeal leukemia given through subcutaneous cerebrospinal fluid reservoir. JAMA, 28:523-528, 1973.

28. Pitman SW, Parker LM, Tattersall MHN, et al. : Clinical trial of high dose Methotrexate (NSC-740) with citrovorum factor (NSC-3590)-toxicologic and therapeutic observations. Cancer Chemother Rep Part 3, 6:43-49, 1975.

29. Seeger DR, Smith JR, Hultguist ME : Antagonist for Peteroyl glutamic acid. J Am Chem Soc, 69:2567, 1947.

30. Skipper HT, Schabel Jr FM : Quantitative and cytokinetic studies in experimental tumor models. In Cancer Medicine, 2nd

ed. (Ed by Holland JF, E Frei III) Lea and Febiger, Philadelphia, pp 663-684, 1982.

31. Smith JM Jr, Cosulich BD, Hultquist ME, Seeger DR : Chemistry of certain pteroyl glutamic acid antagonists. Tr New York Acad Sci Special Number, Series II,10:82, 1948.

32. Tanigawa N, Katch H, Kan N, Mizuro TH, Satomurak H : Effect of vitamin E on toxicity and antitumour activity of adriamycin in mice. Jpn J Cancer Res, 77:1249-55, 1986.

33. Ura H, Denda A, Yokosa Y, Tsutsumi M, Konishi Y : Effect of vitamin E on the induction and evolution of enzyme altered foci in the liver rats treated with diethylnitrosamine. Carcinogenesis, 8:1595-1600, 1987.

34. Volger WR, Huguley CM Jr, Kerr W : Toxicity and antitumor effect of divided doses of Methotrexate. Arch Intern Med, 115:285-293, 1965.

35. Woutersen RA, Van G, Hoctner A : Inhibion of dietary fat promoted development of (pre) neoplastic lesions in exocrine pancrease of rats and hamsters by supplemental vitamins A, C and E. Cancer Lett, 41:179-189, 1988.

36. Yasunaga T, Ohgaki K, Inamoto T, Kan N, Hikasa Y : Protective effect of vitamin E against immuno suppression induced by adriomycine, mytomycine C and flourouracyl in mice. Nippon Geka Hokan, 52:591-600, 1983.

Correspondence:
Mohammad T. Aftab
Department of Pharmacology,
Faculty of Pharmacy,
University of Karachi,
Karachi-75270, PAKISTAN.