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Metronidazole Effect on Lowering Blood Plasma Lipids Level of Iranian Hyperlipidemic Patients

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Abstract: Atherosclerosis is the most common cause of mortality worldwide with hyperlipidemia as an important cause. Finding suitable and effective treatment which can both control the level of blood lipids and solution. Metronidazole is the drug that is only used as an antibacterial and antiprotozoal agent. Researcher have found that oral dose of 750 mg/day has a suitable absorption, is widely distributed in the tissues and reaches to a serum level of 4-6 g mL^{-1}. Despite its suitable efficiency, a few clinical trials have been conducted in this regard. This study was designed as such to examine effect of Metronidazole on lowering serum lipids.The present research was performed as a clinical trial without control, on 150 patients. The tests of LFT; BUN, creatinin, SGOT and SGPT were done on these subjects. Metronidazole was given with a daily dose of 750 mg for 2 weeks. 100 of these patients who had less complaint continued their drug consumption for another 14 days. Lipid parameters such as serum total lipid, total cholesterol and triglyceride were measured in both groups. The data were collected and analyzed with Student t-test using SPSS software. Measuring the serum level of lipids indicated that the mean total serum lipid and total cholesterol decreased significantly compared to their levels before taking the drug ($p<0.05$). The findings also showed a similar decrease in serum TG level ($p<0.001$). In the patients who continued the drug consumption, the serum level of lipids under study showed more decrease compare to first 2 week significantly ($p<0.01$). Metronidazle can be suggested a probable candidate as a new drug in the short life treatment of hyperlipidemia. As the drug for this disorder should be used for a long time, therefore it requires more investigations.

Key words: Atherosclerosis, CAD, Hyperlipidemia, Metronidazole

INTRODUCTION

Antibiotics can move across biological membranes only in the unionised state; this movement depends on pKa of the molecule, pH of plasma. As for all similar systems, this is governed by the Henderson-Hasselbach equation: pH = pKa + log (base/acid). Since the pH of plasma(7.4) is moderate[1], weakly acidic drug molecules like sulphonamides accumulate in higher concentration in plasma,[2] whereas weakly basic drugs such as erythromycin and metronidazole in not tend to concentrate. Thus the degree of ionisation of a drug determines to a large extent its propensity for being in plasma.

Metronidazole (MTZ, 1-[2-hydroxyethyl]-2-methyl-5-nitroimidazole and antiprotozoal drug that has been in use for over 35 years. It is effective in the therapy against trichomonas Vaginalis, amebiasis and giardisis[3]. It is one of the most used drugs world wise and it appears in the essential drug list of the WHO[4]. MTZ was introduced as a trichomonicide in Europe in 1959 by Cosar and Julous[5]. Since then, its clinical application has been growing and it is now the principal treatment for Helicobactor pylori and Crohn's disease. The two principal metabolites of MTZ result from oxidation of side chains[6].Both the parents compounds and its oxidation product can be conjugated with glucuronic acid. It is activated when reduced through electron donation from ferredoxid or flavodoxin that were themselves reduced by pyruvate: Ferredoxin oxidoreductase[7] (POR), possibly forming an hydroxylamine[8]; this process occurs only under strongly reducing conditions[9]. MTZ is also activated in hydroxic cells of animals and hence it has been applied as a radiosenditizer of human tumors[10,11]. Activated MTZ is thought to interact directly with DNA and resultant complex can no longer function as an effective primer for DNA and RNA polymerases. This is the most widely held explanation of its toxic action on cells[12], but it has not yet been properly proved.

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Metronidazole is generally well tolerated, with patients suffering few or no side effects due to standard regimens. Common adverse reactions include nausea, vomiting, headache, insomnia, dizziness, drowsiness, rash, dry mouth and metallic taste (the last two generally being associated with oral metronidazole only). These side effects are usually mild, although some patients do have reactions severe enough to necessitate halting metronidazole therapy. More serious side effects are rare but include eosinophilia, leukopenia, palpitation, confusion and peripheral neuropathy. Side effects have been found to be temporary and resolve on cessation of the therapy[13].

MTZ is readily absorbed in the intestinal mucosa. Its oral bioavailability is higher than 90% and the maximal plasma concentration(Cmax) after an oral dose of 500 mg range from 45 to 75 μmol L^{-1}, approximately[14]. There are some reports of concentrations as 240 μmol L^{-1}[15], but with higher and multiple doses.

MTZ is widely distributed in the human body, with steady-state volumes of distribution of 0.55-0.76 L kg^{-1}[16,17].It crosses the blood-brain barrier[18]. MTZ's half life is about 8 hrs and its volume of distribution is approximately that of total body water.

The liver is the main site of metabolism and it is excreted primarily through the kidney, which accounts for over 50% of systemic clearance of metronidazole[19]. Antibiotic usage is not fairly common among hyperlipidemic patients extensive literature search could not reveal any data on the hypolipidemic effect of metronidazole in patients suffering from dyslipidemia, particularly in Iran.

The putative effect of metronidazole on plasma lipids was noted in a 45-year old female who was taking 1500 mg of MTZ in three divided dose for fascicola hepatitis. A significant decrease in total cholesterol (17%) was observed, which was followed by a randomized open trial of 250 mg 3 times daily in 30 subjects without LFTchecking[20].

The present study was designed to evaluate these phenomena in a clinical trial without control with a reportable number of its toxic action on liver.

MATERIALS AND METHODS

Ardabil city health center affiliated to Ardabil University of Medical Sciences, Iran; is the main center to admit the majority of hyperlipidemic patient who was referred to be under controled.The present drug trial was carried out on 150 subjects who voluntarily agreed to participate in this trial. They were 93 males and 57 females between the age limit of 48-70 years.

All the subjects were given formal information about probable common adverse reactions include nausea, vomiting, headache, insomnia, dizziness, drowsiness, rash, dry mouth and metallic taste. Forms to be confessed their agreements were filled out. Strict exclusion and inclusion criteria were taken into consideration in an attempt to avoid any contraindication and confounding factors that could alter the biochemical endpoint studied "as far as Possible".

Exclusion criteria for the study were CHD and congestive heart failure, pregnancy, central nervous system disease, cirrhosis and abnormal LFT. Those who were in use of drugs such as Phenobarbital, carbamazepine walfarin, beta-blockers, diuretics and so on also were included.

Before the trial laboratory investigations including CBC, ESR, important metabolic profiles urea, electrolyte, Liver Function Tests (LFTs); SGOT and SGPT were performed. Along with the above investigation total lipids, cholesterol and triglyceride were taken under investigation.

Blood heparinized samples for the above investigations were taken after 12-14 hrs fast on first day of treatment before administration of MTZ, all previous administered drugs for hyperlipidemia was stopped. The biochemical analyses were also conducted at the point on first day in the Ardabil central clinical laboratory under direct professional supervisor using standard Iranian commercial kits, supplied by the zistchemie.

This trial was performed in two stages of 28 day (4 weeks).A group of 150 subjects used 750 mg of MTZ tablets per day for 14 days (250 mg three times).Second group of 100 subjects who had least complaint; such as stomach discomfort ability, headache, rashes on skins etc. continued to consume the dose as previous for two more weeks. Patients' complaint was followed in intensive care by expert nurses.

After 2 and 4 weeks of taking drug, the second blood heparinized samples were collected to test for plasma lipids and LFTS to compare them with first stage to get the result of drug challenge.

According to standard Health System Research Methodology and with the help of SPSS using student t-test the collected data were analyzed.

RESULTS

After 15 days, patients treated with MTZ, no pathological changes were noticed in other parameters of blood tests including CBC and LFTs. Results of the hematological and biochemical assays performed on the day before treatment, after two week treatment are shown in Table 1.

Table 1: Changes in serum total lipid, cholesterol and TG. Along with LFTs of 150 hyperlipidmic patients before treatment and one week treatment with metronidozole

		Mean		SD				
Parameters	No. of Patients	Before treatment	one week treatment	Before treatment	one week treatment	Pvalue	Tvalue	Df
Total lipid	150	730.2	568.9	127.4	139.7	<0.01	7.38	49
Total cholesterol	150	307.1	253.8	71.9	62.4	<0.05	13.5	49
Triglyceride	150	555.6	415.1	172.2	160.5	<0.001	14.4	49
BUN	150	20.1	19.1	4.2	3.6	<0.1	4.4	49
Creatinin	150	0.76	6.71	0.14	0.13	<0.8	2.2	49
SGOT	150	17.4	17.1	3.9	3.7	<0.15	1.4	49
SGPT	150	27.1	26.7	4	3.7	<0.15	1.8	49

Table 2: Changes in serum total lipid, cholesterol and TG. Along with LFTs of 100 hyperlipidmic patients one week and two weeks treatment with metronidazole

		Mean			SD					
Parameters	No. of Patients	Before treatment	One week treatment	Tow weeks treatment	Before treatment	One week treatment	Tow weeks treatment	Pvalue	Tvalue	Df
Total cholesterol	100	297.1	247	225.6	43.1	43.2	42.6	<0.01	14.1	19
Triglyceride	100	550.9	401.35	334.5	159.6	146.9	128.9	<0.001	13.5	19
BUN	100	18.5	-	20.7	3.3	-	2.6	<0.05	3.1	19
Creatinin	100	0.82	-	0.85	0.12	-	0.14	0.39	1.5	19
SGOT	100	17.5	-	17.25	4.1	-	2.9	6.66	0.45	19
SGPT	100	27.9	-	27.6	3.79	-	2.5	0.44	0.45	19

Determination of lipids concentration showed that therapy with 750 mg MTZ daily delivered a reproducible reduction of about 22% in mean total lipid ($p<0.01$); about 16% in mean total cholesterol ($p<0.05$) and about 25% in mean triglyceride ($p<0.001$).Mean triglyceride levels showed highly significant decreases compared to the other lipid profiles (Table 1).

A face to face interview was taken from patients about drug tolerance, stomach comfortablity, vomitiry, headache, diarria, weakness and diziness and checked for the rashes on skin.100 individuals including all females were volunteers for the second two weeks.

Mean total cholesterol level decreased significantly by another 9% as compared to first two weeks of treatment ($p<0.01$) and triglyceride level showed appropriately about 19% reduction ($p<0.001$).Laboratory investigation on the day after 4 weeks showed slight increase in BUN.

DISCUSSION

Disease secondary to atherosclerosis is the most common cause of mortality world wide.Hyperlipidemia is one of the causes of premature coronary artery disease[21]. Intervention with diet and drugs to reduce lipid profiles has been proven to decrease the risk of subsequent cardiovascular events, including total mortility[22]. Based on the wealth of clinical trial data, it is widely agreed that virtually all patients with coronary artery disease (CAD) should be on lipid lowering drug therapy to reduce their risk of subsequent event[23, 24, 25, 26].

MTZ was once proposed as hyperlipidemic agents although the mechanism is unclear, despite broad implications, including providing an alternative approach to cholesterol reduction, with potential relevance for current trials of MTZ to reduce cardiovascular disease and possible confounding of routine diagnostic serum lipids measurements. The effect on serum lipids of MTZ with possible mechanisms was therefore explored.

The oxidation of the aliphatic side chain of MTZ in human is the principal metabolic pathway, the nitro group of metronidazole accept electron from electron transport proteins such as flavoproteins in mammalian cells. It is metabolized by liver through the cytochrome P450 (CYP) family of enzymes[19, 27, 28]. There are very few reports about the hepatic biotransformation of MTZ, the last articles have been published 10 years ago by Loft[26, 27]. In these studies he observed that the biotransformation of MTZ yields two principal metabolites: a hydroxylated one (HM) which represents 40% of the urinary excretion of a dose of MTZ and an acetylated metabolite (AAM) which accounts for 15%. There was an interindividual variation of 14% in the amount of metabolites formed. It was also found that the ingestion of ethanol correlated with a higher hydroxylation of MTZ, a fact that might involve the cytochrome P450 as a possible metabolizer, since this (CYP) is induced by ethanol.

Steady-state peak and trough concentrations of metronidazole and its metabolites were measured in the sera of 54 surgical patients who were on intravenous metronidazole, 500 mg every 8 h. These patients had no significant renal or hepatic impairment that corrobating

our trial showed no significant change in LFTs indicating lack of renal or hepatic impairment[29]. Data on hospitalized or critically ill patients with normal hepatic and renal functions indicate that the disposition of metronidazole may be different from that in healthy volunteers[30,31]. There is some controversy as to whether the clearance of metronidazole is impaired in elderly individuals[32,33]. Furthermore, in a recent study Earl *et al.*[34] found that in 23 surgical patients treated with 500 mg of metronidazole every 12 h, the mean serum metronidazole trough concentration was 6.7 mg L^{-1}. They concluded that because the trough concentration was well above the MIC for the majority of the anaerobes, a dosage regimen of once every 12 h was adequate for treatment.

Devis[35] (1983) described the lipid-lowering effect of Metronidazole on normolipimic volunteers which is a positive correlation with our study in which total lipids were declined significantly ($p<0.01$).

In another similar study on five patients with Crohn's disease log-term therapy with MTZ with a dose of 400 mg b.i.d. was followed by a significant reduction of total serum cholesterol from 179 mg dl, to 156 mg dl after 2-4 months administration, to 134 after 6 month and 143 mg dl after 9-12 months, respectively that corroborate with the present study in which triglyceride reduction was significant ($p<0.001$) and in regular order and least theraputical duration might have been due to correct follow up the patients[31].Von Bergman et al in this study showed that billary secretion of cholesterol and bile acids were reduced by 13 and 20% ($p<0.05$) respectively in his investigation which might suggest a decrease in sterol synthesis.Thus,a possible decrease in sterol synthesis and reduction of cholesterol absorption might be responsible for the serum-cholesterol-lowering effect of metronidazole which was matched with loft's study[26,27]. Result of present trial showed that 750 mg metronidazole daily in divided doses for 4 weeks lower total cholesterol and triglyceride significantly which is correlates with shamkhani's study[20].

As Bedesky et al suggested the oxidative metabolite of the drug is responsible for the carcinogenesis, the metabolism of the drug by an individual will be very important. This is so, because approximately 40% of drug metabolism dependent on CYP's is carried out by polymorphic enzymes. Since hydroxylated metabolite (HM) is more genotoxic than MTZ itself, the broad interindividual differences in MTZ metabolism could in part account for individual susceptibility[19, 37].

In the present study also 2/3rdof volunteer wished to continue the therapy due to possible individual susceptibility to MTZ metabolism.

Jenkins *et al.*[33] recently showed twenty-two men and women took antibiotics for 10 days, metronidazole and ciprofloxacin with low-fat diets throughout the study. Blood samples and blood pressure were obtained on days 0 and 10. Their results indicated that metronidazole markedly reduced low-density lipoprotein cholesterol, oxidized low-density lipoprotein and the apolipoprotein B/A-I ratio whereas the reduction with ciprofloxacin was less pronounced which is in keeping and direct agreement with the present study that suggests MTZ or its derivatives may from a new class of lipid lowering compound.

CONCLUSIONS

Treatment for patients in whom there is a less urgent need to reduce risk of hyperlipidemia such as those with a mild elevation of LDL-C who are otherwise at low risk, may be started with lifestyle measures. These measures include dietary steps to correct obesity and to control the amount and type of dietary fat. They also include advice to ensure adequate physical activity. MTZ can be added to the regimen if the lifestyle approach does not result in achievement of the desirable LDL-C target.

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