Evaluation of the effect of omega-3 fatty acids on the induced hepatotoxicity of methotrexate in acute lymphoblastic leukemia children

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Aim of the work

With the enlarged spectrum of clinical use of methotrexate , its toxicity on the liver has gained much more importance **(Suleyman and Veysef ., 2008).**

Methotrexate associated hepatotoxicity is a significant clinical problem that affect the compliance with methotrexate containing treatment regimens. Hydrogen peroxide molecules were reported to act as mediators both in the therapeutic and toxic effects of some antineoplastic agents including MTX **(Zhang et al., 1992).**

A growing body of evidence is emerging which suggest that oxidative stress and reactive oxygen derived free radicals play a crucial role in pathogenesis of MTX induced liver damage **(Ohta et al ., 2007).**

Omega -3fatty acids are a type of unsaturated fat that are classified as an essential fatty acid .A large body of evidence exists to suggest that omega -3 fatty acids have considerable health benefits including anti-inflammatory effects , antioxidant effects and beneficial effects on cholesterol levels and improving non-alcoholic fatty liver disease **(Begin et al., 1986).**

The aim of the study :

1) To study the role of oxidative stress in MTX induced hepatotoxicity.
2) To evaluate the possible hepatoprotective effect of omega-3 fatty acids on methotrexate associated liver injury as evidence by both clinical and biochemical parameters.
3) Patient education about disease and drug.

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List of Abbreviations

AA	Arachidonic acid .
ACCP	American college of clinical pharmacy.
ADE	Adverse drug effect .
ADR	Adverse drug reaction .
AGE	Advanced glycation end product.
AICAR	5-aminoimidazole-4-carboxamide ribonucleotide.
AICART	5-aminoimidazole-4-carboxamide ribonucleotide transformylase enzyme.
ALA	Alpha linolenic acid .
ALE	Advanced lipoxidation end product.
ALL	Acute lymphoblastic leukemia.
ALT	Alanine amino transferase enzyme.
AML	Acute myleoid leukemia.
BM	Bone marrow.
CAT	Catalase
CBC	Complete blood count.
CCG protocol	Children Cancer Group
CMF	Cyclophosphamide , Methotrexate , 5-flurorouracil.
CNS	Central nervous system.
CPDG	Glutamate carboxypeptidase
CSF	Cerebro spinal fluid .
DAMPA	4-amino-4-deoxy-N-methylpteroic acid.
DHA	Docosahexaenoic acid .
DHFR	Di-hydrofolate reductase enzyme.
DHLA	Dihydro lipoic acid.
DMARDs	Disease modifying anti-rheumatic drugs.
DNA	Deoxy nucleic acid.
DPA	Docosapentaenoic acid .
DRP	Drug related problems .
dTMP	Deoxythymidine monophosphate.
dUMP	Deoxyuridine monophosphate .
EBV	Epstein Barr virus.
EPA	Eicosapentaenoic acid.
FAB	French - American - British system .
FAICAR	5-formyl aminoimidazole-4-carboxamide ribonucleotide.

FBP	Folate binding protein **.**
FDA	Food and drug administration .
FGAR	Formylglycinamide ribonucleotide.
FH2	Dihydrofolate.
FH4	Tetrahydrofolate.
FPGs	Foly polyglutamate synthase enzyme.
GAR	Glycinamide ribonucleotide.
GGH	Gamma glutamyl hydrosylase.
GHMT	Glycine hydroxymethyl transferase enzyme.
GI tract	Gastrointestinal tract.
GPX	Glutathione peroxidase .
GR	Glutathione reductase .
HDL	High density lipoprotein .
HDL-C	High density lipoprotein cholesterol.
H2O2	Hydrogen peroxide .
HIV	Human immune deficiency virus.
IT	Intra thecal.
IUPAC	International union of pure and applied chemistry.
IV	Intravenous.
JRA	Juvenile rheumatoid arthritis.
LA	Linoleic acid , Lipoic acid.
LNA	Linolenic acid.
LCFA	Long chain fatty acid .
LDL	Low density lipoprotein .
LDL-C	Low density lipoprotein cholesterol.
MAG	Monoacylglyceride.
MCFA	Medium chain fatty acids.
MDA	Malondialdhyde.
MRD	Minimal residual disease .
MTHFR	Methylene tetrahydrofolate reductase enzyme.
MTX	Methotrexate.
MUFAs	Monounsaturated fatty acids.
NADP	Cytosolic nicotinamide adenosine diphosphate dependant dehydrogenase enzyme.
NCI	National cancer institute.
O_2	Oxygen.
PCR	polymeraze chain reaction.
PL-OH	Phospholipid hydroxide.

PLOOH	Phospholipid hydroperoxide.
PMNs	Polymorphonuclear neutrophils.
P.O.	Per oral.
PPAT	Amido phosphoribosyl transferase enzyme.
PUFA	Poly unsaturated fatty acid.
Rb	Retinoblastoma protein .
RFC	Reduced folate carrier.
RNA	Ribonuclic acid.
ROH	Hydroxyl fatty acid.
ROOH	Lipid peroxides.
ROS	Reactive oxygen species.
SAM	S- adenosylmethionine.
SCFA	Short chain fatty acid.
SOD	Superoxidedimutase.
$T_{1/2}$	Half life.
TAC	Total antioxidant capacity .
TCR	T-cell receptor.
THA	Tetracosahexaenoic acid .
TYMS	Thymidylate synthase enzyme.
UKCS	United Kingdom childhood cancer study.
U.S	United states .
Vd	Volume of distribution.
VLCFA	Very long chain fatty acids .
WBC	White blood cell.

Abstract

Background: Previous studies have demonstrated that patients receiving oral methotrexate in maintenance phase of acute lymphoblastic leukemia suffer from many side effects, the most is hepatotoxicity . In the other hand there was different studies demonstrated the protective effect of Omega-3 fatty acids on liver cells .

Objective: To evaluate the protective effect of Omega-3 fatty acids on the induced hepatotoxic effect of oral methotrexate in childhood acute lymphoblastic leukemia .

Methods: A prospective, randomized, open labeled, controlled clinical trial, was conducted on 60 Egyptian children with acute lymphoblastic leukemia in maintenance phase. From June 2012 to March 2014.The patients were included into two groups, **group I**: 30 patients received oral methotrexate only (20mg/m^2), **group II**: 26 patients received methotrexate (20mg/m^2) in combination with omega-3(1000mg/day). Both groups were followed up for six months; data were collected at the beginning of the study and at the end of the study.

Results: At the beginning of the study there was no significant difference between the two groups neither in demographic nor baseline laboratory data, while after six months there was a significant difference (p<0.001) between the two groups regarding signs and symptoms of hepatic toxicity, better compliance and no one of the patients stopped the chemotherapy cycle in second group due to liver toxicity all over the study period.

Conclusion: The use of omega-3 fatty acids provides a significant protective effect on liver cells against methotrexate toxic effect upon liver cells. Improvement in quality of life due to absence of liver toxicity which causes fatigue and pain to the patient. The chemotherapy cycle was completed without any interruptions and no signs of hepatic damage was observed.

Key words: Acute lumphoblastic leukemia, Methotrexate, Omega-3, Oxidative stress, Malondialdhyde, Clinical pharmacy.

Introduction

Acute lymphoblastic leukemia:

Acute leukemia represents 97% of all childhood leukemia's .Acute lymphoblastic leukemia (ALL) accounts for about 75% of all childhood leukemia's, and nearly one third of all cancers of pediatric age ***(Lanzkowsky and philip.,2005).***

ALL is the most common cancer diagnosed in children and represents 23% of cancer diagnosed among children younger than 15 years .There has been a gradual increase in the incidence of ALL ***(Xie et al ., 2003; Ries et al., 2007 and Smith et al., 2007).***

In Egypt, the incidence of childhood cancers overall is approximately 78,010 new patients (as documented in the years 2002- 2005),seen at the NCI ,among which leukemia represents the most common childhood cancer, representing almost 35% of all cases (34.6% boys and 33.3% girls) ***(Elattar et al ., 2006) .***

Bone marrow invasion results in anemia ,causing pallor , fatigability, tachycardia, dyspnea , and sometimes congestive heart failure ***(Lankzkowsky and Philip., 2005).***

There may be neutropenia, causing fever, ulceration of the buccal mucosa, and infection ***(Lankzkowsky and Philip., 2005).***

Among children with ALL , more than 95% attain remission and 75% to 85% survive free of leukemia recurrence at least 5 years from diagnosis with current treatment that in corporatre systemic therapy e.g combination chemotherapy and specific central nervous system preventive therapy (intrathecal chemotherapy) ***(Moricke et al ., 2010; Pui et al., 2004).***

The improved survival for children with ALL reflects the impact of well-designed clinical trials conducted during this period, with treatment based on risk assessment (including response to initial treatment) ***(Schrappe et al ., 2000).***

Methotrexate:

Methotrexate is a weak dicarboxylic acid with pKa 4.8 and 5.5, and thus it is mostly ionized at physiologic pH ***(Widermann et al ., 2004).***

In maintenance therapy of ALL methotrexate is taken orally. Oral administration in tablet form is often preferred when low dose are being administered since absorption is rapid and effective.

ALL in pediatric patients and young adolescents is the most responsive to present day chemotherapy ***(Seibel et al., 2008).*** The length of maintenance therapy is 3 years for boys and 2 years for girls and adults ***(Pui et al .,2004).***

Oral methotrexate is taken once weekly single dose according to the CCG protocol which is used in the Ain shams university blood and oncology clinic .The dose is 20mg / m^2 ***(Hilden et al ., 2006).***

Methotrexate (MTX), a folic acid antagonist, is widely used as a cytotoxic chemotherapeutic agent in the treatment of various malignancies such as acute lymphoblastic leukaemia as well as in the treatment of various inflammatory diseases ***(Uzar et al ., 2006; Cetinkaya et al ., 2006).***

The efficacy of this agent is often limited by its toxicity which causes severe side-effects on liver histology related to MTX use and may lead to conditions such as fatty infiltration , liver cirrhosis, fibrosis of the liver, hypertrophy of the hepatocytes, hepatitis, hepato-cellular necrosis and death ***(Uzar et al ., 2006).***

MTX is known to be an oxidizing agent that suppresses antioxidant system and increases oxygen species in many organs ***(Cetiner et al ., 2005).***

Prolonged use of MTX leads to accumulation of polyglutamate forms of the drug in hepatocytes, which decreases hepatocellular folic acid levels and eventually leads to hepatocyte necrosis ***(Kaplowitz., 2000).***

It is thought that the detrimental effects of MTX is partly due to its direct toxic action by increasing ROS production. It has further been reported that MTX administration induces oxidative stress and significantly reduces antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase in liver, intestinal mucosa and spinal cord tissues of rats ***(Uzar et al ., 2006; Uz et al., 2005).***

Few studies have demonstrated that methotrexate administration causes cell injury and reduction in the activities of antioxidant enzymes ***(Cetinkaya et al., 2006).***

Polyunsaturated fatty acids are much less resistant to peroxidation than monounsaturated or saturated fatty acids **.** Furthermore , oxidative damage to lipids has widespread effect , as lipid peroxidation triggers a complex chain reaction involving a range of reactive intermediates that can also cause protein and DNA damage ***(Hulbert et al ., 2007).***

Omega-3 fatty acids:

Essential fatty acids constitute an important component of all cell membranes and influence membrane fluidity and the behavior of membrane-bound enzymes and receptors ***(Das ., 2006).***

These fatty acids can modulate physiological and pathological conditions through multiple mechanisms, such as the inflammatory response ***(Kang and Weylandt., 2008)***, and have received great attention in recent years for their critical role in disease prevention and management ***(Gebauer et al., 2006).***

The liver predisposes to oxidative stress presumably by amplifying the capacity of free radical chain reaction. An obvious sign of hepatic injury is the leakage of cellular enzymes into the plasma due to the disturbance caused in the transport functions of hepatocytes. When liver cell membrane is damaged, a variety of enzymes located normally in cytosol is released into the blood ***(Sgroc Clinard and Ouazrir ., 2002) .***

Possible mechanisms that may be responsible for the protection against liver damage by omega-3-Fatty acids includes membrane stabilizing action on the hepatocytes Although the exact mechanism of cytoprotection by omega-3-fattyacids remains unresolved, yet data suggests that this substance could serve as an antioxidant/cofactor that makes the hepatocytes less susceptible to the damaging action of noxious agents ***(Sgroc Clinard and Ouazrir ., 2002) .***

α-Lipoic acid (LA), a naturally occurring sulphydryl compound found in virtually all plants and animal species, is a potent antioxidant with high efficacy of chemoprotection. It is also involved in the chelation of metal ions, regeneration of exogenous and endogenous antioxidants and repair of oxidized proteins ***(Kolgazi et al ., 2007) .***

It appears that LA exerts its beneficial effects in conditions where oxidative stress plays a critical role in the induction of cellular damage/target organ toxicity and hence it was considered for the present study and an attempt has been made to evaluate the possible protective effects of LA against MTX-induced toxicity ***(Bajin et al ., 2006).***

It may cause gas, bloating, belching, nausea and diarrhea , very high doses may cause some but not so undesirable side effects as fishy body odor and/ or fishy breath (***Buckely et al 2004***),Care should be taken with patients taking blood thinning medications as aspirin and warfarin because these patient when using omega-3 fatty acids may bruise easily or suffer from bleeding disorders (***Buckely et al., 2004***).