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METHOTREXATE INDUCED HEPATOTOXICITY AND ANTIOXIDANTS

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Review
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Abstract

Methotrexate (MTX) is an antifolate and antimetabolite group chemotherapeutic agent that has been used in the treatment of many diseases since 1948. MTX treatment has various dose- dependent side effects. The occurrence of these side effects prolongs the treatment process and reduces the success of the treatment. One of the important side effect of MTX treatment is on liver and named as hepatotoxicity. In studies for many years, it has been found that the formation of hepatotoxicity is caused by the disruption of the cellular antioxidant defense mechanism. When the antioxidant defense mechanism is damaged, reactive oxygen radicals increases and causes oxidative damage in hepatocytes. It is thought that antioxidant combination with MTX may reduce these side effects. The purpose of this review is to investigate various antioxidants used to reduce the severity of MTX induced hepatotoxicity.

Key Words: Antioxidant, Hepatotoxicity, Methotrexate.

Özet

Metotreksat (MTX), 1948'den beri pek çok hastalığın tedavisinde kullanılan anti metabolit grubu kemoterapötik bir ajandır. MTX tedavisinin doza bağlı çeşitli yan etkileri vardır. Bu yan etkilerin ortaya çıkması tedavi sürecini uzatır ve tedavinin başarısını azaltır. MTX tedavisinin önemli yan etkilerinden biri hepatotoksisitedir. Uzun yıllardır yapılan çalışmalarda, hepatotoksisitenin oluşumuna hücresel antioksidan savunma mekanizmasının bozulmasının neden olduğu bulunmuştur. Antioksidan savunma mekanizması zarar gördüğünde reaktif oksijen radikalleri artar ve hepatositlerde oksidatif hasara neden olur. MTX ile antioksidan kombinasyonunun bu yan etkileri azaltabileceği düşünülmektedir. Bu derlemenin amacı, MTX kaynaklı hepatotoksisitenin şiddetini azaltmak için kullanılan çeşitli antioksidanları araştırmaktır.

Anahtar Kelimeler: Antioksidan, Hepatotoksisite, Metotreksat.

1. Introduction

Methotrexate (4-amino-10-methylfolic acid) is a chemotherapeutic antagonist of folic acid (Chan & Cronstein, 2013). The absorbitation of MTX from the gastrointestinal tract is at doses of less than 25 mg/m2; intravenous (IV) use is preferred for the doses which are bigger than 25 mg/m2. After IV use of MTX, the drug eliminates from plasma in a three stepped way. Approximately 50% of MTX binds to plasma proteins. The urine contains nearly 90% of MTX excrete (Gilman, 2018). MTX is generally metabolized in the liver and excreted through the kidney. Within 24 hours of ingestion, MTX is excreted as 7-hydroxymethotrexate by renal filtration and tubular reabsorption. 7-hydroxymethotrexate is the main metabolite of MTX. A little proportion of MTX can be eliminated through the bile (Bedoui et al., 2019; Morrison, 1987).

MTX has been used in treatment of cancer, inflammatory, autoimmune, dermatological and rheumatological diseases for many years (Cetinkaya et al., 2006). MTX shows its efficacy on the S phase cell group in the cell cycle. It inhibits the tetrahydrofolate synthesis by binding to dihydrofolate reductase. Tetrahydrofolate requires for production of purine and pyrimidine (Chabner et al., 1985). This inhibition blocks DNA and RNA synthesis (Hyoun et al., 2012). MTX reduces nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) levels. NADP is very essential for glutathione reductase (GSSG-R) enzyme.

Continuity of reduced glutathione (GSH) is provided by GSSG-R. GSH is antioxidant and its very important. The decrease in GSH level causes a downgrade in the effectiveness of antioxidant defense systems that protect cells against oxygens reactive radicals. These radicals are named as hydroxyl radicals, superoxide anions, peroxide of hydrogen and hydrochloric radicals (Cetinkaya et al., 2006).

The liver continuously synthesizes and destroyes the oxygen's reactive products such as hydrogen peroxide, superoxide, free hydroxyl radicals and. Oxidative stress, reactive oxygen products and various cytokines causes damage to Kupffer and Ito cells in hepatocytes. Fibrosis and cirrhosis is caused by using the MTX with chronic and smaller dose. Rapid rise up in liver function tests levels is caused by using the MTX in high doses (Cassiman et al., 2001; Conway & Carey, 2017; De Minicis et al., 2006).

MTX treatment have various side effects; nephrotoxicity, suppression of bone marrow, mucosal damage in the gastrointestinal system, pneumonia, testicular and ovarian toxicity, teratogenicity and pulmonary toxicity (Dhokarh et al., 2012; Mahmoud et al., 2018). Dyspnea, cough, fever, pleural effusions, vomiting and diarrhea are also the other side effects (Tousson et al., 2014).

Hepatotoxicity is a common side effect. It is reversible but causes treatment discontinuation in patients. Hepatotoxicity is related to the dose of taken. When hepatotoxicity occurs the cancer treatment is negatively affected (Al-Ali et al., 2005). The mechanism of MTX hepatotoxicity has not been completely identified (Cetinkaya et al., 2006). Oxidative stress is thought to be responsible for the hepatotoxicity. Previous studies in the literature observed that oxidative stress is a reason for MTX's hepatotoxicity, nephrotoxicity and GIS toxicity (Devrim et al., 2005; Uz et al., 2005).

In histopathological analysis with hematoxylin-eosin stain, hepatocyte degeneration, picnotic nuclei, sinusoidal dilatation, mononuclear cell infiltration and vascular congestion may be seen in MTX group (Şener et al., 2006). In analysis with Periodic acid Schiff stain the Glycogen storage in hepatocytes will be decreased compared to control group. This finding indicate the hepatotoxicity of MTX. (Akbulut et al., 2014).

2. Materials and Methods

This review is organized by searching Pubmed, Google Scholar and literature. 17 antioxidants investigated which are used for MTX-induced hepatotoxicity. Research articles about these antioxidants were published between 1999 and 2020.

3. Results and Discusion

Various antioxidants were used for treatment of the hepatotoxicity induced by MTX. Gallic acid (GA) is a phenol and its generally found in plants. Green tea, grape and red wine contains GA. It has antioxidant, anti-inflammatory and antiapoptotic efficacy (Olayinka et al., 2015). GA was used as an antioxidant for investigating MTX induced hepatotoxicity and nephrotoxicity. It was observed that the hepatic levels of glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), (38.3%, 34.0%, 47.8%) were lower in MTX group, and GA improved the antioxidant system in the liver compared to MTX group. They reported that MTX induced lipid peroxidation by increasing the free radicals. GA ameliorated this effect with its free radical scavenging properties. GA may be used with MTX chemotherapy for its hepatotoxicity and nephrotoxicity (Olayinka et al., 2016).

In a different study Vitamin A and Glutamine used for MTX hepatotoxicity. A significant rise in GSH levels was found in MTX group. In the MTX + Vitamin A group the glutathione (GSH) level were decreased. It is observed that in MTX + Glutamine group the GSH level were decreased again as Vitamin A group. They reported that MTX reduced antioxidant defence capacity and increased lipid peroxidation. Vitamin A and Glutamine reduced lipid peroxidation by their antioxidant effects (Zeiny, 2018).

Ferulic acid (FA) is a naturally found antioxidant and phytochemical. Tomatoes, sweet corn and rice bran contains FA. It's antioxidant activity is very strong (Srinivasan et al., 2007). In a recent study FA was investigated as an antioxidant against MTX induced hepatotoxicity. In the MTX group reactive oxygen species (ROS), lipid peroxidation, malondialdehyde (MDA) and nitric oxide (NO) were increased. In the MTX + FA group; ROS, MDA and NO levels were reduced. Inflammation levels were decreased after using FA. In MTX group Nuclear factor (erythroid-derived 2)-like 2/heme oxygenase-1 (Nrf2/HO-1) and peroxisome proliferator activated receptor gamma (PPARγ) levels were reduced. In MTX + FA group Nrf2/HO-1 and PPARγ levels were improved by FA. FA decreased oxidative stress, and improved all antioxidants in MTX-induced rats. MTX group has a decrease in NAD(P)H-quinone oxidoreductase 1(NQO1), HO-1, and Nrf2

levels but FA reversed this effect. They reported that histopathological analysis of liver sections were normal in control group. In MTX group hepatocyte degenerations, steatosis, activated Kupffer cells, inflammatory cellular infiltration, and cytoplasmic vacuolations were seen and FA prevented this damage. They also observed that in MTX group apoptosis increased and FA decreased apoptosis by its antioxidant effects (Mahmoud et al., 2020).

Another study was examined with virgin coconut oil (VCO). VCO is used with MTX to search its efficacy on hepatotoxicity. In the MTX group SOD, CAT, GSH and glutathione peroxidase (GPx) levels were decreased but MDA and interleukin-6 (IL-6) levels were increased compared to control group. In the MTX + VCO groups; SOD, CAT, GSH and GPx levels increased but MDA and IL-6 levels decreased compared to MTX group. In the VCO groups they observed the protective effect of VCO as an antioxidant compared to MTX group. They reported that MTX toxicity is caused by impairment in antioxidant defense mechanism and this toxicity may be reversed by VCO. VCO administration could be beneficial in MTX chemotherapy by reducing oxidative stress and pro-inflammatory responses (Famurewa et al., 2018).

Montelukast (ML) is an antioxidant. It is an antagonist for leukotriene receptors. ML also have anti-inflammatory efficacy (Coskun et al., 2011; Kose et al., 2012) examined ML for MTX induced hepatotoxicity. In MTX group MDA levels and myeloperoxidase (MPO) levels were increased. They observed that when montelukast was used after MTX injection, MDA levels and MPO levels were decreased. In the histopathological analysis of liver tissue, hepatocytes were normal in control group. In MTX group eosinophilic stained hepatocytes and swelling of hepatocytes were seen. Glycogen storage decreased compared to control group in periodic acid schiff stain. In MTX + ML group they observed that eosinophilic stained hepatocytes and swelling of hepatocytes reduced compared to MTX group but glycogen storage was decreased as similar to MTX group. They reported that ML reduced the oxidative damage in the liver tissue.

Chlorogenic acid (CGA) has a phenolic structure. It is generally found in peach and in prunes. CGA has antioxidant and antihypertensive efficacy (Koriem & Soliman, 2014). In a study, CGA was investigated against MTX induced hepatotoxicity. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and MDA levels were rised up in MTX group as compared to control group. Glutathione reductase (GR) and SOD activities were decreased also hepatic GPx and CAT activities decreased in MTX group compared to control group. In MTX + CGA group, AST, ALT and MDA levels were decreased compared to MTX group. GR, SOD, GPx and CAT activities increased compared to MTX group. They reported that histopathologically control group was

normal. In MTX group hepatocyte degeneration and fatty infiltration were observed. In MTX + CGA group the histologic damage reversed by CGA. They also observed that in MTX group apoptosis increased and CGA decreased apoptosis by its free radical scavenging and antioxidant effects (Ali et al., 2017).

Tempol is a radical scavenger and an antioxidant (Ahmed et al., 2014). There are various articles by using tempol as an antioxidant. (Pınar et al., 2018) investigated tempol against MTX induced hepatotoxicity. In the MTX group AST, ALT, MDA and MPO levels were increased compared to control group. In the MTX + tempol group AST, ALT, MDA and MPO levels were decreased compared to the MTX group. The GPx, CAT and SOD levels were decreased in the MTX group. In the MTX + tempol group GPx, CAT and SOD levels were increased compared to MTX group. They reported that in the histopathological analysis of liver tissue, control group was normal. In MTX group necrosis, inflammation, vascular congestion were observed. In MTX + Tempol group; histologic damage were decreased in hepatocytes compared to MTX group. They also observed that MTX treatment increased oxidative stress, lipid peroxidation and apoptosis in liver. Tempol ameliorated oxidative stress, lipid peroxidation and apoptosis by it's antioxidant effect.

Rhein is an anthraquinone and has hepatoprotective, nephroprotective, anti-inflammatory and antioxidant efficacy (Zhou et al., 2015). In a recent study rhein was tested for MTX induced hepatotoxicity. The levels of mRNA was investigated for the protective and antioxidant effect of rhein on MTX-caused liver injury. In MTX group AST and ALT levels were increased compared to control group. In MTX + Rhein group AST and ALT levels decreased compared to MTX group. They used western blotting (WB) test for observing protein expression levels of Nrf2, glutamate-cysteine ligase catalytic subunit (GCLC) and HO-1 in liver. MTX treatment decreased the Nrf2, HO-1and GCLC levels in liver. In MTX + rhein groups Nrf2, HO-1and GCLC levels were increased when they were compared to MTX group. In the histopathological analysis of liver tissue, control group was normal. In MTX group hepatocellular swelling, necrosis, and intrahepatic hemorrhage were observed. In MTX + Rhein 50mg/kg and 100 mg/kg groups the liver damage was reduced. They also observed that MTX treatment increased oxidative stress, and apoptosis in hepatocytes. Rhein could improve the hepatotoxity of MTX by it's antioxidant effect (Bu et al., 2018).

β-Carotene is an antioxidant. It inhibits free radicals and suppress lipid peroxidation (Leo & Lieber, 1999). In a study β-Carotene was investigated against MTX induced hepatotoxicity. In the MTX group MDA levels were rised up, AST and ALT levels were increased, but on the other

hand SOD levels were decreased and GP-x levels were lower. In MTX + β-carotene group AST and ALT levels were depressed, while MDA SOD and GP-x levels were nearly similar to the control group. They observed that the β-carotene's curative effect and antioxidant efficacy ameliorated MTX depended oxidative hepatic damage. In the histopathological analysis, hepatocytes were normal in control and β-carotene groups. In the MTX group apoptopic cells and sinusoidal dilatation were observed. In MTX + β-carotene group, the damage reduced compared to MTX group. Microscopic damage score was 0.66 ± 0.33 in control group and it increased to 7.0 ± 0.68 in MTX group. In MTX + β-Carotene group the score decreased to 3.33 ± 0.42. They reported that MTX increased free radicals and β-carotene reversed these effect as an antioxidant (Vardi et al., 2010).

Ellagic acid (EA) is an antioxidant and naturally found in raspberry, vegetables and nuts (Zeb, 2018). In a new study EA was investigated against MTX induced hepatotoxicity. MTX increased aminotransferases and alkaline Phosphatase (ALP) levels. Hepatic GSH level was decreased by MTX and so the activities of antioxidant enzymes were decreased in MTX group. In MTX + EA group, the levels of aminotransferases and ALP were decreased by EA treatment. In the histopathological analysis of liver tissue control group and EA group were normal. In MTX group hepatocyte necrosis, hepatocyte degeneration, inflammatory cell infiltration and vascular congestion were observed. In MTX + EA group MTX induced liver damage inhibited by EA. EA ameliorated MTX hepatotoxicity by it's antioxidant effect (Mehrzadi et al., 2019).

Pineal gland produces melatonin. Melatonin regulates the circadian rhythm and has anti-inflammatory and antioxidant efficacy (Reiter et al., 2016). In a study melatonin was investigated against MTX induced hepatotoxicity and nefrotoxicity. In the MTX group MDA and MPO levels were increased, GSH levels were seen as lower. In the MTX + Melatonin group its observed that melatonin reversed the toxic effect of MTX. They reported that melatonin may be used for MTX induced hepatotoxicity and neprotoxicity (Jahovic et al., 2003).

Crocus sativus (saffron) is used in foods for long years. It is a coloring and flavoring agent and also used in cosmetic preparations. It has antioxidant efficacy (Hosseini et al., 2018). In a recent study Crocus sativus (CS) was used against MTX induced hepatotoxicity. In the MTX group AST, ALT, ALP, lactate Dehydrogenase (LDH), MDA and NO levels were rised up; but SOD levels decreased. They reported that in MTX + CS extract group, CS ameliorated liver enzyme levels, oxidative stress marker levels and histological damage signs of the liver (Hoshyar et al., 2020).

Chrysin (CR) is an antioxidant and natural flavonoid. CR also hasf anti-inflammatory efficacy (Pushpavalli et al., 2010). In a different study CR was investigated against MTX and its hepatotoxicity. In the MTX used group ALT levels were increased. Also AST, LDH and MDA levels were rised up. GPX, GR levels, SOD and CAT levels were decreased. Pretreatment of CR improved the level of the enzymes by its antioxidant effect. In the histopathological analysis of liver tissue, hepatocytes were normal in control group. In MTX group fatty infiltration were observed in hepatocytes. Pretreatment of CR reverted these changes in MTX + CR groups. In MTX group p53 expression, Bax protein expression and Cleaved caspases 3 protein expression increased compared to control group. In MTX + CR groups p53 expression, Bax protein expression and Cleaved caspases 3 protein expression decreased compared to MTX groups. They observed that MTX increased apoptosis and oxidative stress and pretreatment with CR reverted these effects (Ali et al., 2014).

Hesperidin (HS) is a natural flavonoid. It has antioxidative, anti-apoptotic, antiinflammatory efficacy (Naewla et al., 2019). In a recent study HS was examined for hepatotoxicity of MTX. In the MTX group serum AST, ALT and MDA levels were rised up compared with the control group. In MTX + HS group, HS decreased AST, ALT and MDA levels. In the MTX group CAT, GSH, and total antioxidant capacity (TAC) levels were lowered as compared to the control but in MTX + HS groups CAT, GSH, and TAC levels were increased compared to MTX group. In the histopathological analysis of liver tissue, hepatocytes were normal in control group. In MTX group pycnotic nuclei, congestion, and lymphatic cells infiltration were seen. In MTX + HS group the damage decreased compared to MTX group. Nrf2/HO-1/Bcl2 signaling upregulated and nuclear factor-kB pathway downregulated. In MTX group oxidative stress, lipid peroxidation and apoptosis increased. HS treatment reversed these effects by it's antioxidant properties (Abdelaziz et al., 2020).

Vitamin E is an antioxidant and found in vegetables and plants (Peh et al., 2006). Major derivate of Vitamin E is alpha (α)-tocopherol (Kurt et al.,2020). In a recent study Vitamin E was investigated against MTX induced hepatotoxicity. In the histopathological analysis of liver tissue; congestion, sinusoidal dilatation, activated Kuppffer cell and mono nuclear cell number were increased in MTX group. In MTX + Vitamin E group, Vitamin E treatment decreased these histopathologic damage compared to MTX group. MTX treatment increased oxidative stress in liver. Vitamin E prevented hepatotoxicity by it's free radicals scavenging properties (Amirfakhrian et al., 2018).

Curcumin (CR) is a polyphenol it has antioxidant and anti-inflammatory effects (Hewlings & Kalman, 2017). In a different study CR was investigated against MTX induced hepatotoxicity. In the MTX group serum AST, ALT and MDA levels were increased. In MTX + CR groups AST, ALT and MDA levels were decreased by CR treatment. In the MTX group CAT, GSH, and SOD levels were lowered as compared to the control but in MTX + CR groups CAT, GSH and SOD levels were increased compared to MTX treatment group. In the histopathological analysis of liver tissue, hepatocytes were normal in control group. In MTX group, vacuolation, sinusoidal dilatation, haemorrhage and lymphatic cells infiltration were observed. In MTX + CR group the damage decreased compared to MTX group. They observed that oxidant activity is induced by MTX and curcumin showed beneficial effects by it's antioxidant properties (Hemeida & Mohafez, 2008).

As a result, MTX is a hepatotoxic chemotherapeutic agent and this hepatotoxicity causes blocage of chemothreapy and treatment. Hepatoxicity that occurs with MTX can be ameliorate with different antioxidants. Combination MTX with antioxidant will be helpful for maintenance of treatment.

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