Review Article

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Methotrexate drug plug and play role in human body

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A B S T R A C T

Purpose: The purpose of this article was to give the detail of MTX related complication. The MTX drug acts as an antimetabolite drug that interferes with folic acid metabolism. It used as an anti-proliferative drug for abnormal cell growth inhibition such as tumor or cancer cell, chronic inflammation, ectopic pregnancy, psoriasis disease, multiple sclerosis disease, Crohn disease, sarcoidosis, rheumatology related disorder, and autoimmune disease. Newly synthesized MTX derivatives via organic Schiff base technique could be prepare and used against different disorders as a treatment agent.

Keyword: Methotrexate

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Introduction

Man strived for well-being, searched mysteries of science, the same story noted in pharmaceuticals field between the 1940s-1950s. The MTX antagonized folic acid that inhibits cell growth by hindering folic acid.1st successful trial reported in 1948.[1] The MTX Glu blocked various synthetic pathway such as tetrahydrofolate enzyme act as cell division arrest. The MTX included WHO essential lifesaving drugs category list, > 90% drug excreted through kidney route.[2] The MTX structure is similar to folic acid except in two ways, a hydroxyl group (OH) replaced with an amino group (NH2) while the methyl group (CH3) substituted with an amino acid. The MTX hinders different enzymes i.e. reductases synthases. The MTX oxidized into 7-hydroxy-MTX in the presence of aldehyde reductase enzyme. Long term MTX medication leads to renal failure.[3] The MTX entered cells via specific transporter and protein known as folate binding protein, a receptor known as folate binding receptor α, β and γ.[4] The MTX polyglutamate process occurred in the cytosol, dynamic for controlling enzyme i.e. FPGS and GGH, glutamate removed from MTX through catalyzing process.[5,6]

The MTX structurally inactive biomolecule, a lower affinity for functional activity, modification required for functional, polyglutamylation active form has a higher affinity towards folic acid metabolizing enzymes such as thymidylate synthase and dihydrofolate reductase resulted in purine and pyrimidine synthesis inhibition. The MTX hinder FPGS enzymatic activity leads to failure of glutamic acid attachment with functional active substance procced to intracellular folate deficiency. The MTX polyglutamate processing, residues of the 5-8 glutamic acid bind with MTX through amide bond linkage to form gamma peptide bond, a moiety of derivatization maintained intracellular concentration by virtue low affinity towards MRP3 in the presence of 3 or more glutamate residues.[7,8]

The MTX mechanism of action not well defined, related to decreased cellular NADPH and GSH level. GSH is a part of host defense protection from cellular injury against cytosolic oxidants. GSH deficiency is unable to protect the cell from ROS and cytosolic antioxidants lead to defense system failure. ROS damage vital tissue which unable to regenerate such as nerve or liver cell, Increased glutathione level which protects hepatocyte from ROS related cell injury and MTX toxicity.[9-11] The MTX increases methylenedioxyamphetamine level, acts as a detectable marker of oxidation stress, induce lipid peroxidation associated with free radicals' metabolites.[12]

MTX Polyglutamylation process

The MTX polyglutamylation process is essential for maintaining intracellular drug concentration with enzyme removal i.e. FPGS and GGH. The MTX higher concentration noted in the cell due to polyglutamylation ([5,6]). The Polyglutamylation is a controlled mechanism, MTX balance depends on the attachment or removal of glutamic acid, 2nd and 3rd generation types of antifolate drugs approved and currently using. The MTX resistance occurred through the following mechanism; Folate transporter failure leads to decrease cellular drug uptake, efflux system overdrive show low drug concentration in the cell, Polyglutamylation related enzyme s' availability play role in drug affectivity, decreased level or inactivation also a resistance cause.[13,14]

The glutamate molecules attached with gamma carboxyl moiety presence of FPGS to form 6-8 glutamate residues chain attached with peptide bond, MTX polyglutamate converted into exporting molecules mono-glutamate, in tumor treatment various derivatives synthesize in the body, most common form is polyglutamate, long term drug taken lead to newly synthesized derivative formation, MTX level fall and derivative level high, derivatives form cannot initiate chemical reaction although targeted enzyme inhibition with specific molecules, polyglutamate derivative has higher affinity to inhibit targeted pathway as compared other. The MTX treatment is sensitive and difficult because it hinders normal cell growth resulted in cell death. The drug designing to kill tumor cells; no control mechanism available to prevent host normal cell death. The Polyglutamate derivative accumulated in the normal cell leads to toxicity the same as abnormal cells, most common normal cell toxicities noted in the bone marrow and intestinal cell. The MTX therapy given through IV route, transported into the tumor cell through the transportation system, converted into polyglutamate MTX, conversion stopped after some time, MTX mono-glutamate synthesis continues along with decreased MTX concentration. The DHFR function and availability for tetrahydrofolate continue until cellular demand accomplish.[15-18]

MTX Indication

The MTX used against cancer and inflammatory disorders. The MTX converted either into a crystal or solid form, bound with natural body atoms for conversion into drug's derivatives such as 7-Hydroxy-MTX or primary metabolite. The drug functional or any other form caused the harmful effect to body lead to damage different parts i.e. kidney cell damage leading to renal failure, bone marrow suppression leading to blood cell lineage suppression, digestive tract mucosal membrane inflammation or ulcer leads to mucositis and nerve cell damaged lead to neurological toxicity. The drug remained effective until target tissue suppressed, uncontrolled growth decreased drug potency. The MTX level reduced by antidote drug named leucovorin is known as folinic acid. The MTX detected in serum, the most common detection technique is radioisotope .[19-21]

In 1956, Li, Hertz, and Spencer did the trail on MTX activity against different types of abnormal pregnancy-related neoplastic disorder i.e. metastatic gestational, choriocarcinoma, trophoblastic malignancy, showed desired outcome against abnormal cell growth inhibition. The MTX used for treating different types of malignancies such as blood-related malignancy i.e. ALL, head and neck cancer, breast cancer, bone-related tumor such as osteosarcoma, Hodgkin and non-Hodgkin lymphoma.[22,23] The MTX administrated against tumor cell or cancer cell, chronic inflammation, psoriasis disease, multiple sclerosis disease, Crohn's disease, sarcoidosis, rheumatology related disorder, and autoimmune disease.[24-26]

MTX side effects

The MTX categorized in those types of drugs which have fewer benefits with high side effects, MTX notable side effect are low WBC count, liver cell toxicity, nephron cells toxicity, myeloid cell suppression, ulcer in the stomach and intestinal mucosal cell line .[27,28] The MTX high dose administration for long period caused complications such as abnormal fat production in the liver known as asteatosis, obstruction or alteration in bile flow identified as liver cholestasis, cirrhosis, and fibrosis.[29] The MTX showed hepatic cellular toxicity, MTX produces oxidative stress through increased lipid degradation resulted in radical's production, adverse side effect includes lipid-related radicals' production. The most observed radical is RO act as superoxide anion, other types of radicals are OH radicals and H2O2, stimulate pro-apoptosis environment. [30]

The MTX severe side effects noted in some patients i.e. anemia disorder, neutropenia, stomatitis, and mouth ulcer. All types of pathological states reverted through folate-related product usage. The MTX toxicities are liver

fibrosis, lung fibrosis, renal dysfunction, tiredness, and weakness.[31] GIT related signs and symptoms included diarrhea, dyspepsia, vomiting, stomach ulcer, and nausea, notable and observed side effect ratio is up to 40%.[32] The MTX related side effect included bone marrow suppuration, alopecia disorder, stomatitis disease, and nodules formation, A1 receptor play part in a different side effect, MTX local area treatment well-known side effect is nodules formation due to an action of adenosine on monocyte cell.[31]

MTX in RBC

The MTX is lethal for erythropoiesis, stopped RBC production at the erythroblast stage. The MTX polyglutamate penetrates immature erythrocyte at the early precursor stage. The MTX taken for many weeks' accumulation, maturation, and migration in the blood noted.[33]

MTX in Psoriasis

Psoriasis disorder is related to the increased turnover of skin cells by pacing the dermal cell cycle. The MTX in psoriasis accepted but the mechanism of its action undefined. The MTX functionally acts as DNA synthesis retardation, renders slow cell division particularly S phase of the cell cycle, decreases cell production of epidermal cells.[34]

The MTX Dose

The MTX formulated into different types of administration routes i.e. IM and oral route. The IM route considered the best route due to the ignorable side effect; the oral route is complicated due to side effects of nausea, vomiting, and other digestive related issues. The MTX by oral routes absorbed in intestinal tract through saturable transporter, folate carrier 1(RFC1) molecule which carried drugs and reached into different parts of body, in liver 10% MTX converted into 7- hydroxy MTX, excreted through kidney and bile route, MTX binding capacity with protein is low or medium, MTX deposited extravascular and drugs level decreased through dialysis. MTX half-life in serum is 6-8 hours which could be detected >24 hours.[35,36] The MTX tablet formulated as MTX disodium form in 2.5 mg concentration while injection formula contains sodium MTX 25 mg/ml concentration, the administration does depend on disease severity.[37]

MTX therapeutic in R.A

The MTX outcome against inflammation is excellent so that is why it helped for the treatment of the rheumatoid disorder. The MTX combined with other biological agents for better functional activity with an excellent outcome. For the treatment of rheumatoid disease special care adopted. The higher toxicity noted in drug combination usage as a compared single agent or drug use.[38]

MTX in Adenosine Releasing

The MTX concentration depends on the administration route, 10% converted into 7-Hydroxy MTX which further derivatized into poly Glu MTX. The MTX and 7-Hydroxy MTX taken through FC1 transporter, intracellular showed AICAR inhibitory action resulted in hinder adenosine degradation pathway, increased adenosine concentration deposited into intracellular and extracellular. Adenosine bind with A2a and A3 types receptor, which are initiator anti-inflammation activity, laterally repressed cellular phagocytic activity i.e lymphocyte cell and anti-inflammatory substance in particular cytokine, TNF, IL-8 and IL12.[39] Invitro study adenosine high level showed decreased neutrophil adhesion. The MTX increased adenosine level, high-level adenosine suppressed leukocyte activity.[40] The MTX released adenosine from the liver cell with high profile side effect i.e. liver cirrhosis and fibrosis. Adenosine released Ethanol. Adenosine binds with A2A receptor in a hepatic cellular fibrogenic cell, enhanced different protein production such as collagen types I and III, diminished metalloproteinase enzyme activity, adenosine bind with A2A receptor stimulate fibrogenic activity, activated receptor speed up wound healing process through matrix production.[41-43] The MTX directly act on different substance, reduced degradation adenine with sugar base or phosphate addition. The enzyme 5-ectonucleotidase helped in AMP dephosphorylation which directly or indirectly increased adenosine level, Adjuvant-induced arthritis treated through adenosine administration.[40]

MTX in Sleeping Sickness

Adenosine act as an A1 receptor initiator activates the lateral hypothalamus perifornical part, controls sleeping, and drowsiness. The MTX treatment fatal in all aged patients, children presented server life-threating complications such as coma and sleeping sickness. The

theophylline is an antidote, used in combination with high dose MTX in children.[44,45]

MTX in Heart Disease

The MTX played a positive impact to control heart disease, atherosclerotic heart disease, not included traditional heart disease. The MTX reversed atherosclerotic heart disease, interferon -γ and different complements which distort the function of two protein named 27 hydroxylases and ABCA1 (helped in the transportation of cholesterol from outside to inside the cell). The MTX obstruct cholesterol transporting protein, excretion outward cell stopped, cholesterol accumulated in macrophages persuade foam cell formation, changed into atherosclerosis plague. The MTX increased 27-hydrolase which helped cholesterol exportation from macrophages. The MTX play a positive effect on bone marrow through adenosine and A2A receptor inhibition, A2A receptor inhibition also protect from cholesterol side effect through controlled transportation mechanism.[46]

MTX used in Ectopic Pregnancy

The MTX medication depends on disease level, single or multiple doses, the single dose used in ectopic pregnancy, improvement rate up to 9-27%.[47-49] The MTX dose is given via different routes i.e. oral or local area drugs administration according to protocol. The single-dose recommended in ectopic pregnancy case, 50 mg/m2 intramuscular drugs given along β-HCG level measured, drug dose correlated with hormonal level.[50,51] The success rate depends on the beta HCG level, gestational age, and regimen of treatment. The MTX play an important role in lowering the beta HCG level.[52-54]

MTX in Malignancy

The MTX widely used for neoplastic disorder treatment i.e acute and chronic lymphoblastic leukemia, osteogenic sarcoma, breast cancer, and choriocarcinoma. The MTX categorized as a cytotoxic agent, inhibits different enzymes such as 5,6,7,8 tetrahydrofolate, tetrahydrofolate dehydrogenases, NADP oxidoreductase (reduce ferredoxin level), EC 1.5-1.3 inhibit dihydrofolate reductase activity.[55]

MTX in ALL

The MTX used against ALL, drug effectiveness relates to WBC count and thiopurine metabolite. Although no technique available to control drug concentration and WBC count, uncontrolled bone marrow destruct condition drug stop.[56]

MTX Detection

The MTX TDM used as a therapeutic indicator, glutamyl synthetase, and hydrolase helped in addition or removal of glutamic acid γ-linked residue MTX and its metabolite. The MTX polyglutamate remained intracellular, the balance of drug determined through monitoring of MTX polyglutamate 7 and 7-γ glutamic acid residue concentration. The MTX long-chain modification remained in the cell for a long period due to higher affinity against folate metabolizing enzymes, high concentrations correlated with a long chain.[57] The MTX half-life in plasma is 4–6 hrs. TDM level measured in RBCs, a diagnostic marker which measured via a blood sample. The MTX polyglutamate detection time >1 week.[58] The MTX detected through different analytic techniques such as Radio Ligand Assay, due to limitation it replaced with liquid chromatography and liquid mass spectrometry assay (LCMS) in 2015 which can differentiate among different types of drug derivatives.[59] The MTX detected through FIA fully automated technique.[60,61]

Discussion

Man, always tried to find out science mysteries. The MTX designed to hinder folic acid metabolism blocked DNA division leads to cell division failure. The MTX consider WHO essential life-saving drug, more than 90% drug excreted via kidney route.[2] The MTX structure is like folic acid except in two ways, the 1st hydroxyl group (OH) replaced with an amino group (NH2) while secondly methyl group (CH3) substituted with an amino acid.[3] The MTX used in safe limits, exceeding limits might be fatal, high potency drug-related to side effects. The MTX metabolite and other derivative forms accumulated in different parts of the body i.e. kidney, bone marrow, digestive tract, mucositis, and nerve cell. The researcher tried to eradicate the side effect and other harmful effects but still failed. Leucovorin is an MTX antidot drug. The MTX detected in serum, most common detection technique is a radioisotope, which is highly sensitive, rapid and accurate.[19-21] The MTX used in an ectopic pregnancy, metastatic gestational choriocarcinoma, trophoblastic malignancy, heart disease, RA, ALL, CLL, head and neck cancer, breast cancer, osteosarcoma, Hodgkin and non-Hodgkin lymphoma treatment.[22,23] The MTX side effects included

low WBC count, liver cell toxicity, nephron cell toxicity, myeloid cell suppression, ulcer in the stomach and intestinal mucosal cell line, diarrhea, dyspepsia, vomiting, stomach ulcer and nausea.[27,28] In earlier MTX was familiar with aminopterin. In 1950, Gubner and his colleague used aminopterin against connective tissue to check anti-inflammatory and anti-proliferative activity. In 1951, MTX 1st historical study published. In previous literature, MTX most used as an anti-inflammatory and anti-proliferative drug. Stoval TG studied to check the MTX effect against unruptured ectopic pregnancy with 3.5 cm or less in size, twenty-three patients conformed previous ectopic pregnancy. A total of 96/100 (96%) received MTX treatment as primary therapy and four (4%) treated until persistent HCG level, the success rate was 80% while five patients (5%) showed failed treatment. [62] Bacci studied osteosarcoma and limb spar surgery, in Italy 164 patients treated with MTX high dose. The average follows up the case was 54 months, 109 patients with 66% success rate become disease-free, 2 patients died due to cardiac toxicity and 52 observed metastases condition, 3 were local repetition, the success rate was more than 60%.[63] Weinblatt et al, selected twenty-six RA disease patients, treated with MTX up to 132 weeks which showed significant results against RA disease. [64] Rehman et al. did a study to check treatment response against skin inflammatory disease, 46 patients selected after conformed atopic dermatitis and psoriasis eczema disease, after treatment completion atopic dermatitis showed 38% and psoriasis eczema showed 83% success ratio.[65] Aka and Kenan studied against ectopic pregnancy complications along with β HCG level, total 65 patients administrated MTX single-dose intramuscular, MTX treatment respondent included 56 patients (86.2%) while non-respondent patients included 9 (13.8%).[66] Svirsky et al, did study among 120 months, a total of 542 women patients diagnosed ectopic pregnancy and treated with MTX drug, 226 female patients later perceived, and 197 females return into the normal physical stable state, 206 babies delivered after MTX successfully treatment. This study gave a new way to treat ectopic pregnancy. [67] Hussa and Pattillo checked MTX effect against human choriocarcinoma invitro. The MTX used in a different concentration such as 0.10 uM and 10 uM, the growth inhibition rate was 83% and 93% respectively. [68] Yang et al, investigated MTX activity against Aspergillus species in-vitro, total 23 clinical isolates of Aspergillus species studied via using microdilution technique to estimate MTX interaction with voriconazole, itraconazole, terbinafine and amphotericin B. The highest rate of synergy was obtained with combination of terbinafine and MTX, combined effect against fungal species was 60.9% while 14 fungal species showed sensitivity out of 23 species. No interaction detected, MTX and itraconazole or amphotericin B combinations show a 95.7% (22/23) success rate, voriconazole with MTX showed indifferent against strains 87% (20/23). MTX antagonism effect founded against strains 13% (3/23), Aspergillus show morphological changes due to MTX and terbinafine combination, inhibition and distortion of growth also founded.[69]

Ghannoum to checked anti-neoplastic agents' activity against T. glabrata growth, MTX inhibits T. glabrata growth, the morphological response observed, enhanced filamentation and influenced cells structures resulted in cytoplasmic materials lost and cell collapse. The MTX also inhibits limited uptake and synthesis of T. glabrata macromolecular.[70] Bush et al, study to checked out Candida albicans growth inhibition through different drugs, MTX showed dihydrofolate reductase inhibition activity by tight binding with C. albicans.[71] MTX antibacterial activity against twenty separate colonies of Staphylococcus aureus isolated from UTI children, out of twenty-ten children were suffering in leukemia and UTI, staph. aureus hemolysin production species growth inhibited against MTX drug.[72]

Kruszewska et al, studied to check MTX anti-microbial activity against Staphylococcus aureus ATCC 6538P, E. coli ATCC 8739, P. aeruginosa ATCC 15442 and C. albicans ATCC 10231. MTX MIC determined by agar dilution method, methicillin resistance Staphylococcus aureus (MRSA-32) MIC 50% was 20 micrograms/ml while MIC 90% was 100->100 micrograms/ml (range: 10-<100 micrograms/ml), methicillin-sensitive Staphylococcus aureus MIC 50% was 10 micrograms/ml while MIC 90% was 20 micrograms/ml (range: 10-20 micrograms/ml).[73]

Hyams et al, studied to an assessed staphylococcal antibacterial activity which showed normal antibacterial activity and decreased metabolic activity in the presence of MTX increased concentration.[74]

Conclusion

The MTX used and management play Vitol role for treating different disorder.

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