Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.6, No.2, 2016

Ameliorative Effect of Folic Acid on the Neurological Changes in Rats Treated with Methotrexate

Hana Khalil Ismail Maha D. N. Kako Eman B. Marie*
Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract
Methotrexate (MTX) is an antimetabolite of the normal folic acid produced by human and animal bodies. MTX antagonizes Folic Acid which is vital for purine and many vital amino acids. Treatment with MTX has been widespread all over the world recently. Physicians usually prescribe folic acid together or following treatment with MTX. Some patients minimize the importance of having folic acid with MTX or after it regarding it as a not essential drug as MTX. From here it comes, the value of this research to clarify the important role of folic acid in minimizing the side effects of MTX. In fact this effect applies on most systems in the human and animal bodies; however, authors prefer to focus on the neurological aspects as references are somewhat scarce especially in the veterinary aspect and found some behavioral changes which coincide with brain histopathological findings.
Keywords: methotrexate, folic acid, neurological changes, rats

1. Introduction

Methotrexate is an antimetabolite and antifolate drug, has been spread in the last years as a cure for large list of diseases in both human and veterinary fields. Therefore, it is important to study its effect on variant systems and organs. From these organs, the nervous system is important to study as its review regarding this drug is somewhat rare in comparison to others.

Methotrexate abbreviated (MTX) and formerly known as amethopterin, is an antimetabolite and antifolate drug. It is used in the treatment of cancer, autoimmune diseases, ectopic pregnancy (Alshimmiri et al., 2003), and for the induction of medical abortions (Vinet, 2013). In addition, it is used for treatment of psoriasis (Kozub and Simaljakova, 2011). Methotrexate has been spread in the last years as a cure for large list of diseases in both human and veterinary fields (especially for pets in case of rheumatoid arthritis (Rheumatrex®) USP.

Medical uses of methotrexate:

Chemotherapy

Methotrexate (MTX) was originally developed and continues to be used for chemotherapy either alone or in combination with other agents. It is effective for the treatment of a number of cancers including: lung, head and neck cancers, leukemia, lymphoma, osteosarcoma, bladder, and trophoblastic neoplasms (Bayram et al. 2005).

Autoimmune disorders

It is used as a treatment for some autoimmune diseases, including rheumatoid arthritis, Juvenile dermatomyositis, psoriasis, lupus, sarcoidosis, and many forms of vasculitis. Although methotrexate was originally designed as a chemotherapy drug (in high doses), in low doses it is a generally safe and well tolerated drug in the treatment of certain autoimmune diseases (Cronstein, 2005).

Because of its effectiveness, low-dose methotrexate is now first-line therapy for the treatment of rheumatoid arthritis. Therefore, methotrexate for autoimmune diseases is taken in lower doses than it is for cancer. Side effects such as hair loss, nausea, headaches, and skin pigmentation are still common.

Folic Acid:

Dihydrofolate reductase (DHFR) is a key enzyme for the biosynthesis of purines, thymidylate, and a number of amino acids. It has strategic location in metabolism has also made it a target for anticancer drugs (Zhang et al. 2004).

MTX acts by inhibition of folic acid synthesis, this is why giving its antagonist (FA), could be the optimum related treatment along with MTX for gaining a minimum side effects.

This experiment was designed to clarify the role of oxidative stress of MTX and the possible protective effect of its antagonist (FA) on the neurological tissue using histopathological examinations.

2. Materials and Methods

15 female Albino rats of 200-250 gm were obtained from the house of animals, Faculty of Veterinary Medicine, University of Mosul. Rats for this experiment divided into 4 groups randomly. Group 1 represents control injected with distilled water 0.6 ml/ rat every week for 4 weeks. Group 2 were given folic acid 1mg/ rat orally by drenching every week for 4 weeks too. Group 3 were injected with MTX intramuscularly 20mg/ kg b.wt. every week for the same period. Group 4 were given folic acid and injected with MTX concomitantly with the same previous doses, periods, and routes of administration. Range of rat weights was as follows:

Rat number	Weight (gm)
1	240
2	245
3	215
4	200
5	235
6	195
7	200
8	215
9	175
10	205
11	195
12	185
13	175
14	205
15	230

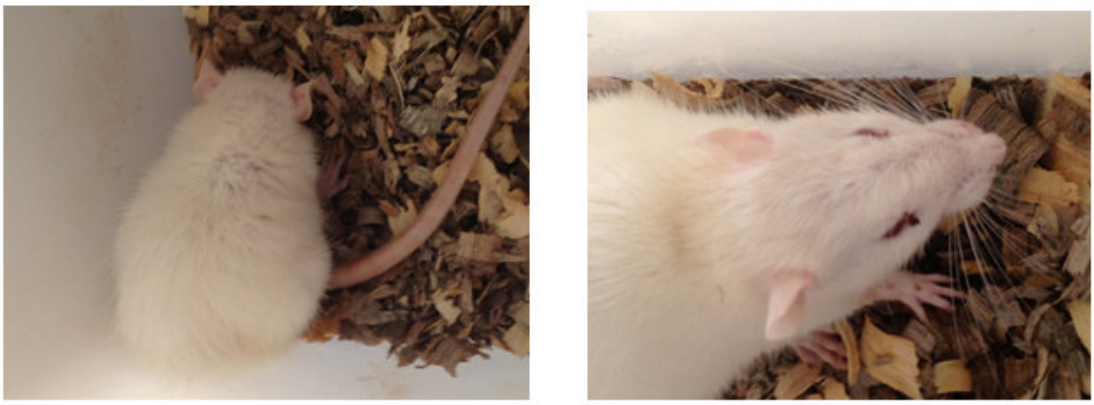
Methotrexate solution (50mg/5ml) (1Flakon) ® is available commercially. For intramuscular injection dose of 0.4 mg/kg for rats was administered by (Fan et al., 2009). For our study, it is preferred to use the dose 20 mg/ kg b.wt. based on a previous related study (Soliman, 2009). Folic acid (FA) was administered orally by needle gavage at 1 mg/ rat once weekly (Fan et al., 2009).

After 4 weeks, rats were sacrificed by merciful killing. Brain and allied Central Nervous System organs were fixed with 10% neutral buffered formalin fixative for routine histopathological sections.

3. Results

Animal behavior:

Rats treated with MTX were dull as revealed in the following photos and suffered from alopecia

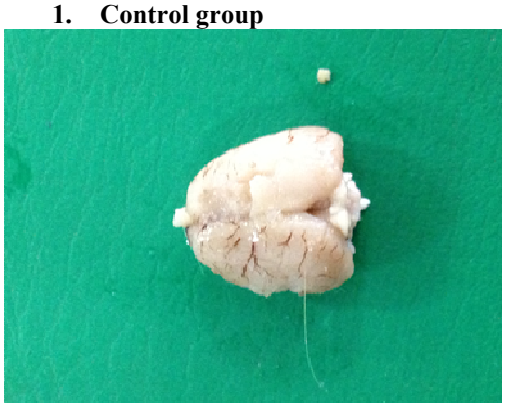


Photos show a dull rat, treated with MTX (Left), another rat suffered from alopecia at face region (Right). This group treated with MTX alone.

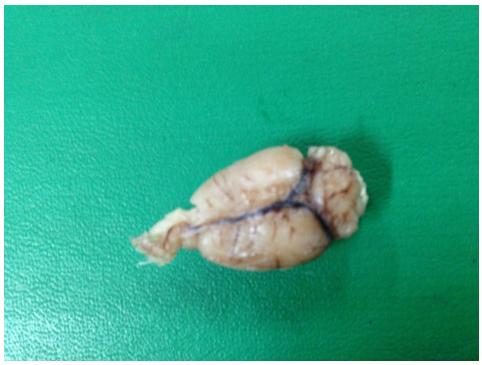
Macroscopic lesions of the brain:

The gross appearance of the brain in the treated group with methotrexate was suffering from severe congestion in comparison to the control group which is similar to the group treated with MTX. The brain of the treated rats with both MTX and FA revealed a better improvement in comparison to the treated group.

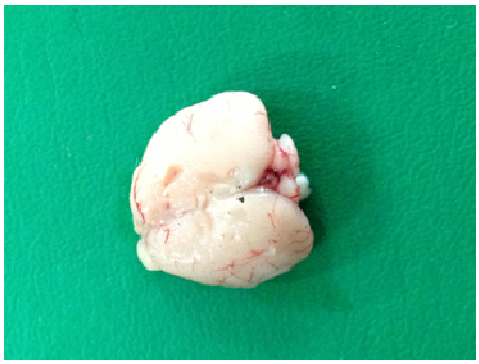
1. **Control group**



2. **Treated with MTX**

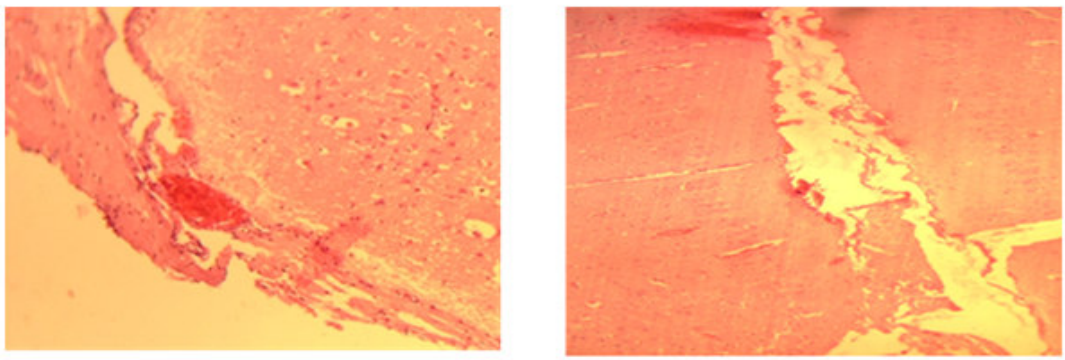


3. **Treated with MTX and FA**

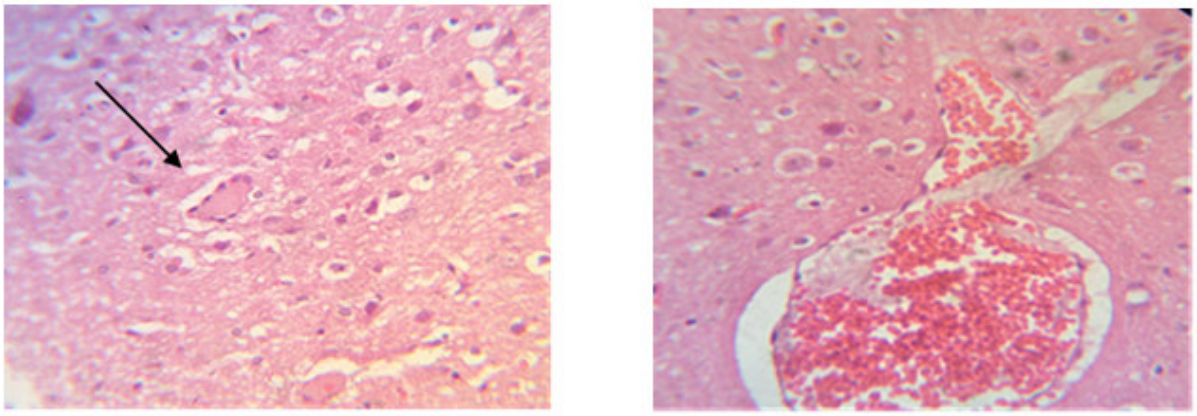


Photos reveal the severe macroscopic congestion of the brain of animals treated with MTX (2) in comparison to control animals (1) and the ameliorative effect of folic acid treated animals (3).

Microscopic lesions of the brain



Photos reveal the congestion of the brain (Left 142X) and the ameliorative effect of folic acid on brain to be as a mild congestion (Right 43X).



Photos (420X) Cross section of a dead neuron surrounded by microglial cells that phagocytize the cell debris (neurophagia) ((left) (420 X) represents a section of brain which reveals the congestion and the beginning of a recent thrombus with areas of vasogenic edema with some spongy change (right)

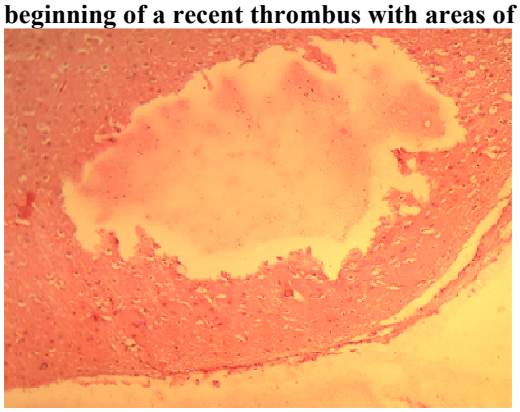


Photo 105X shows the presence of abscess in a region of the brain represented by pus material and cellular debris surrounded by a region of hyperemia and spongiosis.

4. Discussion

MTX acts by inhibition of Folic acid (FA), This is done by inhibition of cellular synthesis of purines, pyrimidines and methionine, methotrexate polyglutamate; RFC1, reduced folate carrier 1; DHFR, dihydrofolate reductase;THF, tetrahydrofolate; TS, thymidylate synthase; MTHFR, methylene tetrahydrofolate reductase; FPGS, folyl polyglutamate synthase;AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AICART'ASE, AICAR transformylase (Cronstein, 2005).

This study was in agreement with some other studies in which their results indicate that high-dose MTX is related with modest toxicity and a radiographic response proportion (74%) was equivalent to more toxic regimens (Batchelor, et. al., 2003). Another study included a patient developed probable MTX-induced leukoencephalopathy, the symptoms included headache and confusion (Gowan, et al., 2002).

However, it was found that there are some studies showed central nervous system (CNS) toxicity from low-dose methotrexate (MTX) has been reported with symptoms consist mainly of dizziness and headache. This study reviewed the records of 25 successive patients treated with low-dose MTX, and found 5 who had spontaneously reported unpleasant cranial sensations (Wernick and Smith, 1989).

Interestingly, cases in which abscess in cerebrum was found in treated group with MTX was in agreement with animal behavior of depression and dullness. This result is scientifically reported (Zachary and McGavin, 2006).

References

Alshimmiri, M.M., Al-Saleh, E.A., Al-Harmi, J.A., AlSalili, M.B., AlAdwani, A., Ibrahim, M.E. (2003) "Treatment of Ectopic Pregnancy with a single intramuscular dose of methotrexate" Arch Gynecol Obstet. 268: 181-183

Batchelor, T., Carson, K., O'Neill, A., Grossman, S.A., Alvi, J., New, P., Hochberg, F., and Priet, R. (2003) " Treatment of Primary CNS Lymphoma With Methotrexate and Deferred Radiotherapy: A Report of NABTT 96–07" American Society of Clinical Oncology.

Bayram, M., Ozogul, C., Dursun, A., Ercan, Z. S., Isik, I., and Dilekoz, E. (2005). Ligth and electron microscope examination of the effects of methotrexate on the endosalpinx. European Journal of Obstetrics &

Gynecology and Reproductive Biology 120, 96–103.

Cronstein, B. N. (2005). "Low-Dose Methotrexate: A Mainstay in the Treatment of Rheumatoid Arthritis". *Pharmacological Reviews* 57 (2): 163–172

Fan, Ch., Cool, J.C., Scherer, M. A., Foster, B.K., Shandala, T., Tapp, H., Xian, C. J. (2009) " Damaging effects of chronic low-dose methotrexate usage on primary bone formation in young rats and potential protective effects of folinic acid supplementary treatment" Bone, Volume 44, Issue 1, January 2009, Pages 61-70

Gowan, G. M., Herrington, J.D., Simonetta, A.B. (2002) " Methotrexate-Induced Toxic Leukoencephalopathy" Pharmacotherapy: 22(9).

Kozub, P. and Simaljakova, M. (2011) "Systemic therapy of psoriasis: methotrexate" Bratsl Lek Listy 112 (112) (7) 390-394

McGavin, M.D., Zachary, J. F. (2006) " Pathologic Basis of Veterinary Diseases" 4th edition

Soliman, M.E. (2009) "Evaluation of the Possible Protective Role of Folic Acid on the Liver Toxicity" *Egypt. J. Histol. Vol. 32, No. 1, June, 2009: 118 – 128*

Vinet, E., Kuryia, B., Pineau, A., Clarke, A.E., Bernatsky, S. (2013) "Induced Abortions in Women With Rheumatoid Arthritis Receiving Methotrexate" Arthritis Care & Research" Volume 65, Issue 8, pages 1365–1369

Wernick, R. and Smith, D. L. (1989), Central nervous system toxicity associated with weekly low-dose methotrexate treatment. Arthritis & Rheumatism, 32: 770–775.

Zhang, Z., Ravi Rajagopalan, P.T., Selzer, T., Benkovic, S. J., Hammes, G.G. (2004) " Single-molecule and transient kinetics investigation of the interaction of dihydrofolate reductase with NADPH and dihydrofolate" Proc Natl Acad Sci U S A. Mar 2, 2004; 101(9): 2764–2769.