



Effect of methotrexate on neurobehavior and cholinesterase in chicks

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Abstract

This reading aimed to determine the therapeutic index of methotrexate (MTX) with a note on the consequence of methotrexate in chicks on neurobehavioral in addition to calculating the activity for cholinesterase in plasma and brain in chicks. Two groups with 10 chicks each were utilized once. The untreated and methotrexate groups were given saline and MTX at dose 40 mg/kg for 5 days, respectively. The methotrexate group obtained considerable amounts of methotrexate 9th day of the start of the experiment for five days. All chicks were exposed to open-field activity and tonic immobility tests. Brain tissue with blood samples were collected to evaluate cholinesterase levels in chicks. ED₅₀ and LD₅₀ for methotrexate in the chicks were 14.36mg/ kg and 102.63 mg/ kg, correspondingly. In addition, Therapeutic index of methotrexate was 7.15. The methotrexate group explained an important enhance in latency to move and tonic immobility evaluated with an untreated group, there is a significant decline in lines crossed, and escape jumps compared with untreated group, and the cholinesterase activity showed a significantly reduced in plasma and brain samples treated with methotrexate and inhibition expressed significantly increased evaluated through the control group. Our findings conclude that methotrexate has a depressant action on the nervous structure and an inhibitory action on the cholinesterase level in the chick's model.

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Introduction

Methotrexate is equally analog to folate, the adversary of folate in addition to DNA formation (1-4), and dihydrofolate reductase inhibited by it, it results in a decrease in change dihydrofolate to tetrahydrofolate (1-6). Anticancer drugs are also used in therapy for different types of cancers (7). They can also be applied only or in combination with these drugs to cure tumors (8,9). The cure of cancer, like leukemia and lymphoma, in children and adultshas an effect via Methotrexate (10). Acute leukemia in infancy is treated via the most important agent MTX (11,12). Numerous situations have been reported characterized by cerebellar toxicity in kids during cure with MTX (11-14). However, the cytotoxicity of MTX, as that has occurred with other chemotherapeutic drugs, was reported to affect many body

organs (15). MTX can result in acute, subacute, and long-term nervous system toxicity (16). Nervous system toxicity occurs through direct nerve cell hurt and disorder of folate homeostasis of the nervous system (17). After expansion for mini-dose intrathecal, oral, or large-dose Subacute neurotoxicity of MTX occurs in 2 to 14 days. The previous information has verified that many sick people can be unharmed with MTX. In contrast, neurotoxicity could reappear in others (18). In animals, it stimulates growth abnormality in the cerebellum in chicken after prenatal MTX exposure (19); a toxic consequence in the cerebellum of guinea pigs and rats has occurred after exposure to methotrexate (20). MTX resulted in death and relapse of Purkinje cells in these animals after intraperitoneal administration (21,22).

Our study aimed to determine ED₅₀, LD₅₀, and therapeutic index for MTX by evaluating the neurobehavioral effect of methotrexate in the chicks. It also evaluated its effect on cholinesterase activity in blood plasma and brain tissues.

Materials and methods

Ethical approval

The paper was completed in the animal house at the College of Veterinary Medicine, University of Mosul, Iraq, with the Institutional Animal Care and Use Committee (IACUC) ethical approval number UM.VET.2022.034 on January 1, 2020.

Animals

Both sexes of Ross broiler chicks were procured starting a limited hatchery. They were divided into groups of 10 chicken, each at 25-30 °C with 24 h light and wood shaving as floor litter, adlib feed, and water.

Preparing medicines

Required doses of methotrexate were provided with distilled water, and the dose volume for methotrexate as well as distilled water was 5 ml/kg.

Determination of ED₅₀ of methotrexate in chicks

This trial used 8 chicks weighing 57- 91g. The initial chick was giving methotrexate at 20mg/kg, IP. Amount of increase with decrease in the dose of methotrexate was a stabilized quantity at 2mg/kg, and through replicating this method amount of dose of methotrexate in support of solitary three animals after the first alter, it was possible to calculate the ED₅₀ for methotrexate (23) via electrostimulation, and using the following equation: $ED_{50} = Xf + Kd$.

Determination of LD₅₀ of methotrexate in chicks

This trial was made with 6 chicks, using the Dixon manner, weighing 50-81g. An initial chick was administered methotrexate at 120 mg/kg, i.p., The enhance and shrink in the dose of methotrexate was a stabilized quantity at 10 mg/kg (23). The purpose of identifying ED₅₀ and LD₅₀ for MTX was to determine the therapeutic index, and the therapeutic index for MTX was 7.15 in chicks.

Determination of choline esterase activity and open field test for chicks

The trial started when the chicks were 9 days. Chicks whose weight body ranged between 84-190 g were used in this experiment for 15 days. Twenty healthy chicks were alienated into two groups; First group obtained normal saline i.p., and second group was i.p. administered methotrexate 40 mg/kg once daily for 5 days. After completion of 15 days from animals old, all chicks were submitted for tonic immobility testing and open-field activity. Plasma and brain samples were isolated and stockpiled until analyzed to determine the cholinesterase level. Cholinesterase action measurement by Michel method (24,25). The inhibition percent of acetylcholinesterase was calculated by $\text{Inhibition \%} = \frac{\text{untreated group ChE activity} - \text{treated group ChE activity}}{\text{untreated group ChE activity}} \times 100$.

Statistical analysis

Statistical analysis of the numbers was made via SPSS. Parametric records were estimated via one way analysis of variance followed via the least significant difference test. In addition to non parametric data were analyzed by man-wittny.

Results

Determination of ED₅₀ of methotrexate in chicks

ED₅₀ for methotrexate in chicks was verifying through using the up and down manner subsequent to administer diverse doses of methotrexate i.p. to 8 chicks via electrostimulation, and it was 14.36mg/kg (Table 1); this result was recorded for the first time in chicks.

Determination of LD₅₀ of methotrexate alone in chicks

LD₅₀ for methotrexate in the chicks was determined after administering different doses of methotrexate i.p. to 6 chicks, and the value of these doses was 102.63mg/kg (Table 2). The rationale of recognizing ED₅₀ and LD₅₀ for methotrexate was to determine the therapeutic index, and the therapeutic index for methotrexate was 7.15 in chicks.

Table 1: Determination for ED₅₀ of methotrexate in chicks

Variables	Results
ED ₅₀ (mg/kg)	14.36
Rate of the doses utilized (mg/kg)	20-12=8
original dose (mg/ kg)	20
finishing dose (mg/kg)	14
Raise or decline in the dose (mg/kg)	2
The extent of chicken involved	(OOOOXOXX) 8
The minimum-maximum voltage that produced pain	5-8 before and 7-11 after MTX administrated

x - analgesia; o - no analgesia.

Table 2: Determination of LD₅₀ of methotrexate in chicks

Variables	Results
ED ₅₀ (mg/kg)	102.63
Rate of the doses utilized (mg/kg)	120- 100= 20
original dose (mg/ kg)	120
finishing dose (mg/kg)	110
Raise or decline in the dose (mg/kg)	10
The extent of chicken involved	(XXOXOX) 6

x - live; o- dead.

Determination of open field activity for chicks

The methotrexate-treated group explained a significant increase in latency to move in addition to tonic immobility was evaluated with the untreated group. That is a significantly decline in escape jumps and lines crossed, compared to an untreated group (Table 3).

Table 3: Open field activity and tonic immobility test for chicks treated with methotrexate

Variables	Control group	Methotrexate
Latency to move (s)	1.39±0.30	4.18±0.57*
Escape jumps	1.30±0.86	0.00±0.00*
Lines crossed	45.71±2.68	9.20±1.36*
Pecking (scores)g	0.00±0.00	0.00±0.00
Distress calls (scores)g	2.6±0.1	2.30±0.56
Defecations	1.60±0.22	0.92±0.72
Tonic immobility (sg)	0.89±0.02	1.55±0.13*

* The data is significantly different from the untreated group.

Determination of cholinesterase activity in chicks

The effect of methotrexate dose on cholinesterase level in brain and plasma were reduced to 0.89 and 0.73ΔpH/30min compared to control group 1.69, 1.24 ΔpH/30min respectively and inhibition present increased to 47.33 and 41.1% respectively compared to control group 0% (Table 4).

Table 4: Cholinesterase activity in plasma and brain samples treated with methotrexate

Treatment	Plasma		Brain	
	ΔpH/30min	Inhibition%	ΔpH/30min	Inhibition %
Control group	1.69±0.037	0.00%	1.24±0.03	0.00%
Methotrexate (40 mg/kg)	0.89±0.011*	47.33%	0.73±0.06*	41.10%

means ± SE (Ten chicks/ group).

Discussion

The ambition of this revise was to detect effect of methotrexate in chicks as well as to evaluate the efficacy of the compound in this animal model. At the beginning of the research, we identified ED₅₀ and LD₅₀ by injection i.p., which is considere done of the important criteria in pharmacology, and determining ED₅₀, in general, was a preliminary stage to determine the range of doses used in the study (26,27). The value of ED₅₀ did not agree with (28). This may be because of factors affecting metabolism and mechanism of action. Where the value of LD₅₀ differed from the values of LD₅₀and maximum tolerated dose determined in other studies (29,30), and this may be due to the difference in the type of manufacturer of the drug, as well as the doses used, the method of administering the drug, the age of the chicks, as well as the difference in sex and genetic factors of the chickens (31). The special act on nervous system origin via anticancer medicines vary special types of medicines, via their dose in addition to they are characterized (32). Methotrexate has hurt effects on various regions in the brain as the hippocampus with the cortex can lead to brain injury and cognitive problems (33). methotrexate treated group showed a significantly increased in latency to move and tonic immobility evaluated with the control or untreated group; there are significant minimize in lines crossed and escape jumps; these results are in agreement with works for Jong

(34) who referred to MTX result in nervous system toxicity reduced the creation of progenitor cells along with being able to result in dysfunction of hippocampus, like depression plus cognitive destruction, This effect may occur due to toxic effect of methotrexate on astrocytes leading to neuron death, in addition to adverse effects of the drug on other components for neurons in the nervous system (33,35) with the disorder of folate metabolism (36,37), all these effects can be influenced on behavior with cognitive disorder. As well as these results are in agreement with studies for Koppelmans in addition to Foley (38-40) who referred to anticancer medicines quickly producing long disorders of cognitive dysfunction. modifying the brain barrier infiltration with oxidative irritants' activity may lead to nerve cell inflammation; all these effects occur with antineoplastic therapy (41,42). Methotrexate is a successful antineoplastic medicine that caused toxic consequences on organs; the oxidative irritate was underlying the mechanism of MTX that resulted in the inhibition of cholinesterase enzyme, and this led to an effect on the neurobehavioral activity of chicks (43). In addition, this result is in agreement with reports of Huang (44,45), when the use of antitumor medicine like sunitinib leads to a decrease in AChE action in mice, this occurs due to the hurt action of the anticancer drug on hippocampal and cortical parts in the brain (46).

Conclusion

Treatment of chicks with methotrexate resulted in hurt effects on motor activity, cholinesterase levels in the brain, and plasma in chicks. Methotrexate resulted in depression and cognitive destruction in chicks.

Conflict of interest

The authors have no conflicts of interest regarding this investigation.

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تأثير الميثوتركسيت على السلوك العصبي وخميرة الكولين استريز في الأفراخ

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الأدوية، كلية الطب، جامعة الموصل، الموصل، العراق

الخلاصة

هدفت الدراسة الحالية إلى تحديد المؤشر العلاجي للميثوتركسيت في الأفراخ مع ملاحظة تأثير الميثوتركسيت في الأفراخ على السلوك العصبي عن طريق اختبار نشاط الميدان المفتوح واختبار عدم الحركة الشدي بالإضافة إلى حساب نشاط أنزيم الكولين استريز في البلازما والدماغ في الأفراخ. تم استخدام مجموعتين تحتوي كل واحدة منهما على ١٠ أفراخ. عوملت المجموعات الغير معاملة والمعاملة بالميثوتركسيت بالمحلول الملحي الفسلجي والميثوتركسيت بجرعة ٤٠ ملغم/كغم لمدة ٥ أيام على التوالي، حصلت مجموعة الميثوتركسيت على جرعة متعددة من الميثوتركسيت في اليوم التاسع من بدء التجربة لمدة خمسة أيام. ثم تم إخضاع جميع الأفراخ لاختبار نشاط الميدان المفتوح واختبار عدم الحركة الشدي. تم جمع عينات من أنسجة الدماغ والدم لتقييم مستويات الكولين استريز في الأفراخ. وكانت قيم الجرعة الفاعلة الوسطية والجرعة المميتة الوسطية للميثوتركسيت في الأفراخ ١٤,٣٦ و ١٠٢,٦٣ ملغم/كغم على التوالي، بالإضافة إلى أن المؤشر العلاجي للميثوتركسيت كان ٧,١٥. أظهرت المجموعة المعاملة بالميثوتركسيت زيادة معنوية في اختبار عدم الحركة الشدي وبدء الحركة مقارنة مع المجموعة الغير معاملة (مجموعة السيطرة)، وهناك انخفاض كبير في الخطوط المقطوعة، والقفز مقارنة مع مجموعة السيطرة أيضاً، أظهر نشاط الكولين استريز انخفاضاً كبيراً في عينات البلازما والدماغ المعاملة بالميثوتركسيت، كما زاد تثبيط الإنزيم بشكل كبير مقارنة مع مجموعة السيطرة. نستنتج من النتائج التي توصلنا إليها أن للميثوتركسيت تأثير مثبط على الجهاز العصبي المركزي وتأثير مثبط على نشاط إنزيم الكولين استريز في نموذج الأفراخ.