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Acute toxicity of metronidazole and its interaction with chlorpyrifos in chicks

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Abstract

Metronidazole is antimicrobial drug for human and animal use, The more characteristic side effect associated with use high dose of metronidazole is neurotoxic signs, some of these signs that recorded in animal represented by ataxia and tremor, there is limited information is available on the pharmacological profile of metronidazole in birds The aim of our study explain some of its neurological effect in chicks by its interaction with one of organophosphorus insecticide chlorpyrifos that have well-known excitatory effect on nervous system. Median Lethal Doses (LD_{50}) of metronidazole and chlorpyrifos were determined depending on up and down method. The intraperitoneal and oral LD_{50} of metronidazole were 516.9 mg/kg, 3061.8 mg/kg respectively. The oral LD_{50} of chlorpyrifos was 13.705 mg/kg, intraperitoneal treatment of metronidazole with Oral treatment of chlorpyrifos in ratio 1:1, 1: 0.5, and 0.5:1, respectively of LD_{50} at the same time increased LD50 for metronidazole and chlorpyrifos and the isobolographic analysis showed that the points of interaction occurred above the diagonal line connecting between LD_{50} of each; while oral treatment of metronidazole and chlorpyrifos in ratio 1:0.5 LD_{50} at the same time decreased LD_{50} for metronidazole and chlorpyrifos and the point of interaction was above the diagonal line connecting between LD_{50} of each in conclusion we found that isobolografhic analysis for metronidazole and chlorpyrifos in different percentages and routs of treatment reveal to antagonist effect despite the similarity in the toxic signs.

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Introduction

Metronidazole is a broad spectrum synthetic antimicrobial drug. That is used in veterinary medicine to treat large and small animals (1). It affected the anaerobic bacteria and protozoa, which it causes many diseases such as giardiasis, amobiasis, bowel disease, peritonitis. used also as a protective treatment after operative surgery (2).

It is a pro-drug that appears in antimicrobial effect through penetration of microorganism by passive diffusion and reduction nitro group. This leads to free radical formation that caused DNA damage and cell death in susceptible microorganisms. Metronidazole is metabolized in the liver by oxidation and by glucuronide formation. It is excreted primarily by the kidneys (3). Metronidazole can cause neurological effects. These effects usually occur if metronidazole is given at high doses or for extended periods of time (4).

Chlorpyrifos is one of the organophosphorus insecticides, widely used in veterinary medicine for insect control (1,5). it kills insects through irreversible inhibition of acetylcholineastrase, which causes the accumulation of excessive acetylcholine in the synaptic cleft thus leads to over stimulation of the nervous system (6,7).

It has good absorption orally and wide distribution through body tissue (8). It metabolizes in liver by

cytochrome-p450 to chlorpyrifos-oxon, which is stronger against the nervous system than chlorpyrifos. the metabolite of chlorpyrifos is easily excreted in urine and feces (9).

Our research aims to study some neurological influence of metronidazole by interacting with chlorpyrifos, which has a well-known stimulating effect on the nervous system

Materials and method

Broiler chicks of both sexes 60 - 136 g were used. it had brought one day of age and put in cages of breeding with condition water food ad libitum, temperature of 30-34ºC with constant lighting, wood shavings as floor litter, until procedure experiments age 7-14 day. The Scientific Committee of the College of Veterinary Medicine at the University of Mosul reviewed and approved the protocol of this study (10).

Drugs preparation

Metronidazole concentration 1000 mg / 10 ml used for injection was prepared by dissolve pure metronidazole in a mixture of (kollidon, lutrol, proplenglygol and distilled water) and modified to final PH to 4.4 by use HCl normality 0.1with use light heat to obtaining complete dissolvent (11). the mixture of distilled water and propylene glygol in ratio 2:3 used as diluted for obtaining the required dose. Metronidazole suspension that used orally was prepared from mixed pure metronidazole base with tween 80 (20%).

A commercial organophosphorus insecticide emulsified solution of chlorpyrifos 48% concentration (Tarkim, Turkey) diluted freshly with distilled water before dosing. The volume of administration was 10 ml/kg B.Wt. administrated orally (12).

Determination of oral and intraperitoneal LD_{50} of metronidazole in chicks by up and down method

Six chicks were used in determination of the oral median lethal dose LD_{50} of metronidazole and their body weight ranged between 60-136 g, firstly metronidazol dosed orally at 3000 mg/kg depending on previous studies, the result was read death X or life O after 24 hour, and the amount of dose increased or decreased was constant 600 mg/kg and repeat dosing up or down for 3 chicks after changing the result death to life and versa.

Calculate metronidazole LD_{50} depending upon the diagram and equation of Dixon (13) LD_{50} =Xf +Kd, in which Xf: last dose, K: diagram value, d: the value of dose increases or decreases.

Determination of the interpertonial median lethal dose LD_{50} of metronidazole used six chicks, firstly metronidazol dosed at 600 mg/kg depending on previous studies, the result was read death X or life O after 24 hours, and the amount of dose increased or decreased was constant 100 mg/kg and repeat dosing up or down for 3 chicks after changing the result death to life and versa and calculate metronidazole LD_{50} depending upon the diagram and equation of Dixon that mentioned previously

Determination of the oral LD_{50} of chlorpyrifos in chicks by up and down method

Eight chicks from both sex were used, firstly chlorpyrifos dosed orally at 25 mg/kg depending on previous studies, the result was read death X or life O after 24 hours, and increased or decreased in dose was constant 5 mg/kg and repeat dosing up or down for three chicks after changing the result death to life and versa and calculate chlorpyrifos LD_{50} depending upon the diagram and equation of Dixon as described before

Interaction of interpertonial LD_{50} of metronidazole with oral LD_{50} of chlorpyrifos in ratio 1:1, 1:0.5 and 0.5:1

In first group five chicks were used, treated chick firstly with 100% of interapertonail LD_{50} of metronidazole and directly dosed orally with 100% of oral LD_{50} of chlorpyrifos that obtain form first and second experiments respectively, while second group which used seven chicks, treated chick firstly with 100% of interapertonail LD_{50} of metronidazole and directly dosed orally with 50% of oral LD_{50} of chlorpyrifos, and the third group used five chicks treated chick firstly with 50% of interapertonail LD_{50} of metronidazole and directly dosed orally with 100% of oral LD_{50} of chlorpyrifos, the results for all groups were read death X or life O after 24 hour, and increased or decreased in dose was constant 10% of LD_{50} and repeat dosing up or down for 3chicks after changing the result death to life and versa and calculate interapertonail LD_{50} of metronidazole and orall of chlorpyrifos depending upon the diagram and equation of Dixon as described before. We subjected the LD_{50s} of both metronidazole and chlorpyrifos to isobolographic analysis. We drew a straight line for the isobolographic analysis between the lethal dose of metronidazole and chlorpyrifos given to chicks either alone or in combination.

The LD_{50} points of metronidazole and chlorpyrifos given alone are represented on the y and X axes, respectively. The straight diagonal line refers to the line of additively (zero interaction) at the LD_{50} values, and the location of the combination points on the left (below)and right (above) side of the additive line refer to synergistic and antagonistic interactions, respectively.

The interaction index was calculated using the equation da/Da+db/Db Da and Db are the individual LD_{50}s for metronidazole and chlorpyrifos respectively whereas da and db are their combined LD_{50}s. An interaction index of 1 means additivity (no interaction), < 1 synergy, and >1 antagonism (14).

Interaction of oral LD_{50} of metronidazole with oral LD_{50} of chlorpyrifos at ratio 1:0, 5

five chicks from both sex were used, firstly metronidazole dosed orally at100% of LD_{50} and directly dosed orally with 50% of oral LD_{50} of chlorpyrifos, the result read death X or life O after 24 hour, and increased or decreased in dose was constant 10% of LD_{50} and repeat dosing up or down for 3chicks after changing the result death to life and versa and calculate orall LD_{50s} of metronidazole and chlorpyrifos depending upon the diagram and equation of Dixon as described above. We subjected the LD_{50s} of both metronidazole and chlorpyrifos to isobolographic analysis also the interaction index was calculated (14,15).

Results

Determination of the oral and intraperitoneal LD_{50} of metronidazole in chicks by up and down method

The acute (24 houre) oral LD_{50} value of metronidazole was 3061, 08 mg / kg body weight and intraperitoneal LD_{50} value was 516, 9 mg / kg body weight (Table 1). with appearance of the toxic signs which represented by quiet, tease feathers, close the eye, ataxia, increase defecation, recumbency and eventually death.

Determination of the oral median lethal dose LD_{50} of chlorpyrifos in chicks by up and down method

The acute oral LD_{50} value of chlorpyrifos in chicks was 13, 705 mg / kg body weight (Table 2), and the signs of toxicity were tremor, salivation, lacrimation, dyspnea, slouch wing, stretchon or both feet, recumbency, finally dead in high toxic dose.

Interaction of interpertonial LD_{50} of metronidazole with oral LD_{50} of chlorpyrifos in ratio 1:1, 1:0.5 and 0.5:1

The intraperitoneal LD_{50s} of metronidazol were 552.312 ; 677.04 ; 292.895 mg / k g body weight and oral LD_{50s} of chlorpyrifos were 14.6306; 10.932; 13.3085 mg / kg body weight, when treated in ratio 1:1 1:0, 5 and 0, 5:1 respectively (Table 3), isobolographic analysis of these LD_{50s} for metronidazole and chlorpyrifos (either alone or in combination) revealed that combined treatment has antagonist effect (Figure 1).

This antagonist effect was established by the location of the points that represent the combined LD_{50s} of metronidazole and chlorpyrifos above the diagonal lines that connect their LD_{50s} when treated alone. Furthermore, the calculated interaction index was 2.136, 2.107, 1.49 in ratio 1:1, 1:0.5, 0.5:1, respectively.

Table 1: Determine LD50 of metronidazole i.p and p.o by up-down method

Measurement	Result	
	IP	PO
Median lethal dose	516.9 mg / kg	3061.8
Range doses	400-600	3000-3600
First dose	600	3000
Last dose	500	3600
Increase and decrease in the dose	100	600
Number of chicks	6 (XXOOXO)	6 (XXOOOX)
Time appear signs	7-20 min.	10-25 min.
Toxicity signs	Quite, tease feathers, close eye, recumbency, death.	Quite, tease feathers, close eye, ataxia, increase defecation, recumbency death

O: chick still life during 24 hours, X: chick dead during 24 hours.

Table 2: determination oral LD50 of chlorpyrifos by up and down method

Measurement	Result
Median lethal dose	13.705 mg / kg
Range dose	25-5
First dose	25
Last dose	10
Increase and decrease in the dose	5
Number of chicks	8 (XXXXOXOX)
Time appear signs	7-10 minute
Toxicity signs	Quite, salivation, lacrimation diarrhea, tremor, dyspnea, slouch wing, stretch on or both feet, convulsion, death

O: chick still life during 24 hours, X: chick dead during 24 hours.

Table 3: determine LD50 of metronidazole i.p and chlorpyrifos p.o at ratio 1:1 1:0, 5 0, 5:1 by up and down method

Groups	LD_{50} mg/kg	First dose	Last dose	No. of chicks	Result after 24h
M	516.9	600	400	6	XXOOXO
Ch	13.705	25	5	8	XXXXOXOX
M+ Ch 1:1	552.312+ 14.6306	516.9+ 13.705	618.9+ 16.305	5	XOOOX
M+ Ch 1:0.5	677.04+ 10.932	516.9+ 6.8525	720.9+ 12.05	7	OOOXOOX
M+ Ch 0.5:1	242+ 13.3085	258.54+ 13.705	258.4+ 13.705.	5	XOOXX

O: chick still life during 24 hours, X: chick dead during 24 hours, M: Metronidazole, Ch: Chlorpyrifos.

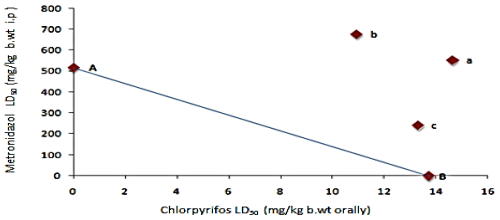

Figure 1: Isobolographic representation of the interaction of metronidazol and chlorpyrifos at LD_{50} level in chicks A, B points represent the LD_{50} of metronidazol, chlorpyrifos respectively when given alone whereas a,b,c points represent isolethal combination of metronidazol and chlorpyrifos in rate of LD_{50} 1:1, 1:0.5, 0.5 :1, respectively.

Interaction of oral LD_{50} of metronidazole with oral LD_{50} of chlorpyrifos in ratio 1:0, 5

When treated metronidazole and chlorpyrifos orally at the same time in ratio 1:0, 5 of LD_{50} the values of LD_{50s} became 3036.1 mg / kg body weight; 6.73742 mg / kg body weight respectively (Table 4). Isobolographic analysis of these LD_{50s} for metronidazole and chlorpyrifos (either alone or in combination) revealed that combined treatment has antagonist effect (Figure 2), this antagonist effect was established by the location of the points that represent the combined LD_{50s} of metronidazole and chlorpyrifos above the diagonal lines that connect their LD_{50s} when treated alone. Furthermore, interaction index was 1.482 (an index of >1 indicates antagonism).

Table 4: Determination of oral LD_{50} of metronidazole and chlorpyrifos at ratio 1:0, 5 by up and down method

Groups	LD_{50} mg/ kg	First dose	Last dose	No. of chicks	Result after 24h
M	3061.8	3000	3600	6	XXOOOX
Ch	13.705	25	5	8	XXXXOXOX
M+ Ch 1:0.5	3036.0876+6.7373	3061.8+6.8525	3061.8+6.8525	5	XOXOO

O: chick still life during 24 hours, X: chick dead during 24 hours, M: Metronidazole, Ch: Chlorpyrifos.

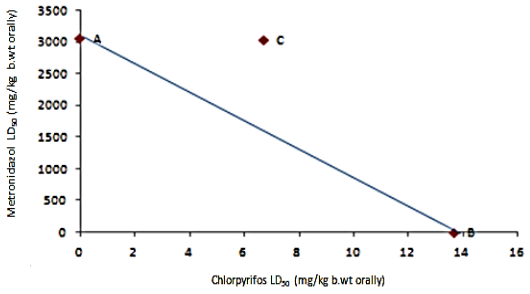

Figure 2: Isobolographic representation of the interaction of metronidazol and chlorpyrifos at LD_{50} level in chicks A, B points represent the LD_{50} of metronidazol, chlorpyrifos respectively when given alone whereas C point represent isolethal combination of metronidazol and chlorpyrifos in rate of LD_{50} 1:0.5.

Discussion

Metronidazole is widely used in veterinary and human medicine, used against protozoa, gram positive and gram negative anaerobic bacterial infection, metronidazole has the ability to cross the blood brain barrier resulting in neurotoxicity effects (16). The more characteristic signs associated with use high dose in animal represented by ataxia, tremor and weakness (17). The acute oral and intraperitoneal toxicity for metronidazole that determined in chicks at present research corresponding well with previous study (18) and the clinical signs represented by tease feather, close eyes, increase defecation, ataxia recumbent and death metronidazole classify as moderately toxic according to this result. The acute oral LD_{50} of chlorpyrifos in chick was 13.705 mg/kg B.Wt, in the previous study the LD_{50} of chlorpyrifos in chicks was 18.14 mg/kg B.Wt and some reported LD_{50} ranged 25-35 mg/kg B.Wt (19) we can attributed this different to variation of chlorpyrifos origin, as well as the solvents, the intermediate vehicles, and the

concentration of the active ingredient in the formula as well as laboratory condition, and animal breed variation (20). It is worthy of mention that chlorpyrifos consider as highly toxic in birds and poultry, the toxic signs represented by cholinergic toxicity syndrome like salivation, defecation, muscle spasm, dispense and death due to a penea.

When given with metronidazole i.p and chlorpyrifos p.o at the same time and in different ratio 1:1, 1:0.5, 0.5:1 in spite of using of double toxic doses that must cause increase the severity of the toxic signs, as well as increased in the death percentage, the results of these ratios increased LD_{50s} for metronidazole and chlorpyrifos that means decrease in toxicity for both. Isobolographic analysis of these LD_{50s} for metronidazole and chlorpyrifos revealed that combined treatment interpretation of antagonist effects these antagonist effect was established by the location of the points represent the combined LD_{50s} of metronidazole and chlorpyrifos above the diagonal lines that connect their LD_{50s} when given alone. Further, the calculated interaction index when treated metronidazole i.p and chlorpyrifos p. o. in ratio 1:1, 1:0, 5, 0, 5:1 were 2.136, 2.107, 1.49 respectively. drug interaction are caused by either pharmacodynamic or pharmacokinetic interaction (21), we can contributed pharmacodynamic interaction to decreased connect of chlorpyrifos with AChE due to connected metronidazole with AChE In competitive and reversible shape as same as mechanism of a reversible cholinergic medication like physostigmin, spontaneous recovery from metronidazol neurotoxicity may confirm this theory As it will explain devolution of metronidazole toxicity compare with chlorpyrifos (22), pharmacokinetic interaction may cause by inhibit effect of metronidazole liver enzyme caused diminished transformation of chlorpyrifos to active form to became less toxic (23,24), Moreover, metronidazole basic drug distributed better than acidic chlorpyrifos which make its effect greater than chlorpyrifos which may be undergo from ion trapping in plasma (25). to more confirmation type of interaction we given metronidazole and chlorpyrifos orally in ratio 1:0.5 from LD_{50} respectively the results of interaction were 3036.8, 6.733 mg/kg B.Wt in spite of decrease LD_{50} for both metronidazole and chlorpyrifos the isobolographic analysis still reveal to antagonism and the interaction index confirm above results which it was 1.482. we can contribute decrease LD_{50} to rapid oral absorption of chlorpyrifos by GIT compare with metronidazole (3,8,25) subsequently apply their effect rapidly.

Conclusion

In conclusion, the toxicity of metronidazol and chlorpyrifos in chicks are decreased when using at the same time in different ratios and route of administration. Further studies must be done to evaluate real clinical significance of these drug-drug interactions.

Acknowledgement

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Conflict of interest

Researchers declare no conflict of interests of the manuscript.

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السمية الحادة للميترونايدازول وتداخله مع الكلوربايرفوس في أفراخ الدجاج

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الخلاصة

الميترونيدازول هو دواء مضاد للميكروبات للاستخدام البشري والحيواني، التأثير الجانبي الأكثر تميزًا المرتبط باستخدام جرعة عالية منه هو علامات سمية عصبية، بعض هذه العلامات التي تم تسجيلها في الحيوان ممثلة بترنح ورجفة، إن المعلومات الدوائية المتاحة للميترونيدازول في الطيور محدودة. الهدف من دراستنا تفسير التأثير العصبي في أفراخ الدجاج من خلال تداخله مع أحد مبيدات الحشرات الفسفورية العضوية الكلوربايرفوس التي لها تأثير مهيج معروف على الجهاز العصبي. تم تحديد الجرعات المميتة الوسطية (الجم.ه) من ميترونيدازول و الكلوربايرفوس اعتمادًا على طريقة الصعود والنزول. كانت الجم.ه عن طريق الحقن في الخلب و التجريع في الفم للميترونيدازول ٥١٦,٩ ملغم / كغم، ٣٠٦١,٨ ملغم / كغم على التوالي، وللكلوربايرفوس ١٣,٧٠٥ ملغم / كغم عن طريق التجريع في الفم، أدى إعطاء الميترونيدازول عن طريق الحقن في الخلب مع الإعطاء الفموي للكلوربايرفوس. بالنسبة ١: ١، ١: ٠,٥، و ٠,٥: ١ من الجم.ه، على التوالي. في نفس الوقت إلى زيادة الجم.ه للميترونيدازول و الكلوربايرفوس وقد اظهر التحليل البياني وقوع نقاط التداخل أعلى الخط القطري الواصل بين. الجم.ه لكل منهما و عند إعطاء الميترونيدازول عن طريق الفم. والكلوربايرفوس بنسبة ١: ٠,٥ الجم.ه في نفس الوقت انخفضت الجم.ه لكل من الميترونيدازول والكلوربيريفوس وكانت نقاط التداخل أعلى الخط القطري الواصل بين. الجم.ه لكل منهما أيضا. استنتجت دراستنا أن تحليل البياني لتداخل الميترونيدازول الكلوربايرفوس بكل النسب وطرق الإعطاء المختلفة تكشف عن التأثير المضاد على الرغم من التشابه في العلامات السامة لكليهما.