An evaluation of neurotoxic effect of metronidazole in rabbits

*Wisam Hussein Al shibani    **Khalil Gazar Ghelab     **Hala Abbas Naji

*Pharmacology Department, Veterinary Medicine College Al Qadisiya University
**Pathology Department, Veterinary Medicine College Al Qadisiya University

Abstract:
The present study was undertaken to assess the neurotoxic effect of metronidazole in rabbits. Eighteen rabbits were randomly divided into three equal groups, first group was injected with metronidazole at the therapeutic dose 20 mg/kg.B.W., second group was injected with metronidazole at double therapeutic dose whereas the last group serve as a control. Each animal in all groups was administered intraperitoneally twice daily for (20) days.

The evaluations markers have used in this study were monitoring of clinical symptoms and determination of histopathological changes in brain and sciatic nerve. The careful observation of clinical signs, reflexes (patellar, cross extensor, and papillary light) and certain responses (pupil size, nystagmus and a menace response) in first and second group showed a remarkable nervous system dysfunction has improved by histopathological examination of brain and sciatic nerve. There were severe vacuolation in brain, where the lesion was characterized by spongiform changes, and degeneration of the nerve bundles in sciatic nerve were detected in rabbits treated with metronidazole at therapeutic dose, where as there is degeneration and selective loss of parkinjii cells with severe congestion in brain as well as demyelination and degeneration in sciatic nerve in rabbits treated with metronidazole at double therapeutic dose. This study revealed that metronidazole has a neurotoxic effect in rabbits (both centrally and peripherally) with a severity depended on its dose and duration of administration.
Introduction:

Metronidazole is a unique antimicrobial agent that has very little effect on most aerobic Gram-positive and Gram-negative bacteria, but is highly effective against anaerobic bacteria (1). It has antiprotozoal properties where it used routinely in treatment of Giardiasis, Trichomoniasis, and Amebiasis (2).

Metronidazole is lipophilic and distributes widely where peritoneal fluid and milk concentrations approach plasma concentrations. It reaches therapeutic concentrations in bones, abscesses, and the CNS. It readily cross the placenta and enters the fetal circulation (1). It is rapidly taken up by obligate anaerobic microorganism and converted into active form by reduction of its nitro group then binding to DNA and prevent nuclic acid formation (2). Metronidazole is metabolized primarily in the liver, both unchanged drug and metabolites are eliminated in urine and feces.

Metronidazole in rabbits has been cited as a treatment of choice for enterotoxaemia caused by Clostridium spiroforme, which is considered one of the most common and dangerous disease in rabbits (3), also it has been found to be effective in preventing abscess formation after experimental septic peritonitis in rabbits (4).

The adverse effects of metronidazole were documented in both human (5,6) and animals (7). In veterinary medicine, in both treated cases and experimental studies, the most commonly
reported side effects of oral administration of metronidazole include lethargy, anoxia, vomiting, and diarrhoea in dogs (8&9), in addition to salivation/ptyalism in cats (10&7). Another study using 14c-labeled metronidazole detected accumulation of the unchanged drug in the cerebellum and hippocampal areas of mice after intravenous administration (11).

The studies concerning the neurotoxicity of metronidazole in rabbits, a popular pet, are still rare where veterinary practitioners should be aware of susceptibility of rabbits and the form of metronidazole neurotoxicity in these species where they still questionable.

Therefore, the present study was carried out to investigate the side effect and neuronal histopathological alteration associated with metronidazole administration in rabbits.

Materials and Methods:
The experiment of this study was conducted in the animal house at veterinary medicine college of Al-Qadisiya university. Eighteen healthy adult of local breed rabbits of either sex with a mean of body weight 2.58±0.44 Kg. All rabbits were clinically examined before the beginning of the experiment, then they were randomly assigned into three groups (six animals per group). Rabbits of all groups were injected intraperitoneally twice daily for (20) days with equal volume as follow:

**Group (A):** injected with metronidazole (flagel) at recommended therapeutic dose in rabbits (20) mg/Kg.B.W. (12).

**Group (B):** injected with metronidazole at (40) mg/Kg.B.W.

**Group (C):** injected with normal saline and serve as control group.

- Parameters: the following parameters were assayed:
  1. Careful observation of general clinical signs showed by animals along the period of experiment.
  2. Assessment of some reflexes and responses that have beneficial role in determination of the severity and location of nerve lesion including:
     - Patellar reflex.
     - Cross extensor reflex.
     - Pupillary light reflex.
     - Eye pupil size.
     - A menace response.
  3. At the end of the experiment rabbits in all groups were then sacrificed, and the brain and sciatic nerve were taken in order to examine histopathological change.

Results:
Clinical observations of the animals along the duration of the experiment explained that metronidazole treated animals showed general signs reflected nervous dysfunction as weakness, lethargy, ataxic, tremor, and marked alteration in response to certain reflexes and external stimuli (table, 1).

The onset of these signs and the response to reflexes and external
stimuli were obviously increased proportional to the dose used and the duration of treatment with mitronidazole (table, 1).

Rabbits treatment with mitronidazole at dose of (20 mg/Kg B.W.I.P.) showed clear nervous signs compared with animal in control group, but it is less prominent as compared with rabbits received (40 mg/Kg B.W.I.P.) dose of metronidazole, where they showed more prominent clinical nervous signs especially in the second ten day period of the experiment as weakness of movement, knuckling, incomplete flexion, extension cause wobble, ataxia, falls down easily, difficulty in rising, nystagmus, abnormal pupil size and tetraparesis.

**Table (1): Reflexes and response to external stimuli in rabbits treated with metronidazole for (20) days.**

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Reflexes and Responses</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1-10) Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patellar reflex</td>
<td>Present *</td>
<td>Present *</td>
<td>Reduced in (2) treated animals</td>
</tr>
<tr>
<td></td>
<td>Cross - Extensor Reflex</td>
<td>Present *</td>
<td>Present *</td>
<td>Mild Reduction in (2) animals</td>
</tr>
<tr>
<td></td>
<td>Pupillary Light Reflex</td>
<td>Present *</td>
<td>Present *</td>
<td>Slow Response *</td>
</tr>
<tr>
<td></td>
<td>Pupil Size</td>
<td>Bilateral Normal size *</td>
<td>Bilateral Normal size *</td>
<td>2 Animals showed bilateral miotic pupil size</td>
</tr>
<tr>
<td></td>
<td>A menace Response</td>
<td>Present *</td>
<td>Present *</td>
<td>Present *</td>
</tr>
<tr>
<td></td>
<td>Nystagmus</td>
<td>Absent *</td>
<td>Absent *</td>
<td>Absent *</td>
</tr>
<tr>
<td>(10-20) Day</td>
<td>Patellar Reflex</td>
<td>Present *</td>
<td>Mild Reduction *</td>
<td>greatly reduced*</td>
</tr>
<tr>
<td></td>
<td>Cross - Extensor Reflex</td>
<td>Present *</td>
<td>Mild Reduction *</td>
<td>greatly reduced*</td>
</tr>
<tr>
<td></td>
<td>Pupillary Light Reflex</td>
<td>Present *</td>
<td>Slow *</td>
<td>slow and consensual response*</td>
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<td></td>
<td>Pupil Size</td>
<td>Bilateral Normal size *</td>
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<td></td>
<td>A menace Response</td>
<td>Present *</td>
<td>Reduced *</td>
<td>reduced *</td>
</tr>
<tr>
<td></td>
<td>Nystagmus</td>
<td>Absent *</td>
<td>Present in (2) animals</td>
<td>Present *</td>
</tr>
</tbody>
</table>
Group A: rabbits treated with metronidazole at dose of (20)mg/Kg.B.W.I.P. Twice daily.

Group B: rabbits treated with metronidazole at dose of (40)mg/Kg.B.W.I.P. Twice daily.

Group C: rabbits injected I.P. with normal saline twice daily.

* In all animals in the group.

Microscopic examination of brain sections from animals of both treated groups was much different from the histological section of control group (fig.1). In sections taken from brain of rabbits treated with metronidazole at dose of (20) mg/kg. B.W.I.P at multiple sites there were several fairly well demarcated areas of necrosis and increased cellularity, affecting different regions of brain within the necrotic foci, there was no apparent preservation of neurons, astrocytes or oligodendrocytes (fig.2&3).

Brain lesions also were characterized by spongiform changes due to cytoplasmic vacuolation within the neurons (fig.4&5).

Histopathological examination of brain specimens from animals treated with 40 mg/kg B.W. of metronidazole showed more prominent degeneration (swelling and vacuolation) compared with animals received metronidazole at dose of (20) mg/kg B.W. and also there was selective loss of purkinji cells with severe brain congestion (fig.6&7).

The histopathological changes in sciatic nerves sections from rabbits treated with metronidazole at therapeutic dose for (20) days were swollen axons with degeneration in bundles of nerve fibers (fig.9&10), where as the nerves in sciatic nerves of animals received double therapeutic dose for (20) days undergo demyelination and severe vacuolation and degeneration of axons (fig. 11&12). Compared with microscopic alterations in sciatic nerve sections from both treated groups there were no evidence of detected histopathological alteration in sciatic nerve sections from rabbits in control group (fig.8).
Figure (1): Brain. Control group. There is normal brain tissue and normal neurons (double arrows) and presence of normal pyramidal cells (arrows). 50X H&E.

Figure (2): Brain. There were several fairly well demarcated areas of necrosis (arrows). 50X H & E.

Figure (3): Brain. There were several fairly well demarcated areas of necrosis (arrow) and increased cellularity, affecting different regions of brain (double arrows). 50X H & E.

Figure (4): Brain. There are sever vacuolation in the brain, the lesion was characterized by spongiform changes due to cytoplasmic vacuolation within the neurons (arrows). 50X H & E.
Figure (5): Brain. There are severe vacuolation in the brain, the lesion was characterized by spongiform changes due to cytoplasmic vacuolation within the neurons (arrows). 200X H & E.

Figure (6): Brain. There is degeneration (swelling and vacuolation) and selective loss of purkinji cells with severe brain congestion (arrow). 50X H & E.

Figure (7): Brain. There is degeneration (swelling and vacuolation) and selective loss of purkinji cells with severe brain congestion (arrow). 200X H & E.

Figure (8): Sciatic nerves. Control group. There are normal axons with normal bundles of nerve fibers (arrow). 50X H & E.
Figure (9): Sciatic nerves. There are swollen axons (arrows) with degeneration in the bundles of nerve fibers. 200X H & E.

Figure (10): Sciatic nerves. There are swollen axons (arrows) with degeneration in the bundles of nerve fibers. 50X H & E.

Figure (11): Sciatic nerves: there is demyeliation with vacuolation and severe degeneration of axons. 50X H & E.

Figure (12): Sciatic nerves: there is demyeliation with vacuolation and severe degeneration of axons. 200X H & E.
**Discussion:**

The obvious reduction in sensation and motor activity in metronidazole treated rabbit was manifested by weakness in limbs movement, decrease in peripheral reflexes (patellar reflex and cross-extensor reflex) could be attributed to the degenerative lesion in peripheral nerves (particularly axonopathies and /or peripheral myelinopathies) as it showed by the present study .Furthermore, as the axons degenerate and /or the disruption of myelin is diffusely occurred generate a global neurological deficit, when they are limited to peripheral nerves produces the symptoms of peripheral neurpathy (13&14), in which impairment in sensation and motor strength are first impaired in the most distal extent of the axonal process particularly in the fore and hind limbs (13).The axanopathy recorded in this study come in agreement with that referred by (15) where metronidazole causes sensory and peripheral neuropathy.

The results of the present study showed that treated animals suffered from weakness of movement ,ataxia ,falling down easily ,difficulty in rising (tetraparesis) ,nystagmus and abnormal of the pupil size and reflexes raised the possibility of a central function lesion as it was confirmed by histopathological examination in this study .It was documented by (16) that nystagmus that is usually occurred with impaired consciousness, abnormal pupils and opithotonus originated from cerebralar –potine area to midbrain area injury and increased intraocular pressure.

The result of CNS dysfunction that recorded in this study was consistent with previous studies in dog (8& 9), cat (7) and human (5& 6).

The mechanism of toxic effects of metronidazole has not been identified (17) .The neurotoxic effect of metronidazole may be due to one or more of the following speculations:

The first proposed that the mechanism of toxicity is well linked to the fact that metronidazole and it's reduced metabolites ,bear close structural resemblance to the antineuritic nutrient ,thiamine ,where metronidazole toxicity may be due ,wholly or in part ,to its conversion to thiamine analogue and consequent vitamin B1 antagonism (18) Consistent with this hypthesis , the drug is accepted as substrate for the thiaminase (18) so conversion of metronidazole to an analog of thiamin may mimic nutrition deficiency neuropathy (18&19) .

Vitamin B1 is an essential co enzyme in the mitochondrial metabolism of α-ketoglutarate and pyruvate ,where they are part of biochemical pathways that resulted in generation of ATP, a major form of energy for the cell(20).Also pyruvate dehydrogenase is involved in the production of acetylcholine, and for myelin synthesis (21) ,and
this may explain the demyelination that observed in histopathological sections in this study. In consistency with this speculation we will start a study focusing on using of vitamin B1 to ameliorate the neurotoxicity of metronidazole.

Second speculation, the intermediate metabolites of metronidazole are thought to be capable of binding to and disrupting cellular DNA and induce cell death in anaerobic microorganism (22&23). In mammalian cell it has been proposed that metronidazole and/or its metabolites may bind to RNA instead of DNA (22), so the neuronal protein synthesis may be inhibited by metronidazole-mediated RNA binding, which causes axonal degeneration (24&25).

Another speculation, postulated that nitroradical anions and semiquinone generated during reactions between catecholamines and metronidazole contribute to metronidazole neurotoxicity (26).

Further speculation for metronidazole neurotoxicity based on metronidazole affinity for the GABA receptor site was based on the similarity of both the chemical structure and clinical signs of toxicity of metronidazole and benzodiazepine antagonist flumazenil, which also known to attach to the GABA receptor (27), where stimulation of these receptors results in CNS depression, somnolence, fatigue, lethargy, ataxia and muscular incoordination (28&29) which also may explain the clinical signs observed in this study.

A dose-dependent and duration-dependent relationship between central and peripheral neurotoxicity induced and metronidazole administered was demonstrated in rabbits.

Further studies are required to overcome or ameliorate the neurotoxic effect of metronidazole as our future study that raise a question about using of thiamine in alleviation of neurotoxicity induced by metronidazole.

References:


5. Alvarez, R.S., Richardson, D.A., Bent, D.A. and Ostergard. D. R.


