STUDY OF SOME PATHOLOGICAL CHANGES IN MICE GROUPS INDUCED BY *MYCOBACTERIUM TUBERCULOSIS* AND TREATED WITH ETHAMBUTOL

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(Received 26 June 2010, Accepted 5 October 2010)

**Keywords:** histopathological, mycobacterium tuberculosis, granulomatous

**ABSTRACT**

The main objective of this study is to demonstrate the histopathological changes and the efficacy of ethambutol of treatment mice infected with *mycobacterium tuberculosis*.

Thirty of white Swisrland mice is 6-8 weeks age, weighted 20-25gm were used they were randomly divided into 3 groups contain of 10 animals for each group.

- The 1st group (infected animal group) were inoculated with 0.1 ml of bacterial suspension contain \(1 \times 10^8\) cfu/ml intraperitonially.

- The 2nd group (group of infected-treated animal) were also infected as the fist group but after 30 day of infection were treated with 0.1 ml of ethambutol was given orally for 1 30 days.

- The 3rd group maintained as a control and were inoculated with 1 ml of sterial normal saline intraperitonealy.

At 60 day post infection all animals were sacrificed and samples from different organs (liver, lungs, kidncys, spleen, intestine) were isolated for that histopathological examination.

The result showed sever pathological lesion such as granulomatous lesions in lungs and livers of infected animals, with depletion of white pulp of spleen and ongestion with degenerative changes seen in kidneys and intestine with infiltration of inflammatory cells. While the infected-treated animals show mild or no pathological lesion in their internal organs.
INTRODUCTION

Tuberculosis is common and often deadly infectious disease caused by Mycobacterium, usually Mycobacterium Tuberculosis in humans(1).

A third of worlds population are thought to be infected with M. tuberculosis(2) and new infections occure at rate of about one per second(3).

The primary site of infection in the lungs is called the ghon focus, and in generally located in either the upper part of the lower lobe, or the lower part of the upper lobe, tuberculosis infection begins when the mycobacterium reach the pulmonary alveoli where they invade and replicate within the endosomes of alveolar macrophages(4), tuberculosis is classified as one of the granulmatous inflammatory conditions, macrophage, T cell, and B cell and fibroblast among the cells that aggregate to form agranuloma, with lymphocyte surrounding in infected macrophages, T lymphocyte was secrete cytokines such as interferon gamma, which activate macrophages to destroy the bacteria which they are infected(1) (4).

The infectious dose of tuberculosis is very low and inhaling less than 3 bacteria may cause an infection(5). Effective tuberculosis treatment is difficults due to unusual structure and chemical composition of mycobacterial cell wall which makes many antibiotics in effective and hinders the entry of drugs (6,7).

This microorganism have multidrug resistant. (8) There for the aim of this study is to demonstrate the effect of ethambutol on mice infected with Mycobacterium tuberculosis.
MATERIALS AND METHODS

Bacterial isolates: Mycobacterium tuberculosis were isolates obtained from tuberculosis institute in Baghdad, the biochemical test were done to these isolates to confirm their diagnosis and identification.\(^9\)

**Culture media:**

Lowenstein Jensen media which is a special media for Mycobacterium tuberculosis prepared according to the production manualls.

**Determination of challenge dose:**

Preparation of bacterial suspension of the counting was made by using Mcfarlands tubes according to.\(^{10}\)

**Drug used for treatment:**

Treatment commenced 4weeks after infection aperiod which corresponds to the peak in primary lesion, using ethambutol which prepared in 50% sucrose and estimated according to.\(^{11}\)

**Experimental design:**

Thirty of white swissland mice, 6-8 weeks age were randomly divided equally into 3 groups and each group co 10 animals treated as the following:

1- The 1\(^{st}\) group (group of infection) were inoculated intra peritonealy with 0.1 ml of bacterial suspension contain 1x10\(^8\) CFU of Mycobacterium tuberculosis

2- The 2\(^{nd}\) group (group of treatment): Were treated as the 1\(^{st}\) group but were given 0.1 ml (5mg/kg body weight ethambutol orally daily after 30 day post infection.

3- The 3\(^{rd}\) group: were maintained as acontrol and were inoculated with 1ml of sterial normal saline intraperitonily all animals of all groups were sacrificed after 60 days post infection.

Post mortem examination done to all groups recording any gross lesion and pieces (1x1x1cm) from internal organs (liver, lungs, kidneys, spleen, intestine) were isolated fixed in 10% normal buffered formaline for 72hrs, then used the routine procedure for histopathogical section preparation according to.\(^{12}\)
RESULTS

- Pathological changes:

Conjestion of the most examined organs the main gross lesion in both infected and treated groups with multiple focal granulomatous lesion in the liver and lungs of infected non treated group.

Histopathological examination:

Infected-non treated group:

- Liver: multiple focal granulomatous lesion in liver parenchyma especially around of central vein consist mononuclear cell aggregation and vacular degeneration of hepatocyte (figure. 1) also showed multifocal coagulative necrosis with infiltration of inflammatory cells.

- Lung: large granulmatous lesion consist of macrophages aggregation in lung parenchyma (figure 2).

- Spleen showed depletion of white pulp with conjestion of red pulp(figure 3)

Kidney: area of conjestion of blood vessel with infiltration of inflammatory cells in their lumen (figure4) and showed together with acute cellular degeneration charactrized by vaculation of cytoplasm of epithelial cells lining renal tubule.

Intestine: heavy infiltriation of inflammatory cells (usually lymphocyte) between mucosal glands with sever conjestion of blood vessels.

2- Infected-Treated animals:

- Liver: histopathogical section showed conjestion of central vein with mononuclear cell in their lumen (figure 5) also proliferation of kupffer cell (figure 6)

- Lung: The lung showed no pathological lesion except conjestion of blood vessel with few inflammatory cell in their lumen (figure 7) and inter alveolar septa (figure8)

- Spleen: clear histopathological lesion was showed.

- Kidney there is moderate cell degeneration of epithelial cells lining renal tubule with neutrophil infiltration (figure 9)

- Intestine microscopic section revealed few inflammatory cells between mucosal glands (figure10).
The table showed the mean and standard error of both infected and treated animals, and the efficacy of ethambutol in treatment.

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<th>Second group (treated animal)</th>
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<tr>
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<td>1(+)</td>
</tr>
<tr>
<td>2.</td>
<td>3(+++)</td>
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<tr>
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<td>3(+++)</td>
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<tr>
<td>8.</td>
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<td>10.</td>
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<table>
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<th>N</th>
<th>Std. Deviation</th>
<th>Std. error Mean</th>
<th>t-test</th>
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<td>0.13333</td>
<td>15.057</td>
<td>Hs</td>
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<tr>
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<td>10</td>
<td>0.52705</td>
<td>0.16667</td>
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</table>

Hs= 0.05

+++ = 3 sever lesion
++  = 2 medium
+   = 1 less effect lesion (simple lesion)
-   = 0 no lesion
Fig. (1): Histological section in liver of infected non-treated group showed multiple focal granulamatous lesion in liver parenchyma especially around central vein consist of aggregation of mononuclear cells (H & E 10X).

Fig. (2): Histological section in lung of infected non-treated group showed large granulomatous lesion consist of aggregation of macrophages in lung parenchyma. (H & E 40X)
Fig. (3): Histological section in spleen of infected-non treated group showed depletion of white pulp with congestion of red pulp. (H & E 40X)

Fig.(4): Histological section in kidney of infected-non treated group showed congestion of blood vessel with infiltration of inflammatory cell in their lumen. (H & E 10X)
Fig. (5): Histological section in liver of treatment group with thambutol showed congestion of central vein ( ) with mononuclear cell in their lumen. ( ) (H & E 40X)

Fig.(6): Histological section in liver of treatment group with ethambutol show proliferation of kupffer cell. ( ) (H & E 40X)
Fig.(7): Histological section of lung of treatment group revealed but conjestion of blood vessel with few inflammatory cell in their lumen. (            ) (H & E 40X)

Fig.(8): Histological section of lung of treatment group showed aggregation of mononuclear cell in the interalveolar septa. (            ) (H & E 40X)
Fig. (9): Histological section of kidney of treatment group revealed cellular degeneration of epithelial cell lining renal tubule (a) and neutrophil infiltration in the interstitial tissue (b) (H & E 40X)

Fig. (10): Histological section of intestine of treatment group revealed few inflammatory cell between mucosal glands (H & E 10X)
DISCUSSION

In this study we have shown that intra peritoneal infection does result in chronic TB infection in mice organs similar to that observed during low dose aerosol infection mentioned by.(13)
The present study revealed that the main histopathological in the examined lungs and livers of inflected animals were granuloma and this observation agreed with.(14)
The absence of necrosis and langhans type cells makes the tuberculosis granulomas of mice histologically different from those arising in guinea pigs, rabbits and humans and this result supported the investigation that mentioned by (15), these differential features have been related to the stronger immunological response elicited in mice, which consequently tend to sustain lesser degrees of systemic dissemination of mycobacterial infection than guinea pigs or rabbits.(16).(17)
The Mycobacterion tuberculosis spread through the blood stream to other tissues and organs and so all parts of body can be affected by the disease. ( (18) and this agree with our result, that the Micro Organsim reach to different organs (lungs, liver, kidneys, intestine and spleen).

Virulence is the measure of pathogenicity of amicroorgansim as determined by it, ability to invade host tissue and produce disease, and after thirty days of infection bacilli had disseminated resulting in microscopically visible lesions in liver, spleen, lungs, and these result supported by the observation.(19)

Tuberculosis is classified as one of the granulomatons inflammatory conditions, macrophage, T cell, B cell and fibroblasts are among the cells that aggregate to form granuloma with lymphocytes surrounding the inflected macrophages, T lymphocyte secrete cytokines such as interferon gamma which activate macrophages to destroy bacteria.(20)
The treatment with ethambutol resulted in significant reduction in pathological lesion in different organs and our result is agreed with (21) Who use aguinea pig as amodel of infection.

Ethambutol inhibits arabinosyl transferase-an enzyme that is important for the synthesis of the mycobacterial arabinogalactan cell wall.(22)
دراسة بعض التغيرات المرضية لمجاميع الفئران المصابة بجرثومة السل البشري والمعالجة

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الخلاصة

لعرض دراسة التغيرات المرضية النسجية وتعرف تأثير عقار الإيثاميبيتول على إعصاب الفئران المصابة بجرثومة السل البشري، mycobacterium tuberculosis استخدم 30 من الفئران السويسرية البيضاء ومن كلا الجنسين والتي تتراوح اعمارها بين 6-8 اسبوع وبازان 20-25 غم، قسمت هذه الحيوانات عشوائيا الى ثلاث مجاميع ضمت كل منها 10 حيوانات.

المجموعة الأولى: (مجموعة الإصابة) تم إصابتها بجرثومة السل البشري بطريقة الحقن داخل الخلب بجرعة 0.1مل من العالع الجرثومي الحاوي على 10^8 CFU من الجرثوم.

المجموعة الثانية: وهي مجموعة (الإصابة والعلاج) تم إصابتها كما في المجموعة الأولى وبعد مرور 30 يوم على الإصابة عولجت بعقار الإيثاميبيتول بجرعة 5 ملغ/كم برمياً عن طريق التجريج بالفم.

المجموعة الثالثة: عدت بوصفها مجموعة سيطرة للمجاميع المصابة حيث حققت 1 مل من محلول الفسلي المعقم داخل الخلب.

وبعد مرور 60 يوماً من الإصابة تم قتل حيوانات المجموعات الثلاث واخذ عينات من الأعضاء الداخلية (الكبد، الريتين، الكلى، الطحال، الامعاء) لعرض دراسة التغيرات المرضية.

أظهرت النتائج حدوث افتس مرضية شديدة تميزت بالانفاذ الحبيبي المنتشر في إكبار وراث اليوانات المصابة إضافة للاحتقان والتغيرات للتنكسية وارتياح الخلايا الالتهابية في الطحال والكلى والامعاء.

أما مجموعة الإصابة والعلاج فقد أظهرت تغيرات وافقت مرضية تراوحت بين الشدة الاقل والشفاء التام.

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