Pathogenesis of Mycobacterium Tuberculosis in Rabbits (Experimental Model for Human Disease)
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ABSTRACT
Experimental model using adult rabbits to investigate pathogenesis and ill effect of M.tuberculosis was worked up. Three groups of rabbits used each group eight in number. Animals in groups one and two were injected intraperitoneally with 1mg/ml/animal of the bacterium containing 2x10 CFU/ml. The third groups were left as control. Pathological changes were seen after sixty days of infection in the visceral organs in both groups infected. Immediately following the appearance of lesions, animals of groups one treated with Isoniazide and Rifampicine for two months. Sacrification of rabbits after 120,130 & 140 days post infection of both groups envisages no typical lesions of tuberculosis in group one in contrast to the formation of clear and evident typical tubercles in group two. Histopathology revealed well established allergic inflammation and histiocytic granulomas of atypical tuberculosis infection predominantly in some internal organs (lung, liver, spleen & lymph nodes) in group one which is treated with TB-regimen while typical TB-granulomas were markedly established in various internal organs of group two infected with M.tuberculosis only. The non typical tuberculomas and inflammatory changes with tissue reaction in group one treated rabbits indicate a paradoxical lesion which gives such a non familiar tuberculous lesion behavior. Rabbits as an experimental animal model proved to be an attractive model in study tuberculosis especially in the aspect of pathogenesis or treatment response because rabbits have disease manifestations and pathology similar to those noted in humans. Establishment of a rabbit model to study M.tuberculosis infection and treatment with anti-tuberculosis drugs can allow an important opportunity to investigate the mechanisms underlying susceptibility to tuberculosis of a currently increasing prevalence.

Keywords: experimental model, rabbit’s tuberculosis, anti TB-drugs, M.tuberculosis

1. Introduction

In most industrialized nations, tuberculosis (TB) declined throughout the 20th century (Davies, 2003; anonymous, 2008) but it has recently reemerged as an important infectious disease (Waaler, 2002). The increased prevalence is apparently due to; (i) a relaxation of public health tuberculosis control efforts (ii) human immunodeficiency virus infection (AIDS), which
greatly increases susceptibility to tuberculosis (Davies, 2006; anonymous, 2005) (iii) increased poverty and homelessness in parts of the industrialized world; (iv) the emergence of multi-drug-resistant cases (Wales, 2008). In developing countries, tuberculosis has always been a common disease, and the above factors, as well as civil war and famine, have increased its prevalence (WHO, 2006).

Tuberculosis is an infectious granulomatous disease caused by acid fast bacilli of the genus mycobacterium. The disease is a major risk problem for the human beings and domestic animals. Zoonotic behaviors by transport and infection promote ingestion and inhalation (Radostits et al., 2008).

Three types of tubercle bacilli are recognized, human tuberculosis (M. tuberculosis), Bovine tuberculosis (M. bovis) and avian tuberculosis (M. avium). The three types differ in cultural characteristics and pathogenicity. All three types may produce infection in host species other than their own (Hirsh et al., 2009). *Mycobacterium tuberculosis* is most specific. In man it has a worldwide distribution, occurs in every country in the world and it has been accounted for eight million cases of clinical disease with three million deaths annually on zoonotic tuberculosis with the participation of other immunosuppressive diseases (FAO, 1993). Tuberculosis is a well-known disease and at the same time a forgettable disease. In developed countries, because of high living standard and introduction of strong anti-tuberculosis therapy under efficient tuberculosis control programmed schemes (Nijland et al., 2006), the incidence and mortality of tuberculosis has steadily decreased. Some countries are about to enter the stage of elimination of Tuberculosis (WHO, 2007). In Yemen as in any developing countries, tuberculosis is still one of the major public health. The diagnosis of tuberculosis in laboratory animals is difficult on the ground of clinical examination. That was the reason to investigate the pathologic effect on treated and non treated tuberculosis infected rabbits by postmortem and/ or histopathology. Then the observations on disease followed up and its segueale after following a treatment regimen. The aim of this work is to compare the pathological alteration in infected rabbits by the bacterium M. tuberculosis with the group of rabbits infected by the same bacterium and treated with same TB-regimen. The designed experimental model used to be marked as , animal model of human disease.

2. Methodology

The bacterium (Mycobacterium tuberculosis): Two strains of Mycobacterium tuberculosis was provided by tuberculosis center in Sana’a of a code C T 2605 and C T 2508. The two strains isolated from patients with tuberculosis infection. Classified and identified according to Grange et al. (1996), both strains were sub cultured on modified Ogawa semisolid media. Subjected to Niacin test to confirm the typical type of any of the strains, the strains used in the experiment are the typical one.

Culture media and infective inocula; 2% modified Ogawa media with glycerin were used for reisolation of the infecting mycobacterium organisms from tissue of sacrificed rabbits. The
concentration of infective inocula was 1mg/ml of the typical mycobacterium strain suspended in a sterile saline solution.

Bacteriological Reisolation: Reisolation of infecting mycobacterium for the purpose of localization of bacterium in situ were attempted from the control, infected and treated animals especially from lesions identified and described during postmortem examination according to Grang et al.(1996).

Laboratory animals used; New Zealand White breed Rabbits of age between 6-8 months were used in the experimental work. Animals were kept in experimental animal cages and feed green and particulate diets.

Experimental design; Three groups of rabbits were divided into eight animals each (Table.1). The first and second groups were exposed to the bacterium Mycobacterium tuberculosis by intraperitoneal injection. The third group left as a control. The first group treated for 60 days after experimental infection by a TB-regimen for 60 days. Isoniazid and Rifampicin, referring to table.2 (Katsung, 2001; William, 2002; Lund, 1994) used by oral route. The second group is left untreated. All animals in both groups were sacrificed on days 60, 70 and 80 post treatment.

<table>
<thead>
<tr>
<th>Methods of infection and treatment.</th>
<th>Group one</th>
<th>Group two</th>
<th>Group three</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection with M. tuberculosis.</td>
<td>+</td>
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<td>-</td>
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<tr>
<td>Treatment with INH, 60 days Orally Post infection.</td>
<td>+</td>
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<td>Sacrificed animals on days 120, 130 and 140 post infection</td>
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</table>

+ Infected or treated or sacrificed,
- Not infected or not treated or not sacrificed,
INH Isonizid (bactericidal),
Rif Rifampicin (bactericidal).

The animals in group one after scarification examined for any pathogenic effect on tissue alterations after treatment, compared with typical lesions seen in group two. Tissue samples were taken from the organs affected in both groups to compare the pathological entities. Samples also were taken from the rabbits in the control group.

Table 2: Demonstrating the mechanism of action and activity of drugs in TB-regimen used in the experimental study as first line agents.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of Action</th>
<th>Activity Against M. tuberculosis</th>
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<tbody>
<tr>
<td>Rifampicin</td>
<td>Inhibits bacterial RNA synthesis by binding to the β subunit of bacterial DNA-dependent RNA-polymerase (DDRP) Inhibition of DDRP leads to inhibition susceptible organisms at concentrations of less</td>
<td>Inhibits susceptible organisms at concentrations of less</td>
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</tbody>
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blocking of the initiation chain formation in RNA synthesis. One of the most effective antituberculosis agents available and is bactericidal for intra- and extra-cellular bacteria.

| Isoniazid (INH) | Most active drug for the treatment of TB caused by susceptible strains. Is a pro-drug activated by katG, which exerts its lethal effect through inhibition of synthesis of mycolic acids, an essential component of mycobacterium cell walls, through formation of a covalent complex with an acyl carrier protein (AcpM) and KasA, a beta-ketoacyl carrier protein synthetase. | INH inhibits tubercle bacilli at a concentration of 0.2 μg/ mL. than 1 μg/ mL. |

Drug treatment of rabbits with Isoniazid and Rifampicin; Rabbits were given the drugs through oral medication. They were habituated. to receive 2 ml of raspberry-flavored syrup by 3 ml syringe daily for 3 weeks prior to drug treatment. Isoniazid (INH) (10 mg/ ml) and Rifampicin (Rif) (10 mg / ml) (Table.2) (WHO, 2003). The drug were prepared and offered orally in raspberry syrup. The mixture of drugs solution was prepared and used within 15 minutes of mixing with syrup. The dose were given 3 times weekly and located for 60 days as a whole treatment course period.

3. Results

Rabbits in group one and two have shown distinct clinical features related to pulmonary tuberculosis which appeared after (40) day’s post infection. Group one animals after treatment with Isoniazid and Rifampicin regimen that is started (60) days post infection revealed mild clinical manifestations in breath and behavior. Two animals developed perennial and abdominal enlargements which was soft in consistency and pelvic swelling. Group two rabbits (infected not treated) resist infection after development of severe clinical manifestations till the day of scarification. One animal died after (4) weeks post infection due to severe illness by tuberculosis infection. Both animals in group one and two lost weight about 25-30 g /body weight and look emaciated after 30 days post infection. The range of body weight before infection and start of experiment was 1200-1000g/ b/ w to all eight rabbits in each group. The range of body weight after (60) days from post infection was 1050-800 g/ body weight.

Reisolation of M.tuberculosis from specimens of organs taken after scarification of rabbits in both groups one and two on days 120, 130, 140 post infection clearly indicated the dissemination of the bacterium in treated and non treated groups of rabbits. An extensive dissemination of M.tuberculosis in group two was found in different organs at different intervals post infection (Table. 3). The dissemination of bacterium in group one was less pronounced especially in lymph glands in all intervals post infection. Staphylococcus and other bacteria were identified,
suspected Mycobacterium mucogenicum were isolated from the abscesses seen in the pelvic and perineal regions in rabbits treated with TB-regimen.

Table 3: Showing the dissemination of *M. tuberculosis* after infection of rabbits in various organs in treated and non treated rabbits with TB-regimen.

<table>
<thead>
<tr>
<th>Days</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Heart</th>
<th>Intestine</th>
<th>Kidney</th>
<th>L.N.M</th>
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<td>130</td>
<td>+</td>
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<td>+</td>
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<td>140</td>
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</table>

+ Positive isolation,  
- Negative,  
T Treated with TB-regimen,  
Nt Not treated with TB-regimen,  
Pi Post infection,  
ND Not done,  
LNM Lymph nodes or glands mesenteric,  
LNP Lymph nodes or glands pelvic,  
LNPe Lymph nodes or glands pectoral.

4. Discussion

Because of the complex pathogenesis of the disease, the development of animal models for specific stages of disease such as latency and cavitation remains a priority. The rabbit model offers certain advantages over both the murine and guinea pig models (Al-Sammariae, et al., 2002). When infected with *M. tuberculosis* or *M.bovis*, rabbits have a spectrum of disease that represents many of the specific stages of human disease. In general, rabbits are able to contain disease caused by virulent *M.tuberculosis*. Rabbit model using the aerosol route of infection to produce pulmonary TB (Converse et al., 1996), can be an excellent model for TB in human beings (Dannenberg and Tomashefski, 1997), in that both species of mycobacterium (bovis and tuberculosis) readily form liquefied caseous foci and cavities. A considerable variation in the extent of the disease occurs (anonymous, 2008).The rabbit model of tuberculosis (TB) is important because rabbits develop a disease that is similar to TB in humans, namely, granulomas with caseous necrosis, liquefaction, and cavities. In this study a comparison of infected only and infected-treated New Zealand white rabbits infected by peritoneal injection with *M.tuberculosis* which prove differences in the rate of progress of infection after treatment that seem to be unrelated to differences in the character of inflammatory response result in accord with (Converse et al., 1996).The physical characteristics of the local microenvironment in which M.
tuberculosis resides is an important goal that may allow the targeting of metabolic processes to shorten drug regimens. Currently Pimonidazole hydrochloride (Hypxyprobe) proves an imaging agent that is bioreductively activated only under hypoxic conditions in mammalian tissue. The good in this research work is the trial through an experimental model designed to serve solving a problem of the pathogenic effects in non human primates (rabbits) as a model for human disease through or by infection with mycobacterium bacilli, and apply a treatment by a known antibacterial TB-regimen. A major obstacle to the study of tuberculosis has been the lack of a rapid, reproducible model. According to Finegall and Martin (1982), they mentioned that a definitive proof of tuberculosis infection is provided, only by the demonstration of tubercle bacilli in clinical and postmortem specimens obtained from infected patients. Although bacteriological examination is considered the priority procedure in case-firdig programme (Salem, et al., 1986), the results of reisolation of the bacterium from the tissue organs after sacrfication in both groups (one and two) in this study, showed very logical explanation to the effect of the TB-regimen in its course of treatment especially if we admitted that the course of treatment was shorter than usual and would be more effective in using other route of treatment like paranteral administration, which might stopped the paradoxical complication reaction that the animals suffer in the end of the experiment. The dissemination of bacterium in group one was less pronounced especially in lymph glands in all intervals post infection, which could means that an effect of treatment regimen would have the reason of inhibiting the localization of the bacterium and reflected on the less pathogenic effect recognized in tissue when inspected grossly and examined microscopically (Houben et al., 2006). Certain staphylococcus and other bacterial colonies not identified were isolated from the abscesses seen in the pelvic and perineal regions in rabbits of group one treated with TB-regimen could explain the start of paradoxical reaction in the animals (Marshall, et al., 2008).

5. Conclusion.

The rabbit is a useful experimental animal in studies concerned with animal model for human disease in TB studies. Rabbits develop a disease that is similar to TB in humans. The pathological alterations like granulomas, caseous necrosis, liquefaction and cavitations are the main lesions in non treated rabbits. Rabbits in infected-treated group develop atypical lesions of tuberculosis and a condition similar to a paradoxical reaction. Dissemination of M. tuberculosis after infection by intraperitoneal route in certain organs of the experimental rabbits could be easily reisolated and hence considered as a predilection seats. Follow up studies are needed to study the relationship between the clinical outcome and the immunity status. Further studies are needed to evaluate the clinical usefulness of using TB-regimen drugs induced immunostimulation.
References


