The role of doxycycline as an mmp-9 inhibitor in tuberculosis spondylitis infection in the rabbit model

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Abstract

Background: Tuberculous spondylitis or spinal tuberculosis, which is also known as Pott's disease of the spine is a disease that occurs throughout the world. This disease affects the spine caused by the bacteria Mycobacterium tuberculosis (TB). MMP-9 is a proteolytic enzyme that is thought to play a role in the progressive degradation of extracellular matrix in physiological and pathophysiological processes involving remodeling of spinal tissue. This study wanted to examine the role of doxycycline as an MMP-9 inhibitor in a rabbit model of tuberculous spondylitis infection.
Methods: This type of research is an experimental study using a randomized controlled design method with a One Group Posttest Only design. We conducted this research from March – June 2020 at the Animal Hospital of the Bogor Agricultural University (IPB). The population of this study was adult New Zealand rabbits (*Oryctolagus cuniculus*) weighing 1.8-2 kg with 40 rabbits divided into 12 groups.
Results: In this study, there was a significant difference between the delta values between the inoculation duration groups, both in the treatment group without doxycycline, doxycycline 1 mg/kgBW, and doxycycline 5 mg/kgBW. Regarding the effect of doxycycline administration, only Group D (decrease) and Group H (increase) showed a significant effect of doxycycline administration on MMP-9 levels in experimental animals with TB spondylitis.
Conclusion: MMP-9 will still increase even though doxycycline intervention has been given considering that the occurrence of a chronic process in which inflammatory factors will continue to increase even though antibiotic therapy has been given to suppress the number of these inflammatory factors.

Keywords: *Tuberculous spondylitis, MMP-9 inhibitor, Doxycycline, Rabbit model*

1. Introduction

Tuberculous spondylitis or spinal tuberculosis which is also known as Pott's disease of the spine is a disease that occurs throughout the world. This disease affects the spine caused by the bacteria Mycobacterium tuberculosis (TB). Approximately 3 million deaths occur each year due to this disease. Indonesia is the third-largest contributor after India and China, with the discovery of 583,000 recent cases per year, 262,000 infectious TB cases, and 140,000 deaths per year. Incidence of extrapulmonary TB is around 4000 cases every year in America. The most commonly affected place is the spine, which is almost half of the incidence of extrapulmonary TB affecting the bones and joints. Extrapulmonary tuberculosis can occur in 25%-30% of children infected with TB. Bone and joint TB occurs in 5%-10% of infected children, and most often occurs within 1 year, but can also occur 2-3 years later (Paramarta, 2008).

In 2005, the World Health Organization (WHO) estimated that the largest number of new TB cases was in Southeast Asia (34% of TB incidence globally) including Indonesia. We expect the number of patients with tuberculous spondylitis to continue to increase along with the increasing number of patients with gained immunodeficiency syndrome (AIDS). One to five percent of people with TB develops osteoarticular TB. Half of osteoarticular TB is tuberculous spondylitis. In developing countries, we know young TB patients to be more susceptible to TB spondylitis than old age, while in developed countries, the age at which TB spondylitis appears is usually in the fifth to sixth decades. Osteoarticular TB is commonly found in HIV-positive patients,

immigrants from countries with a high prevalence of TB, the elderly, children under 15 years of age, and other immune-deficient conditions. In HIV-positive patients, we know the incidence of TB to be 500 times higher than in the HIV-negative population. On the other hand, approximately 25 – 50 percent of new TB cases in the United States are HIV positive (Paramarta, 2008., Leibert et al., 2017).

Matrix metalloproteinases are a large group of enzymes capable of degrading various components of the extracellular matrix (ECM). Under normal conditions, MMPs are only formed at the place and time of tissue remodeling, such as during embryonic development, the wound healing process, the transition from cartilage to true bone in the process of ossification, and the process of trophoblast invasion of the endometrium. However, MMP can also be produced in several pathological conditions such as heart failure, periodontitis, tumor cell invasion, angiogenesis, and chronic inflammatory conditions. One of the MMPs that play a role in the pathogenesis of tuberculous spondylitis is MMP-9. MMP-9 is a proteolytic enzyme that is thought to play a role in the progressive degradation of extracellular matrix in physiological and pathophysiological processes involving remodeling of spinal tissue (Camillo, et al., 2008).

Another study stated that there was an increase in MMP-9 levels in pulmonary tuberculosis infection; however, until now there is no literature that specifically discusses MMP-9 expression in tuberculous spondylitis in Indonesia, so researchers are interested in knowing the level of MMP-9 expression in rabbits exposed to tuberculous spondylitis (Hrabec, et al., 2002). Doxycycline, a Food and Drug Administration (FDA) approved tetracycline, was used in an in vivo tuberculosis model for temporal control of mycobacterial gene expression. Many tetracyclines, including DOX, have anti-inflammatory properties mediated by the suppression of tumor necrosis factor (TNF-α) and matrix metalloprotease (MMP). In vitro, DOX inhibits MMP secretion induced by TB infection at 5 mg/liter and higher (Gengenbacher, et al., 2020). Based on the above background, this study wanted to see the role of doxycycline as an MMP-9 inhibitor in the rabbit model of tuberculous spondylitis infection.

2. Methods

All This type of research is experimental research, which is studying a phenomenon in causal correlation, by giving a treatment to the research subject, then seeing and studying the effects of the treatment. This study used a randomized controlled design method with a One Group Posttest Only design. We conducted this research from March – June 2020 at the Animal Hospital of the Bogor Agricultural University (IPB). The population of this study was adult New Zealand rabbits (Oryctolagus cuniculus) weighing 1.8-2 kg. We obtained this rabbit from the Bogor Agricultural University (IPB) Animal Hospital. From the research population, we will choose samples randomly based on the calculation of the estimated sample size and obtained a minimum sample size of 36 research samples with 3 rabbits divided into 12 groups. To expect dropout and failure cases, 4 additional rabbits were prepared with 40 rabbits, because we fear the morbidity and mortality of the group that will be subjected to the Mycobacterium tuberculosis bacteria inoculation procedure to be quite high.

After obtaining approval from the ethics committee to conduct the study, we collected rabbits for inoculation with Mycobacterium tuberculosis bacteria. We divided rabbits into 12 groups where each group consisted of 3 rabbits with different research periods. Groups 1-3 are rabbits with a study period of 2 weeks, groups 4-6 with a study period of 4 weeks, group 3 with a study period of 7-9 weeks, and groups 10-12 with a study period of 8 weeks. Each rabbit to be inoculated was weighed using a scale and the scales were recorded. We carried out the inoculation procedure in all groups. Before the inoculation procedure, clinical and radiological examinations were carried out on rabbits. Under general anesthesia using ketamine anesthetic at a dose of 44 mg/kg, the backs of the rabbits at the T13-L1 level were shaved and aseptic and antiseptic we performed procedures using 70% alcohol and betadine and then put on a sterile cloth. The rabbit was prepared in a side-facing position with the left-back facing the surgeon, the 12th thoracic was identified by palpating the 12th rib and then traced to the

transverse process. A transverse incision was made to reach the spine at the T12 level, starting from the spinous process 3-5 cm wide to the left lateral through the cutis and subcutis. We separated the paraspinal muscles by the 12th rib, transverse process, and 12th thoracic plate.

Then a drill is used to make a hole at the midpoint of the 12 thoracic body (+ 5 mm from the transverse process) 6 to 10 mm deep using a 1.5 mm drill bit. 0.2 mL of Mycobacterium tuberculosis bacterial suspension was inoculated with a quantity of 1 x 108 CFU/mL aseptically into the hole made in the body, then exposed for 5 minutes to open air and then closed by sewing the fascia, muscle, and subcutis. The lesion material obtained from the debridement procedure was divided into 2 parts: we sent 1 pot for smear examination and culture on the same day to the laboratory.

The surgical wound was then closed layer by layer and the skin was sutured one by one using 3.0 vycril thread and covered with a bandage. AFS examination was carried out by taking 1-2 oses of material that had been ground with physiological NaCl/liquid material samples then made a thin layer on a slide, briefly fixed on a Bunsen fire, dripped with 0.3% carbol fuxin solution (BD, TB Carbol Fuchsin ZN) onto the thin layer, then heated on a hotplate. For 5 minutes until smoke comes out but not boils or dry. The preparation is then allowed to cool for 5-7 minutes, then the excess dye is removed and washed with running water. 3% acid-alcohol solution was poured (BD, TB Decolorized) for 2-4 minutes, then washed with running water for 1-3 minutes. 0.1% methylene blue solution (BD, TB Methylene Blue) was dripped to cover the entire surface of the preparation, left for 1 minute and then washed with running water, then dried with blotting paper for further observation under a microscope with 1000 x magnification using immersion oil. Mycobacterium tuberculosis bacteria will appear as smooth red rods and slightly curved, singly, in pairs or groups on a bluish background. The reading starts from the left edge to the right or vice versa up to 100 visual fields, according to the IUATLD (International Union Against Tuberculosis and Lung Diseases) assessment.

Culture examination was carried out by grinding the tissue samples until smooth using a mortar, then put into a centrifugation tube to a limit of 2 mL and mixed with sterile distilled water in a ratio of 1: 1 or up to a limit of 4 mL. The mixture was vortexed until homogeneous and allowed to stand for 10 minutes, placed in a rotor adapter, and weighed to achieve balance. The mixture was centrifuged at 3000 g for 15 minutes then the supernatant was removed to a limit of 2. The acidity of the solution was adjusted to pH 6-7 with the addition of NaOH or HCl solution, then the solution was allowed to stand for 10 minutes. The sediments were ready to be re-prepared for Ziehl Nielsen staining and inoculated into Lowenstein-Jensen (LJ) bottles for 100 L culture, duplicate. Then 100 L of sediment was pipetted into LJ medium and made in duplicate. The bottle is closed but not too tightly, then the bottle is moved so that it is evenly distributed and placed on a shelf with a slope of 30ºC for 24 hours in an incubator at a temperature of 35-37ºC. After 24 hours, the culture bottle caps were tightened and the bottles were placed on a tube rack in an upright position for 6 weeks of incubation. After 6 weeks, the culture results were readout.

We then returned the rabbits to the cage to be incubated for 2, 4, 6, 8 weeks according to the group and given ketoprofen at a dose of 3 mg/kg intramuscularly every 12 hours for 3 days. The rabbits were given food and drinks in the form of pellets and rabbit feed and clinical examinations were carried out, namely observing daily activities, signs of infection (appetite, paralysis, sinus) and measuring temperature and body weight every three days, as well as the control rabbits. Doxycycline 1 mg/kg BW/day, 5 mg/kg BW/day, and we administered the control group without doxycycline for 4 weeks based on the group for the 2nd, 4th, 6th, and 8th week of inoculation.

We adjusted posttest blood sampling according to the study period from each group. Blood was drawn from the lateral vein and put into an EDTA tube which was then sent to the laboratory. Assessed the results of MMP-9 levels after inoculation of Mycobacterium tuberculosis bacteria. Then performed AFB examination, culture, PCR, and histopathology Mycobacterium tuberculosis. After all we obtain the research results, then they are processed and analyzed statistically. We arranged data on the success of Mycobacterium tuberculosis bacteria

inoculation in a frequency distribution table. In bivariate data, the normality test will be carried out using the Shapiro-Wilk test. If the normality test results obtained p> 0.05, it means that the data is normally distributed, then the data will be analyzed using the Paired T-test. If the data is not normally distributed, then the data analysis will use Wilcoxon. Data analysis using SPSS version 15.

3. Results

Based on the effect of doxycycline administration on tuberculous spondylitis infection in experimental rabbits, forty treated and control rabbits that had been inoculated with Mycobacterium tuberculosis bacteria and were given doxycycline treatment for 4 weeks were incubated in individual cages in one large room. During the treatment period, the rabbit's appetite does not decrease, the rabbit can defecate and urinate normally, remain active, and can respond well to the environment. Of the forty experimental animals, 1 died in group B 1 died in group C, 1 died in group E, and 1 died in group K. The mortality rate was 10.0% (4/40).

Table 1. Experiment Group Division

No	Group	Treatment I	Treatment II
1	Group A	Mycobacterium tuberculosis, incubation 2 weeks	Without doxycycline for 4 weeks
2	Group B	Mycobacterium tuberculosis, incubation 2 weeks	Doxycycline 1 mg/kg/day for 4 weeks
3	Group C	Mycobacterium tuberculosis, incubation 2 weeks	Doxycycline 5 mg/kg/day for 4 weeks
4	Group D	Mycobacterium tuberculosis, incubation 4 weeks	Without doxycycline for 4 weeks
5	Group E	Mycobacterium tuberculosis, incubation 4 weeks	Doxycycline 1 mg/kg/day for 4 weeks
6	Group F	Mycobacterium tuberculosis, incubation 4 weeks	Doxycycline 5 mg/kg/day for 4 weeks
7	Group G	Mycobacterium tuberculosis, incubation 6 weeks	Without doxycycline for 4 weeks
8	Group H	Mycobacterium tuberculosis, incubation 6 weeks	Doxycycline 1 mg/kg/day for 4 weeks
9	Group I	Mycobacterium tuberculosis, incubation 6 weeks	Doxycycline 5 mg/kg/day for 4 weeks
10	Group J	Mycobacterium tuberculosis, incubation 8 weeks	Without doxycycline for 4 weeks
11	Group K	Mycobacterium tuberculosis, incubation 8 weeks	Doxycycline 1 mg/kg/day for 4 weeks
12	Group L	Mycobacterium tuberculosis, incubation 8 weeks	Doxycycline 5 mg/kg/day for 4 weeks

Based on table 2 regarding the results of the examination of healing treatment without doxycycline, the inoculation time of 2 weeks with AFB examination showed 2 samples with positive smear, 2 samples with a

positive culture, 3 samples with positive PCR, and 1 sample with positive histopathology. Meanwhile, for 4 weeks inoculation, 0 samples showed positive smear results, 2 samples each showed positive culture, positive PCR, and positive histopathology. At 6 weeks of inoculation, 1 sample each showed positive smear and culture results, 0 samples showed positive PCR results and 3 samples showed positive histopathological results. At 8 weeks of inoculation, 0 samples showed positive smear results, 2 samples showed positive culture results, 1 sample showed positive PCR results and 0 samples showed positive histopathological results.

Based on table 3 regarding the results of the examination of healing with doxycycline 1 mg/kgBW, the inoculation duration of 2 weeks with AFB examination showed 1 sample with positive smear, 3 samples with a positive culture, 2 samples with positive PCR, and 1 sample with positive histopathology. Meanwhile, for 4 weeks inoculation, 2 samples showed positive smear results, 2 samples each showed positive culture, positive PCR, and 3 samples showed positive histopathological results. At 6 weeks of inoculation, 0 samples showed positive smear results, 2 samples showed positive culture results, 0 samples showed positive PCR results and 2 samples showed positive histopathological results. At 8 weeks of inoculation, 0 samples showed positive smear results, 2 samples showed positive culture results, 0 samples showed positive PCR results and 3 samples showed positive histopathological results.

Based on table 4 regarding the results of the examination of recovery from doxycycline 5 mg/kgBW, the inoculation duration of 2 weeks with AFB examination showed 1 sample with positive smear, 3 samples with a positive culture, 3 samples with positive PCR, and 3 samples with positive histopathology. Meanwhile, for 4 weeks inoculation, 0 samples showed positive smear results, 3 samples each showed positive culture, 1 sample showed positive PCR results and 0 samples showed positive histopathological results. At 6 weeks of inoculation, 0 samples showed positive smear results, positive culture, positive PCR, and positive histopathology. At 8 weeks of inoculation, 0 samples showed positive smear results, 1 sample showed positive culture results, 0 samples showed positive PCR results and 0 samples showed positive histopathological results.

In table 5, regarding the results of the healing examination with the duration of inoculation, it can be seen that in the smear examination, the p-value shows the number 0.407 and the r-value is 0.283. Meanwhile, in the culture examination, the p-value shows several 0.259 and the r-value of 0.079. PCR examination showed the number 0.259 with an r-value of 0.154 while the histopathological examination showed a p-value of 0.223 and an R-value of 1,000. It can be interpreted that from all examinations when compared with the duration of inoculation, the histopathological examination has a strong correlation regarding the identification of cure outcomes in models with TB spondylitis, as shown in Figure 1.

Table 2. Examination Results of Treatment Without Doxycycline

Examination	Inoculation duration			
	2 weeks	4 weeks	6 weeks	8 weeks
AFS	2 (n=12)	0 (n=12)	1 (n=12)	0 (n=12)
Culture	2 (n=12)	2 (n=12)	1 (n=12)	2 (n=12)
PCR	3 (n=12)	2 (n=12)	0 (n=12)	1 (n=12)
Histopathology	1 (n=12)	2 (n=12)	3 (n=12)	0 (n=12)

Table 3. Results of Healing Examination of Doxycycline 1mg/kgBW

Examination	Inoculation duration			
	2 weeks	4 weeks	6 weeks	8 weeks
AFS	1 (n=12)	2 (n=12)	0 (n=12)	0 (n=12)
Culture	3 (n=12)	2 (n=12)	2 (n=12)	2 (n=12)
PCR	2 (n=12)	3 (n=12)	0 (n=12)	0 (n=12)
Histopathology	1 (n=12)	1 (n=12)	2 (n=12)	3 (n=12)

Table 4. Results of Healing Examination of Doxycycline 5mg/kgBW

Examination	Inoculation duration			
	2 weeks	4 weeks	6 weeks	8 weeks
AFS	1 (n=12)	0 (n=12)	0 (n=12)	0 (n=12)
Culture	3 (n=12)	3 (n=12)	0 (n=12)	1 (n=12)
PCR	3 (n=12)	1 (n=12)	0 (n=12)	0 (n=12)
Histopathology	3 (n=12)	0 (n=12)	0 (n=12)	0 (n=12)

Table 5. Results of Healing Examination with Long Inoculation

Examination	Inoculation duration				*p-value*	*r value*
	2 weeks	4 weeks	6 weeks	8 weeks		
AFS	4 (n=36)	2 (n=36)	1 (n=36)	0 (n=36)	0,407	0,283
Culture	8 (n=36)	7 (n=36)	3 (n=36)	5 (n=36)	0,259	0,079
PCR	8 (n=36)	6 (n=36)	0 (n=36)	1 (n=36)	0,259	0,154
Histopathology	5 (n=36)	3 (n=36)	5 (n=36)	3 (n=36)	0,223	1,000

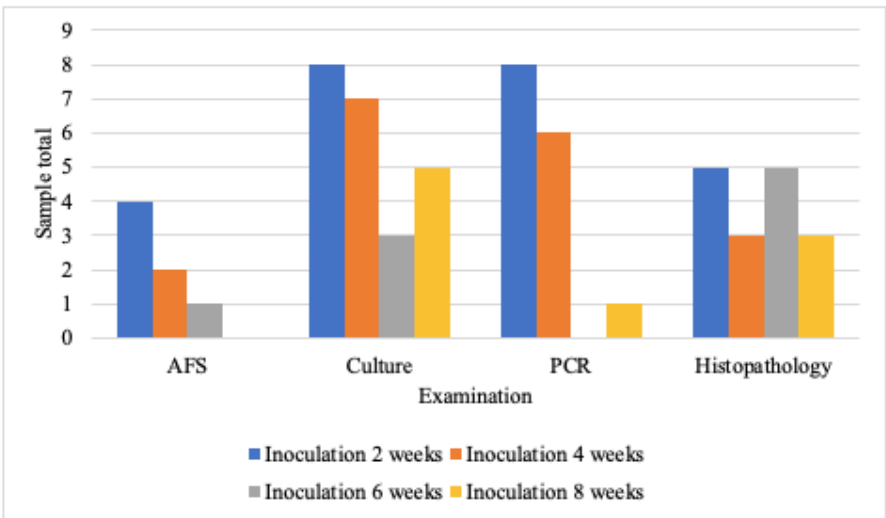

Figure 1. Healing Examination with Inoculation Time

In testing the difference between the MMP-9 values before and after the intervention using the ANOVA test, it showed a p-value of 0.08 for the MMP-9 value before the intervention and a p-value of 0.17 on the MMP-9 value after the intervention. This means that the MMP-9 value between groups has a significant difference. Associated with the value of the difference between before and after shows a p-value of 0.379 which indicates that there is no statistically significant difference related to the MMP-9 value when compared before and after the intervention when compared within each group.

Regarding the effect of doxycycline administration, after statistical testing with a paired t-test, only Group D with MMP-9 levels before intervention was 659.82 + 63.492 compared to after intervention 525.02 + 22.260

with a p-value of 0.043 (p <0.05). and Group H with MMP-9 levels before intervention 447.02 + 35.701 compared after intervention 553.17 + 77.805 with a p-value of 0.050 (p<0.05) which indicates a significant effect of doxycycline administration on MMP-9 levels in experimental animals with spondylitis TB in Table 6.

Table 6. Effect of Doxycycline on MMP-9 levels

Group	Intervention	Mean ± SD MMP-9	P-value*
Group A	Before	562,47 ± 58,153	0,57
	After	497,61 ± 86,091	
Group B	Before	597,11 ± 50,436	0,52
	After	500,55 ± 18,647	
Group C	Before	631,55 ± 4,868	0,126
	After	581,10 ± 35,266	
Group D	**Before**	**659,82 ± 63,492**	**0,043***
	After	**525,02 ± 22,260**	
Group E	Before	638,11 ± 16,267	0,158
	After	564,34 ± 41,824	
Group F	Before	575,26 ± 106,729	0,191
	After	434,48 ± 18,787	
Group G	Before	527,52 ± 15,231	0,112
	After	551,30 ± 30,094	
Group H	**Before**	**447,02 ± 35,701**	**0,050***
	After	**553,17 ± 77,805**	
Group I	Before	575,74 ± 81,069	0,994
	After	576,04 ± 37,636	
Group J	Before	490,55 ± 72,200	0,142
	After	582,10 ± 138,690	
Group K	Before	577,97 ± 61,320	0,414
	After	616,72 ± 83,753	
Group L	Before	572,77 ± 51,128	0,175
	After	674,30 ± 48,867	

*T-paired test

In testing the difference in delta values between treatment groups with inoculation time, it appears that for the treatment without doxycycline with an inoculation period of 2 weeks, the average delta was 64.86, the inoculation duration of 4 weeks had an average delta value of 134.7933. 6 weeks has an average delta value of -23.7733, while the 8-week inoculation time has an average delta value of -91.5467 with a p-value of 0.001 which means that there is a significant difference between the delta values between groups of inoculation time and treatment without doxycycline.

For treatment using doxycycline 1 mg/kgBW with an inoculation duration of 2 weeks has an average delta of 96.56, a 4-week inoculation time has an average delta value of 73.7667, an inoculation duration of 6 weeks has an average delta value of -106, 1467, while the 8-week inoculation time had an average delta value of -38.7467 with a p-value of 0.05, which means that there is a significant difference between the delta values between the inoculation time groups and the treatment using doxycycline 1 mg/kgBW.

For the treatment using doxycycline 5 mg/kgBW with an inoculation duration of 2 weeks having an average delta of 50.4533, a 4-week inoculation period having an average delta value of 140.78, an inoculation duration of 6 weeks having an average delta value of -0.3, while the 8-week inoculation time had an average delta value

of -101.5267 with a p-value of 0.042 which means that there is a significant difference between the delta values between the inoculation time groups and the treatment using doxycycline 5 mg/kgBW.

Table 7. Differences in Delta Values Between Treatment Groups with Inoculation Duration

Treatment	Inoculation duration				P-value
	2 weeks	4 weeks	6 weeks	8 weeks	
Without Doxycycline	64.86	134.7933	-23.7733	-91.5467	0.001*
Doxycycline 1 mg/kgBW	96.56	73.7667	-106.1467	-38.7467	0.05*
Doxycycline 5 mg/kgBW	50.4533	140.78	-0.3	-101.5267	0.042*

**One-Way ANOVA* test

4. Discussion

In this study, the success of Mycobacterium tuberculosis bacteria inoculation in the vertebral bodies of rabbits was assessed based on the results of AFB staining, culture, histopathology, and PCR of lesion tissue samples. AFS staining is said to be positive if 1 or more AFB is found in 100 visual fields. A positive culture examination was declared if there was bacterial growth in the medium after 9-14 days and continued with Niacin and PNB tests. Positive histopathological examination results are stated if there is a picture of the tissue reaction to Mycobacterium tuberculosis bacterial infection such as the presence of Datia Langhans cells, giant cells, necrotic tissue, cases, and so on. PCR results were declared positive if there was a picture of the DNA band of the bacterium Mycobacterium tuberculosis H37RV, which is the same bacteria that was inoculated into the vertebral body of rabbits.

The highest success rate for inoculation of Mycobacterium tuberculosis bacteria in the vertebral bodies of rabbits was in group 3, namely 7 of 8 samples (87.5%) with an incubation period of 6 weeks, followed by group 2 (75%, with an incubation period of 4 weeks), group 1 (37.5%, with an incubation period of 2 weeks), and group 4 (12.5%, with an incubation period of 8 weeks). This is following the research of Ahmad Jabir Rahyussalim et al, regarding the potential for the spread of Mycobacterium tuberculosis into the environment with the rabbit spinal TB model, that based on PCR examination the inoculation was successful at week 1, and based on histopathological examination the inoculation was successful at week 6 (Rahyussalim et al., 2015).

In this study, the highest results were found at 6 weeks (87.5%) and 4 weeks (75%). This is per the research of Xiaochen Liu et al, using a dose of Mycobacterium tuberculosis 1 x 107 CFU/mL, the results showed that spinal tuberculosis had developed since the 4th week based on CT-Scan and MRI examinations. At week 8, from 15 rabbits with positive MRI and CT-Scan results, 10 rabbits with positive culture were found (Rahyussalim et al., 2015). In this study group 4 (12.5%), with an incubation period of 8 weeks, differed from the results of research conducted by Guangqi et al, regarding the formation of a rabbit spinal TB model, that based on the results of culture after 8 weeks of inoculation, 9 out of 17 rabbits had positive culture (52.9%) (Ashwin et al., 2008). In this study, group 4 with 8 weeks of inoculation did only have 8 samples. The number of samples may be the cause of this discrepancy. However, this study was successful in inoculation of a rabbit spinal TB model with an incubation period of 6 weeks (87.5%).

Histopathology has a much higher diagnostic success rate than culture, with 100% confirmation. Cytology shows characteristic epithelioid cell granulomas, granular necrosis, lymphocytes, and Langerhans giant cells (Khanna et al., 2019). This is also found in this study. In this study, the positive results on histopathological

examination (HE view) were tubercles, macrophages, and epithelioid cells. The study of Liu et al and Geng et al, on the establishment of a rabbit spinal TB model, found suitable histopathological results. Rabbits, with their large lungs that can accommodate multiple lesions without significant health effects, have proven to be very useful in evaluating drug distribution, lesion penetration, and cellular accumulation of drugs in different lesion types using laser microdissection and the MALDI-MRM-MS imaging method. high sensitivity. Through this technology, we have studied the location of bacteria within the lesion despite their lipid content and that MOX may preferentially accumulate in foamy macrophages. In a comparison of fluoroquinolones, Sarathy et al. (2019) demonstrated that MOX: was more active than levofloxacin and gatifloxacin in an ex vivo caseum assay, was superior in sterilizing cellular and necrotic rabbit lesions during in vivo treatment, and was predicted to be more active in the GranSim model.

In a similar study, both RIF and RFP had a penetration coefficient of 1 in cellular lesions compared to plasma, but in cavities, RIF penetrated at ~1, and RFP had only 0.25 penetration coefficient compared to plasma. The lower penetration of the RFP into the cavity is thought to explain why the RFP requires a dose four times higher than the standard to improve outcomes in patients with large cavities compared to patients without cavities. Moreover, this study may have solved the mystery of the contribution of EMB to the INH, RIF, PZA, and EMB (HRZE) regimens. EMB was shown to have sustained accumulation above the MIC in both cellular and necrotic lesions; Likewise, the high penetration potential of PZA may increase its slow but potent activity in necrotic lesions (Yang et al., 2021). TB can cause macrophage dysfunction, anemia, and hypoproteinemia, thus making the patient highly susceptible to other infections (Zou et al., 2018). TB is a lung disease. Lung extracellular matrix biochemistry predicts that matrix metalloproteinases (MMPs) will be the dominant proteases that promote lung matrix destruction in TB. In a zebrafish model, MMP-9 regulates monocyte recruitment to granulomas, suggesting that MMP modulates the immune response against Mycobacterium tuberculosis (Mtb) and triggers pathological conditions (Zou et al., 2018). Many tetracyclines, including DOX, have anti-inflammatory properties mediated by the suppression of tumor necrosis factor (TNF-α) and matrix metalloprotease (MMP). In vitro, DOX inhibited MMP secretion induced by TB infection at 5 mg/liter and higher (Gengenbacher et al., 2020).

Like other body tissues, inflammation of the central nervous system (CNS) also results in increased MMP secretion and can also affect the permeability of the blood-brain barrier (BBB). Recent studies have shown that upregulation of MMP-9 has been observed in brain biopsies of patients with tuberculous meningitis. This increased MMP-9 activity in brain tissue may be involved in the breakdown of the blood-brain barrier (BBB), edema, and exudation of inflammatory cells. Li et al analyzed the expression of MMP-9 in the pathophysiological process of tuberculous meningitis in a mouse model (Sabir et al., 2019). The role of MMP-9 has been reported in modulating cellular recruitment to granulomas and decreased MMP-9 expression results in smaller, virulent M marinum granulomas significantly increasing MMP-9, MMP-13, and MMP-14 expression compared to attenuated strains. These studies demonstrate a major role of MMP activity in the pathogenesis of mycobacterial infections. In addition to this TB model, non-human primates (NHP) such as the cynomolgus macaque have been used to replicate human TB lesions. NHP has a close evolutionary relationship with humans and produces TB disease with clinical findings and lesions very similar to those of humans. Apes develop the type of granuloma seen in humans in the presence of classic caseous pulmonary granuloma. In addition, macaques also exhibit other types of lung lesions such as non-necrotizing granulomas, calcifications, cavitations, consolidations, and interstitial fibrosis (Sabir et al., 2019).

Overall, a promising model for the role of MMPs in TB is that Mtb induces lung tissue remodeling and granuloma formation through MMP upregulation. Whole granulomas are considered beneficial to the host as they keep the pathogens under control and prevent their spread. Reactivation of infection and increased secretion of MMP-1 result in degradation of the lung matrix and cavitation. However, the regulation and role of specific MMPs during different stages of Mtb infection remains to be explored. However, the role of the kallikrein-kinin

system in MMP regulation in Mtb infection is still not fully understood. Recently, most publications, using various animal models of TB, have suggested MMPs as viable therapeutic targets. Additional treatment with MMP inhibitors along with front-line TB drugs including isoniazid and rifampin significantly reduces Mtb survival in the lung by preventing granuloma maturation and also minimizing matrix degradation and cavitary lesions. Current TB treatment regimens require multiple drugs and must be taken for long periods; therefore, they impose other challenges such as non-adherence and the emergence of drug-resistant Mtb strains (Rahyussalim et al., 2015).

5. Conclusion

From the statistical analysis of this study about the role of doxycycline as MMP-9 inhibitor it can be concluded that doxycycline doesn't have an effect on MMP-9 value in rabbit's tuberculous spondylitis.

Acknowledgements

A study with different variant of MMP is required; the same study with different animal model is needed so that the result of this study can be more applicable in clinical practice.

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