Abstract

Models for lapin septic arthritis and cryptic arthritis were developed. Joint infection was determined using several criteria such as sluggishness, loss of appetite, loss of weight, joint swelling, lamness and histopathology. These criteria were applied using two infection routes: the intravenous (IV) and intrarticular (IAI).

Infection through the intravenous route showed marked joint swelling cell wall defective rather than intact state. It failed to induce both, lameness and loss of appetite with minimal weight loss of around 50gm. In the intrarticular route, however, there were appetite loss, weight loss, mild sluggishness and marked joint swelling in CWD rather than intact. Intact Staphylococcus aureus in IV was arthropathic but by IAI was non-arthropathics. Cell wall defective Escherichia coli was arthropathic in both IV and IAI. Both intact and CWD E.coli were arthropathic by IAI.

Thus human arthropathic E.coli isolates showed lapin arthropathy both as intact and cell wall defective but in CWD state there were more severe clinical outcomes, while, human arthropathic S.aureus isolates showed lapin arthropathy in cell wall defective state but not in intact state.

Introduction

Laboratory animal models are useful for several biologic, pathologic as well as immunologic purposes of which mimicking of pathogenesis of infectious agents is one male and female mice as well as male rats were mostly the experimental laboratory animal model used for the demonstration of pathogenesis of septic and cryptic arthritis[1-6]. In the present work, however, it has been tried to put forward a lapin model possibly helpful for the demonstration of septic bacterial arthritis as an analogy to human septic and cryptic arthritis.
Materials and Methods  
1- Arthropathic organisms: 
   Normal and cell well defective  
   *S.aureus* as well as normal and cell  
   wall defective *E.coli* isolates were  
   elected from:  
   1- Clinically proven septic arthritis.  
   2- Marked swollen joints  
   3- Monocytic infiltrate in synovial  
   fluid  
   4- Culture negative.  
2- Preparation of the infectious doses  
   The intact was bacterial  
   suspensions which were prepared and  
   adjusted to the density of 3x10^8/ml(7).  
   Cell wall defective suspensions  
   matching 3x10^8/ml prepared as in  
   8,9,10 and histopathology sectioning,  
   staining, mounting were performed as  
   in (11).  
3- Animals:  

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Laboratory Animals as Regimens</th>
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</thead>
<tbody>
<tr>
<td>Groups /Sub groups</td>
<td>No of animals</td>
</tr>
<tr>
<td>Intravenous</td>
<td></td>
</tr>
<tr>
<td>I.</td>
<td>2</td>
</tr>
<tr>
<td>II.</td>
<td>2</td>
</tr>
<tr>
<td>III.</td>
<td>2</td>
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<tr>
<td>IV.</td>
<td>2</td>
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<tr>
<td>V.</td>
<td>2</td>
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<tr>
<td>intraarticular</td>
<td></td>
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<tr>
<td>I.</td>
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<td>II.</td>
<td>2</td>
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<td>III.</td>
<td>2</td>
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<td>IV.</td>
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<td>V.</td>
<td>2</td>
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Rabbits *O. cuniculus* was brought from local market, adapted for  
housing conditions for two weeks and kept throughout the experimentation  
period and they were grouped into two  
groups each for ten. Then each group  
was subgrouped into five subgroups  
four as a test group and one as a  
control group (Table -1).  
4- Parameters for infectivity  
evolution:  
   These parameters were as in the  
   following:  
   a- sluggishness  
   b- loss of appetite  
   c- lameness  
   d- histopathology  
   e- weight loss  
   f- swelling of joints  
   g- direct examination  
   h- resolution
Results
1- Sluggishness:
Rabbits, infected with CWDB have shown moderate sluggishness. Intact bacteria induced mild sluggishness and controls showed no evidence of sluggishness.

2- Loss of appetite
Apparently, the four infected animals showed no evidence of loss of appetite in those rabbits infected through intra-articular route which showed mild symptoms of loss of appetite.

3- Loss of weight:
Animals inoculated through IV route with both intact and cell wall defective bacteria were showing weight loss of 43-93 gms. Meanwhile, rabbits infected by CWDB through intra–articular route were showing weight loss of ≈ 250 gms and these infected with intact bacteria were ranging around ≈ 100 gms (Tables: 2 and 3).

4- Joint swelling:
4-1- Intravenous infections:
Severe joint swellings were noted among rabbits with cell defective S.aureus with two death instances. In comparison, cell wall defective E.coli has shown in comparison with cell wall defective E.coli has shown moderate joint swelling. Both of which were more severe than those infected with intact S.aureus and E.coli (Table 3).

4-2- Intra – articular infections:
Intact cell wall defective E.coli infected rabbits showed joint swelling, but the more severe was that induced by CWDB. Meanwhile, CWD S.aureus showed moderate swelling of the joints in comparison to the lack of swelling noted among intact S.aureus infected rabbits (Table 3).

5- Lameness:
5-1- Intravenous:
No evidence for lameness can be noted among IV infected rabbits with both of cell wall defective and intact S.aureus as well as E.coli (Table 3 and fig. A and B).

Table 2 Effect of experimental infection on animal weight during experimentation period.

<table>
<thead>
<tr>
<th>Mode of injection</th>
<th>Average of weight for two rabbits before the experiment/gm</th>
<th>Average of weight for two rabbits before the experiment/gm</th>
<th>Average of weight loss/ gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWDEc / IV</td>
<td>1575</td>
<td>1523</td>
<td>52</td>
</tr>
<tr>
<td>CWD Sa / IV</td>
<td>1285</td>
<td>1224</td>
<td>61</td>
</tr>
<tr>
<td>CWDEc / IA</td>
<td>1500</td>
<td>1258</td>
<td>242</td>
</tr>
<tr>
<td>CWDSa / IA</td>
<td>1437</td>
<td>1310</td>
<td>127</td>
</tr>
<tr>
<td>CW Ec / IV</td>
<td>1425</td>
<td>1332</td>
<td>93</td>
</tr>
<tr>
<td>CW Sa / IV</td>
<td>1327</td>
<td>1259</td>
<td>68</td>
</tr>
<tr>
<td>CW Ec / IA</td>
<td>1197</td>
<td>949</td>
<td>246</td>
</tr>
<tr>
<td>CW Sa / IA</td>
<td>1100</td>
<td>934</td>
<td>180</td>
</tr>
<tr>
<td>Control / IV</td>
<td>1345</td>
<td>1302</td>
<td>43</td>
</tr>
<tr>
<td>Control / IA</td>
<td>1580</td>
<td>1530</td>
<td>50</td>
</tr>
</tbody>
</table>

CWD : Cell wall defective
CW: Cell walled  
Ec: E. coli  
Sa: Staphylococcus aureus  
IV: Intravenous injection  
IA: Intra-areicular injection

Table 3 Arthritis determination criteria in experimental animal model

<table>
<thead>
<tr>
<th>Criteria</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>Control</th>
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<tr>
<td></td>
<td>IV</td>
<td>IAI</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>CW</td>
<td>CWD</td>
<td>CW</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>N</td>
<td>N</td>
<td>LA</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>68*</td>
<td>61</td>
<td>180</td>
</tr>
<tr>
<td>Sluggishness</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Joint swelling</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Death</td>
<td>n</td>
<td>D</td>
<td>n</td>
</tr>
<tr>
<td>Lameness</td>
<td>n</td>
<td>n</td>
<td>+</td>
</tr>
</tbody>
</table>

N = Normal appetite  
LA = Loss appetite  
+= Mild  
++= Moderate  
*= Weight loss in grams  
= Normal  
= Doubt

Figure 1-A The control animal without lameness
Figure 1-B The infected animal with lameness state.

5-2- Intra-articular (IAI)

Intact and cell wall defective *E.coli* infected rabbits (IAI) have shown the same degree of lameness. Cell wall defective *S.aureus* induced mild lameness, while intact *S.aureus* fails to induce such reaction.

6- Direct Geimsa and Gram stained synovial fluid films:-

6-1- Intravenous:

Cell wall defective *S.aureus* and *E.coli* infected synovial fluid showed partial and complete defectiveness of the wall along with inflammatory cells. *S.aureus* showed single, diplo and clusters of partially swollen and completely swollen spheres. *E.coli* however, have showed partially swollen and completely swollen cylinders. Intact *E.coli* infected synovial fluid showed intact cells with inflammatory responses, while those for intact *S.aureus* showed no evidence for cocci but with inflammatory cells.

6-2 Intrat-aarticular:

CWD *S.aureus* and *E.coli* IAI synovial fluid showed partial and completely defective bacteria along with inflammatory cells. Rabbits infected with intact *E.coli* showed normal intact rods along with inflammatory cells while there was no evidence for intact normal cocci along with inflammatory cells in rabbit infected with intact *S.aureus*.

6-3 Controls:

Control synovial fluids showed neither inflammatory cells nor bacterial bodies.

7- Histopathology

7-1- intravenous:

Nil intravenous reactions were noted among all of *E.coli*, *S.aureus* as well as control groups. It was noted worthing to mention an intracellular edema noted among CWD *S.aureus* together with vascular congestion in the synovial membranes (figs.2A & 2B).

7-2- Intra-articular:

Most of the tissues of the synovial membranes have shown severe inflammatory cell infiltrate except the synovial membrane of animals infected with intact *S.aureus* (figs .3A and 3B).
8: Reisolation: Cell wall defective from intrarticularly infected rabbits. *S. aureus* and *E. coli* were reisolated mostly.

**Figure 2A** Tissue infected with cell wall defective *S. aureus* revealed simple intercellular odema (intravenously).

**Figure 2B** Tissue infected with cell wall defective *E. coli* revealed blood haemorage and vascular conjustion (intravenously).
Figure 3A Tissue infected with intact *S.aureus* intra-articularly.

Figure 3B Tissue of control rabbits with no changes

**Discussion**

During bacteremia and / or septicemia caused by an infection at site, outside of the joint, organism and/or their toxic products are deposited in or on the synovial membrane and the organisms may proliferate to cause septic arthritis. When they do grow, the infection could spread to the joint space then spread to the bone and cartilage. Such situation, an inflammation of the synovial membrane is quickly established and results in a marked increase in leukocyte in the synovial fluid. The pathogenic findings are varied and depends on the duration of infection, nature of the pathogen and resistance of the host. Early in the infection, only inflammatory changes in the synovium are seen. Late in the course of untreated septic arthritis destruction of joint structures is marked. To make an analogy to this in laboratory animals,
Researchers have put forward murine model of septic arthritis in the present work; lapin model is being reported [15].

The model gave plan for septic and other for cryptic arthritis the former kind by infection with an intact organism via IV and IAI and the latter represented by cell wall defective organism via IV and IAI in rabbits [1-3,15].

The most prominent indications for arthritis are loss of weight, joint swelling and lameness. All of these indications were noted by these lapin models but with variable grades of clinical manifestations [2-4,12-14]. The human arthropathic bacterial isolates were found to have the same potential in lapin but with different grades. Both of infection routes and the organisms were found valid for production of the experimental disease in rabbit but in variable grades [14]. CWD isolates were showing more sever clinical manifestations than the intact organisms, a finding which came in agreement with that of some workers [2,3,12,14]. Chocks postulates were fulfilled and the infectious organisms resulted in pathogenic changes in the synovial membranes are concomitant with septic arthritis and cryptic arthritis respectively [2,5,9,15,17].

Thus in conclusion one may point out the followings:
1- Experimental infected animals should have both joint swelling and lameness in variable degrees.
2- The experimental cryptic arthritis infections were of more severe than septic arthritis model
3- Human arthropathic E. coli & S. aureus were proved of lapin arthropathic potentials
4- The infection course of clinical manifestation, histopathology changes as well as the resulting studies support Koch’s postulation on one hand and septic as well as cryptic arthritis on the other hand in a lapin model.

References
3- Hultgren , O.; kopf , M. & Tarkowski,A.(1998 b) Staphylococcus aureus induced septic arthritis and septic death is Decreased in IL -4deficient mice: Role of IL-4 as promotor for bacterial growth. J. Fmmunol.160; 5082 ,5087.