Comparison of Animal Models with Soft Tissue Infection by Different Bacilli

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ABSTRACT. In clinical medicine improved diagnostic methods for the detection of infection are needed. A good infectious animal model is very important for the development of a new diagnostic method or drug. The purpose of this study was to establish a good animal model with soft tissue infection. Twenty-four SD rats were divided into four groups (6 in each group). Various bacilli including *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*), and *Escherichia coli* (*E. coli*) were injected intramuscularly into the left caudal thighs of three groups of rats to create soft tissue infection. In addition, normal saline was injected into the left caudal thighs of ten rats which were used as controls. Before and 48 hr after inoculation of the bacilli, a blood sample (0.5 ml) was taken from each rat and analyzed to determine the white blood cell count and differentiated cell count. In addition, 48 hr after the inoculation, 0.2 mCi of gallium-67 was injected via the tail vein. Gallium scan was performed at 24 hr and 48 hr after administration of the radiotracer. The dorsal view of both hind legs was imaged and analyzed by computers to calculate the lesion-to-normal (L/N) ratio. After imaging, all rats were sacrificed and specimens from portions of the infected thigh muscle were sent for histopathologic investigation to confirm the infection. The increase in both the WBC counts and the segmented polymorphonuclear leukocytes (PMNs) were most significant in the *S. aureus* group, followed by the *S. pneumoniae* group, *E. coli* group and normal control groups. The rats with *S. aureus* infection had significant gallium uptake at the site of infection and the highest L/N ratio of 2.14 on the 24-hr image and 2.0 on the 48-hr image. The rats with *S. pneumoniae* had the second highest L/N ratio (1.41 at 24 hr, and 1.48 at 48 hr). The L/N ratio for the *E. coli* group was 1.27 at 24 hr and 1.35 at 48 hr. No obviously abnormal gallium uptake was demonstrated in the normal controls. We conclude that all three bacilli induced a soft tissue infection in SD rats. *S. aureus* resulted in the most significant infectious signs.

KEY WORDS: bacilli, gallium-67 scan, infectious animal model, SD rats.

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Since a major consideration in the treatment of patients with focal infection is drainage, prompt identification of the site of involvement is extremely important. Early in the evolution of an inflammatory lesion, before formation of an abscess, radiological methods such as plain x-ray, computed tomography and ultrasound cannot readily localize the area of involvement. In addition, in a case with unknown fever, a whole body survey is always needed to locate the infectious source. Radionuclide techniques allow for early diagnosis of infection as well as whole body scanning, and are widely used in the detection of infection in patients with unknown fever. Gallium-67 citrate (gallium) scan and leukocyte scan are the two most common techniques used in nuclear medicine departments but at least 48 hr is required to obtain the final results of the gallium scan, which is a major drawback in its use for the detection of infection. In addition, significant physiological bowel activity in the gallium image becomes an obstacle when applied to patients with suspected intra-abdominal infection [3, 5, 10, 13, 14, 23]. Labeled leukocytes provide more accurate and quicker diagnosis than gallium imaging but In-111 is not available in many countries, Tc-99m HMPAO labeling of leukocytes

is expensive and time consuming, and Tc-99m HMPAO scan may also show intense physiologic bowel activity. Again, this limits its application in patients with suspected intra-abdominal infection [3, 14, 16, 20]. In addition, Tc-99m labeled human IgG have also been used in the detection of infection [7, 21]. Therefore, there is an urgent need in nuclear medicine to develop a new drug or new technique to detect infectious foci [22, 24].

In order to develop such a drug or technique it is important to establish a good and reliable animal infectious model. In the literature, different kinds of micro-organisms, including *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Streptococcus pneumoniae* (*S. pneumoniae*) have been used to produce infectious foci in different animals such as mice, rats and rabbits [2, 4, 6, 8]. There are no comparative data on the abilities of these micro-organisms to induce infectious foci. In this study, we evaluated the ability of different kinds of micro-organisms including *S. aureus*, *E. coli*, and *S. pneumoniae* to induce infectious foci in rats and established a good and reliable infectious animal model.

MATERIALS AND METHODS

Animals: Twenty-four male Sprague-Dawley (SD) rats (Charles River Breeding Laboratories, Burlington, MD, U.S.A.) weighing 150 g were used in this experiment, and

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were divided into four groups (six in each group). Single clinical isolates of *S. aureus*, *E. coli*, and *S. pneumoniae*, were used to induce infectious foci. The appropriate microbial strain was incubated overnight on trypticase soy agar plates at 37°C. Individual colonies were diluted with sterile normal saline to produce a turbid suspension containing approximately 5×10^9 organisms/ml. The rats were anesthetized with ketamine, and 0.2 ml of a suspension containing 1×10^9 organisms was injected into the left caudal thigh of the rats. In addition, the six rats in the control group were injected with 0.2 ml of normal saline.

Evaluation of infection

Blood Cell Counts: Before and 48 hr after inoculation with the bacilli, a blood sample (0.5 ml) was taken from each rat and analyzed to determine the white blood cell count (WBC) and segmented polymorphonuclear leukocytes (PMNs).

Gallium-67 scintigraphy: All rats were injected with 0.2 mCi gallium-67 citrate via the tail vein 24 hr after the injection of bacilli or normal saline into the left caudal thigh. The rats were anaesthetized and tied to a board before imaging, then they were placed in the prone position under the camera with the legs stretched out and fixed. A planar dorsal view of the hind legs with a total count of 300K was obtained at 24 and 48 hr after administration of the radioactive agents with a large field-of-view gamma-camera (Elscint APEX 415 W) with an APC-3 (low energy, medium resolution, medium sensitivity) collimator. The images were obtained in a 256x256 matrix.

Calculation of uptake ratio: Regions of interest (ROIs) were drawn over the infected and non-infected thighs to determine radioactivity at the site of infection (Fig. 1). The edge of the ROI over the infected thigh was defined manually when a threshold of 30% of maximum counts in the lesion was reached. The threshold was easy to define in a color image shown on the computer. The ROI over the non-infected thigh was defined empirically as of a similar size to that of the right thigh. It was not necessary for both ROIs to be exactly the same size since mean counts per pixel were used for calculation. The formula used to calculate the lesion-to-normal ratio (L/N ratio) for the ROIs of each rabbit is shown below:

$$\text{L/N ratio} = \frac{\text{Mean counts per pixel of ROI1}}{\text{Mean counts per pixel of ROI2.}}$$

Where

ROI1=ROI over infected caudal thigh

Fig. 1. Dorsal view of a rat injected *S. aureus*. Regions of interest (ROIs) were drawn over the infected left caudal thigh (ROI1) and non-infected right caudal thigh (ROI2) to generate the mean counts per pixel. The L/N ratio was calculated with the following equation: L/N ratio = mean counts per pixel of ROI1/ mean counts per pixel of ROI2.

ROI2=ROI over non-infected caudal thigh

The L/N ratio was taken as an index of the ability of the radiopharmaceutical to localize the infection.

Pathologic examination: After finishing the gallium imaging, the infected animals were sacrificed and specimens from portions of the infected caudal thigh muscle were sent for histopathologic investigation to confirm the infection.

Statistical analysis: Mann Whitney U test were used for testing differences. The test results were considered statistically significant when p value was < 0.05. Data were analyzed using the Statistica for Windows Release 4.5 package (StatSoft, Inc., OK, US).

Table 1. Hematologic data after intramuscular injection of various organisms into the left caudal thigh of rats

	WBC (count/ml)			PMNs (%)		
	Baseline	48 hr	% Increase	Baseline	48 hr	% Increase
Control	8124 ± 903	9024 ± 1933	11.07	18.8 ± 4.15	17.6 ± 13.3	- 1.2
<i>S. aureus</i>	7638 ± 1722	12682 ± 796	66.03	10.6 ± 1.9	39.7 ± 14.5	29.1
<i>E. coli</i>	8838 ± 1656	10816 ± 1127	22.38	15.2 ± 5.2	14.8 ± 6.1	- 0.4
<i>S. pneumoniae</i>	7024 ± 1532	10476 ± 1762	49.14	11.2 ± 3.2	27.2 ± 9.5	16.0

PMNs: segmented polymorphonuclear leukocytes.

Fig. 2. Macroscopic examination of the injected thigh of the rats. (A) Normal control: No redness, swelling or abscess formation was observed. (B) The rat with *S. aureus* injection: Abscess formation was observed (arrows).

Fig. 3. Microscopic pathologic examination. (HE stain, 100 ×) (A) Normal control. Intact muscular structure with no significant inflammatory cells infiltration. (B) *E. coli* group. Mild inflammatory cells infiltration between the muscle fibers. (C) *S. pneumoniae* group. Obvious infiltration by inflammatory cells in the muscle with destruction of the muscular structure. (D) *S. Aureus* group. Significant infiltration of the inflammatory cells with abscess formation (arrows). Destruction of the muscular structure was also significant.

Table 2. The ratio of gallium uptake between infectious tissue and normal tissue after intramuscular injection of various organisms into the left caudal thigh of rats

	L/N Ratio	
	24 hr	48 hr
Control	1.01 $\pm$ 0.05	1.04 $\pm$ 0.04
<i>S. aureus</i>	2.14 $\pm$ 0.29	2.00 $\pm$ 0.40
<i>E. coli</i>	1.27 $\pm$ 0.16	1.35 $\pm$ 0.14
<i>S. pneumoniae</i>	1.41 $\pm$ 0.30	1.48 $\pm$ 0.34

Shown as MEAN $\pm$ STD.

RESULTS

Hematologic results: The hematologic data after intramuscular injection of various micro-organisms into the left caudal thigh of rats are shown in Table 1. Intramuscular injection of these bacilli resulted in obvious change in the WBC population in the rats. The increase in WBC counts was most significant in the *S. aureus* group. The WBC counts increased from 7638 to 12682 counts/ml 48 hr after the injection. The increase in the WBC population was as high as 66%. As for the other three groups, the increase was 49.1% for the *S. pneumoniae* group, 22.3% for the *E. coli* group and only 11% for the normal controls. The increase in PMNs was most significant in the rats with the *S. aureus* injection. The percentage of PMNs increased from 10.6% to 39.7% 48 hr after injection of *S. aureus* (an increase of 29.1%). The rats with *S. pneumoniae* injection also showed an increase in the PMNs percentage (16%) but no increase in PMNs was noted in either the *E. coli* or the control group.

Results of pathologic examination: Macroscopic analysis indicated infection of the injected sites in the three groups receiving bacilli, especially the *S. aureus* group. Abscess formation was noted, although no swelling or abscess formation was observed in the normal controls (Fig. 2). The microscopic pathological analysis showed that infiltration by inflammatory cells occurred in all three groups receiving the bacilli, but not in the control group. The infiltration by inflammatory cells was most significant in the *S. aureus* group and less significant in the *E. coli* group (Fig. 3).

Scintigraphic results: The L/N ratios after intramuscular injection of various organisms into the left caudal thigh of the rats are shown in Table 2. The rats with *S. aureus* injection had significant gallium uptake at the infection site and the highest L/N ratio of 2.14 on the 24-hr image and 2.0 on the 48-hr image (Fig. 4). The rats with *S. pneumoniae* had

the second highest L/N ratio (1.41 at 24 hr and 1.48 at 48 hr). The L/N ratio for the *E. coli* group was 1.27 at 24 hr and 1.35 at 48 hr. No obvious abnormal gallium uptake was demonstrated in the controls.

Statistical results: The statistical analysis of the hematological data and the ratio of gallium uptake in infected rats caused by various organisms is shown in Table 3. The 48-hr WBC counts in the *S. aureus* group was significantly higher than those of the control, *E. coli* and *S. pneumoniae* groups. In addition, the *E. coli* and *S. pneumoniae* groups also had significantly higher 48-hr WBC counts than the control group. The 48-hr PMNs percentage in the *S. aureus* group was significantly higher than those of the control and *E. coli* groups but not statistically higher than the *S. pneumoniae* group. The 48-hr PMNs percentage in both *E. coli* and *S. pneumoniae* groups showed more statistical increase than that of the control group. The L/N ratios for the *S. aureus*, *E. coli* and *S. pneumoniae* groups were significantly higher than that of the normal control on both 24-hr and 48-hr images. In addition, the *S. aureus* group also had a significantly higher L/N ratio than the *E. coli* and *S. pneumoniae* groups.

DISCUSSION

Ideally, an animal model should be inexpensive and easy to establish. SD rats are cheap, easy to obtain and easy to take care of, which makes it a good candidate for the creation of an infectious animal model. According to our findings, both *S. aureus* and *S. pneumoniae* were clearly highly invasive in SD rats. Both bacteria are gram positive but *E. coli*, a gram-negative pathogen, was less invasive in the SD rats. Among these three bacteria, *S. aureus* resulted in the most significant symptoms and signs of infection. The formation of an abscess on the site of injection, the increase in WBC and proportion of PMNs in the blood were significantly increased in this group compared to the controls. PMNs are mature phagocytes that migrate through tissues to destroy microbes and respond to inflammatory stimuli; their presence is always indicative of an acute infection. *S. aureus* infection is very common in hospitals. This pathogen can produce many toxins that cause illness in humans, sometimes leading to death [11, 12, 19]. *S. aureus* can adhere to the surface of endothelial cells and damage host cell membranes. This bacterium is of particular interest to microbiologists and public health officials because of outbreaks of antibiotic-resistant infections [11, 12]. Therefore, *S. aureus* is an ideal bacterium for the creation of an animal

Fig. 4. Gallium scan (dorsal view) of the rats injected with various organisms. The first row is for the *S. aureus* group: (A) On the 24-hr image, significantly increased gallium uptake (arrows) at the infected left thigh with an L/N ratio of 2.26. In addition, physiologic bowel activity is also noted in the abdomen (arrow heads) (B) On the 48-hr image, the uptake of gallium in the infected thigh showed no apparent change with an L/N ratio of 2.35 (arrows). The second row is for the *S. pneumoniae* group: (C) On the 24-hr image, increased gallium uptake (arrows) in the infected left thigh with an L/N ratio of 1.69, (D) On the 48-hr image, the uptake of gallium in the infected thigh showed no apparent change with an L/N ratio of 1.65 (arrows). The third row is for the *E. coli*: (E) On the 24-hr image, increased gallium uptake (arrows) in the infected left thigh with an L/N ratio of 1.42, (F) On the 48-hr image, the uptake of gallium in the infected thigh showed no apparent change with an L/N ratio of 1.41 (arrows).

Table 3. Statistical analysis of the hematological data and the ratio of gallium uptake in infected rats caused by various organisms

	Control	<i>S. aureus</i>	<i>E. coli</i>	<i>S. pneumoniae</i>
WBC at 48 hr				
Control	—	0.016	0.075	0.347
<i>S. aureus</i>	0.016	—	0.028	0.028
<i>E. coli</i>	0.075	0.028	—	0.916
<i>S. pneumoniae</i>	0.347	0.028	0.916	—
PMNs at 48 hr				
Control	—	0.047	0.753	0.251
<i>S. aureus</i>	0.047	—	0.015	0.174
<i>E. coli</i>	0.753	0.015	—	0.027
<i>S. pneumoniae</i>	0.251	0.174	0.027	—
L/N ratio 24 hr				
Control	—	0.009	0.016	0.016
<i>S. aureus</i>	0.009	—	0.009	0.009
<i>E. coli</i>	0.016	0.009	—	0.464
<i>S. pneumoniae</i>	0.016	0.009	0.464	—
L/N ratio 48 hr				
Control	—	0.009	0.009	0.047
<i>S. aureus</i>	0.009	—	0.047	0.047
<i>E. coli</i>	0.009	0.047	—	0.464
<i>S. pneumoniae</i>	0.047	0.047	0.464	—

model for investigating new infectious diagnostic methods or therapeutic drugs. The purpose of the study was to develop a small animal model of soft tissue infection without mortality which mimicked the clinical condition in humans.

Gallium imaging has been used for decades to detect infection clinically [3, 5, 10, 13, 14, 23]. As gallium binds to transferrin, leukocyte lactoferrin, bacterial siderophores and inflammatory proteins, it can be used to localize foci of infection. In most infection areas, accumulation of gallium is always significant. In our study, the rats with the *S. aureus* injection had the highest gallium uptake at the infection site (thigh) when compared to those with *S. pneumoniae* and *E. coli* injection. These findings also corresponded to the results of blood tests and histopathologic examinations. Gallium citrate is not only accumulated in an infectious lesion but also in an inflammatory lesion [11, 12]. In addition, Ga-67 citrate has been used as a tumor seeker for more than two decades and proven to be able to localize in a wide variety of tumors [1, 17]. The uptake of gallium in the infection, inflammation or malignancy is not specific. Therefore, sometimes it is difficult to differentiate malignancy from infection or infection from inflammation only based on a gallium scan.

Moreover, physiological excretion of gallium into the intestines can also be a problem. The physiological bowel activity is sometimes difficult to differentiate from an intra-abdominal infection and could cause a false positive gallium scan. There are two methods which may decrease the interference of normal gallium bowel activity during interpreta-

tion: (1) cathartics and enemas, (2) serial imaging. Bowel preparation before gallium imaging has been reported to be useful in decreasing physical accumulation of gallium in the bowel [3, 8, 18]. Serial imaging is a useful method for distinguishing normal bowel activity from intra-abdominal infection. Normal gallium activity in the bowel will move with time, whereas an infectious lesion or abscess will appear unchanged.

In our study, all three pathogens, *S. aureus*, *S. pneumoniae* and *E. coli*, resulted in varying degrees of soft tissue infection in SD rats whereas the *S. aureus* had the best invasive ability in SD rats. We conclude that injection of *S. aureus* into the thigh of a SD rat is a good method to create soft tissue infection.

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