

STAPHYLOCOCCAL CATALASE REGULATES ITS VIRULENCE AND INDUCES ARTHRITIS IN CATALASE DEFICIENT MICE

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Abstract : To figure out whether *in vivo* expression of *Staphylococcal* catalase could correlate with the virulence and pathogenicity of the bacteria in the catalase deficient Swiss albino mice. 3 Amino 1, 2, 4 triazole (ATZ) (2 mg/g body wt) treated catalase deficient mice were infected with virulent *S. aureus* and bacterial burden, antioxidant enzyme levels were estimated after 3, 5 and 10 days of infection. Arthritic scores and levels of serum uric acid in mice were also determined. ATZ treatment was found to have slowed down the clearance of bacteria from blood and their rapid elimination from spleen. Increased tissue catalase activities in the spleen and liver of ATZ pre-treated mice even after 5 days of infection suggested its bacterial origin. It was further verified by zymographic analysis. Increased swelling of joints was observed after 5 days of infection. Uric acid level was found lesser in ATZ treated mice. ATZ treatment slowed the bacterial passage from blood with a lower tissue anti-oxidant enzymes leading to induction of joint inflammation.

Key words : intracellular survival *Staphylococcus aureus*
murine peritoneal macrophages arthritis
catalase deficient mice

INTRODUCTION

Staphylococcus aureus (*S. aureus*) has always been considered a human pathogen with a wide array of disease conditions, ranging from minor skin abscesses to life threatening endocarditis, osteomyelitis, and pneumonia (1). Once staphylococci gained access to the tissues, the bacteria must find there a favorable environment; the opportunity to grow and multiply is of

immediate importance. Circumstances favorable to the staphylococci are contributed by local conditions in the tissues where they lodge (2). In order for *S. aureus* to initiate invasive infection, it must gain access to internal tissues or vasculature of its host (3). Once inside the host, *S. aureus* likely undergoes a shift in gene expression resulting in controlled production of virulence determinants that facilitate infection (4). The bacterial components

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and secreted products that affect the pathogenesis of *S. aureus* infection are numerous (5). The complex relationships between host immunity and microbial pathogenesis, the balance between protective immunity and immuno-pathology and strategies to exploit the many networks that are involved in antimicrobial strategies have been reported (6). *S. aureus* is able to use a number of immuno-avoidance strategies during infection (7). However, the organ specific immune response to *staphylococcal* infection is not well defined. Experimental evidence indicated that the production of reactive oxygen intermediates (ROI) represent one of the primary mechanisms utilized by host macrophages for limiting the intracellular replication of the staphylococci (8). Accordingly, the staphylococci appear to be well equipped to deal with the exposure of ROI to encounter during their intracellular residence in host macrophages (9). Staphylococci like many other organisms rely on antioxidant defense mechanisms for protection against oxidative damage (10). A prerequisite for the success of human pathogenic staphylococci is their ability to defend against ROI elicited by host effector cells during the course of an infection (11).

Catalase is known to detoxify hydrogen peroxide (H_2O_2) and was proposed to be a major virulence determinant in *S. aureus* (12). Thus oxidative stress resistance is an important factor in the ability of *S. aureus* to persist in the hospital environment and so contribute to the spread of human disease (13). Several recent studies have greatly expanded our understanding of the contribution of the enzymatic constituents of *S. aureus* antioxidant defense system to protect against oxidative damage (9). In a

recent study we have demonstrated the intracellular survival of pathogenic *S. aureus* in murine macrophages correlating the production of catalase and super oxide dismutase (SOD). Interestingly, we found that *staphylococcal* catalase and SOD combat ROI enabling *S. aureus* to persist within macrophages, inducing local inflammation, causing greater induction of serum tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) (14). Whether inactivation of host catalase (by 3 amino 1, 2, 4 triazole i.e. ATZ) in mice resulted in enhancement of virulence, increased colonization of the spleen or blood of mice is a pertinent question. The present study was undertaken to investigate the role of catalase in the pathogenesis of *staphylococcal* infection, its establishment and persistence leading to induction of arthritis in mice.

MATERIALS AND METHODS

Bacterial strain

Pathogenic *Staphylococcus aureus* (ICH-757) was obtained from Institute of Child Health, Kolkata and was maintained in our laboratory. ICH-757 was catalase positive and coagulase negative. *S. aureus* were cultured in nutrient broth at 37°C overnight in an orbital rotary shaker. Fresh bacterial cultures were made by diluting the overnight bacterial culture 1:100 in fresh nutrient broth and grown at 37°C with tumbling for 3 h.

Animals

Male Swiss albino mice, 6–8 weeks of age with body weight 20±4 g were purchased from local registered animal suppliers to our department. Upon arrival, mice were randomized into plastic cages with filter

bonnets and saw dust bedding, followed by a one-week quarantine period. Mice were housed 8 per cage with food and water ad libitum. Animal holding rooms were maintained at 21 to 24°C and 40–60% humidity with a 12h light dark cycle. Animals were divided into 4 groups containing 8 mice in each group; (A) Control, (B) ATZ treated, (C) *S. aureus* infected, (D) ATZ treated and *S. aureus* infected. All experiments used mice as accredited by the Institutional Animal Ethical Committee.

Chemicals and fine reagents

RPMI-1640 and FBS were purchased from Hyclone, 1725 South Hyclone Road, Logan, Utah 84321. ATZ was purchased from ICN Biomedicals, Inc. 1263 South Chillicothe Road, Ohio 44202. BSA was purchased from Sigma Chemicals, St. Louis, MO 63178, USA. All other reagents were of analytical grade.

Isolation of peritoneal macrophages from normal mice

Murine peritoneal macrophages were isolated as according to a method described elsewhere (15). Briefly, peritoneal fluids of the mice, previously injected intraperitoneally with 3% starch in 0.9% NaCl, 3 days prior to the sacrifice, were collected and centrifuged. The cell pellets were re-suspended in RPMI 1640-FBS (5%) and were allowed for plastic adherence. Adherent cells were collected and finally suspended in RPMI 1640-FBS (5%).

Preparation of virulent *S. aureus* (ICH-757) from the intracellularly survived bacteria

Intracellular killing of *S. aureus* by macrophages was determined as described by Leigh et al (16). Briefly, equal volumes

of a suspension of 10^7 macrophages/ml and 10^7 bacteria/ml in Hank's balanced salt solution (HBSS)-gelatin were incubated for 1h at 37°C under slow rotation. Phagocytosis was stopped by transferring the tubes to the crushed ice and the non-ingested bacteria were removed by differential centrifugation for 4 min at 110 xg and two washes at 4°C. A suspension of 5×10^6 macrophages/ml that had ingested *S. aureus* was re-incubated at 37°C under slow rotation. At different time after re-incubation, intracellular killing was terminated by transferring the tubes in crushed ice and spinning the cells at 4°C. The macrophages were disrupted by adding 1 ml of distilled water containing 0.01% bovine serum albumin (BSA), to the cell pellet and vigorously shaking the suspension on a vortex mixture for 1 min. The number of viable bacteria was then determined by plating 10 fold serial dilutions of the suspensions into agar plates. Serial 10 fold dilutions in saline was made over a range assuming that at least one dilutions will contain between 100 and 1,000 viable bacteria per ml. Aliquots (0.1 ml) of the 3 highest dilutions were pipetted onto each of 2 agar plates, the plates were incubated at 37°C for 18–24 h, and the number of colonies were counted. The number of viable bacteria per ml was calculated from the mean numbers of the colony counts of duplicate plates of the two highest dilutions, providing the plates contained <500 colonies.

The bacteria that were survived after time dependent phagocytosis as obtained from the plates of respective re-incubation times (plates of intracellular viable *S. aureus* from the above experiment) were further cultured in nutrient broth.

***in vivo* infection to mice**

Intracellularly survived clinical isolates of *S. aureus* (ICH-757) recovered after 90 min re-incubation with macrophages were further cultured and injected intravenously (10^6 cfu/mouse of average body weight of 20 g) via the tail vein in 0.1 ml saline to each of the mice. Control mice received 0.1 ml of sterile saline through the tail vein. The mice were monitored daily.

Treatment of mice with 3 amino 1, 2, 4 triazole (ATZ) and infection with *S. aureus*

Mice used in these experiments were wild type Swiss albino mice and were made catalase deficient by giving intraperitoneal injection of 2 mg of ATZ in 0.1 ml saline per g of body weight (17). After 24 h of ATZ treatment, animals were infected as before. Because of the somewhat subjective nature of the observations to be made, animals and treatment solutions were provided coded to the investigator who did the scoring. The code was made known only after the observations were recorded.

***in vivo* clearance of bacterial load from blood and spleen**

At 0, 3, 5, 10 days post infection blood was collected and organs were dissected aseptically. The blood from each infected mouse was diluted and plated onto nutrient agar. Spleens were excised, weighed, homogenized, diluted in saline and plated in nutrient agar. The plates were maintained at 37°C for 48 h and bacterial colonies were counted. Results were expressed as the number of bacterial cfu/ml of blood and spleen homogenate (18).

Tissue homogenization

Spleen or liver tissues were separately

homogenized in 10 volumes of 50 m.mol/L phosphate buffer (pH-7.4) on ice for 30 s using a power driven polytron homogenizer. The homogenate was transferred into centrifuge tubes and centrifuged at 9000 xg at 4°C for 20 min. The supernatant was used to measure antioxidant enzyme activity and the amount of protein present. The cell free homogenate was collected and stored at -80°C until further use.

Assay of catalase activity

Catalase activity in the cell free homogenate was determined spectrophotometrically (Analab, Japan) by measuring the decrease in H_2O_2 concentration at 240 nm. At time zero, 10 μ l of homogenate was added to 2.89 ml of 50 mM potassium phosphate buffer (pH 7.4) taken in a quartz cuvette. To it 100 μ l of 300 mM H_2O_2 was added and the reading was taken at 240 nm for 3 min at an interval of 20s. Catalase activity was expressed in terms of m.mole/min.mg tissue protein (19).

Assay of superoxide dismutase (SOD) activity

100 μ l of tissue homogenate was mixed with 1.5 ml of Tris EDTA-HCl buffer (pH 7.4), then 100 μ l of 7.2 mmol/L pyrogallol was added and the reaction mixture was incubated at 25°C for 10 min. The reaction was terminated after the addition of 50 μ l of 1 m.mol/L HCl and measured at 420 nm. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as U/mg protein (19).

Electrophoresis and in situ staining of catalase

Electrophoresis was performed on non-denaturing acrylamide gels using the Bio-Rad Mini system with Tris/glycine buffer.

Splenic homogenates of ATZ treated or non-treated infected mice recovered after 5 days and 10 days of infection were applied to 10% bisacrylamide gels prepared with 1.5 M Tris-HCl, pH 8.0. Electrophoresis was carried out at 200 V. Catalase activity was visualized on non-denaturing acrylamide gels following the methodology as described by Woodbury et al (20). After electrophoresis, gels were washed three times in distilled water for 20 min and soaked in a solution of 0.015% H₂O₂. Activity was then visualized by transferring the gels to a solution of 1% ferric chloride, 1% potassium ferricyanide. Regions corresponding to catalase activity were determined as yellow bands on a green background.

Clinical evaluation of mortality and arthritis

Mice were given intravenous injection (via the tail vein) with *S. aureus* (10⁶ cfu/mouse of average body weight of 20 g) as described previously. Their limbs were inspected visually everyday. Arthritis was defined as visible joint swellings of at least one joint. To evaluate the intensity of arthritis a clinical scoring system of 0–4 for each limb was used (21). 0: no swelling, 1: mild swelling, 2: moderate swelling, 3: marked swelling, 4: maximum swelling.

Estimation of serum uric acid level

Protein free filtrate from the serum of different groups was allowed to react with the uric acid reagent containing phosphotungstic acid which upon reaction with the uric acid gives blue colored complex in presence of sodium carbonate solution. The coloured product formation is due to reduction of phosphotungstic acid by uric acid in alkaline medium. The reaction of uric acid in alkaline solution with phosphotungstic

acid reagent oxidizes uric acid to allantoin and itself is reduced to tungsten blue which was measured at 620 nm (22).

Statistical analysis

One-way model I ANOVA was performed to analyze the difference between mean intracellularly survived bacteria for the experiments involving cfu count, for *in vivo* clearance of bacterial load from blood and spleen. For SOD and catalase assay, serum uric acid level one tailed Student's t-test was performed to analyze the significant difference between means. A level of 0.05 is considered to be significant.

RESULTS

Number of intracellularly survived bacteria after one hour phagocytosis followed by time dependent re-incubation

Number of *S. aureus* (ICH-757) survived after initial 1 h phagocytosis by peritoneal macrophages followed by time dependent re-incubation at 37°C was estimated by determining bacterial cfu count. Number of bacteria survived intracellularly inside macrophages after 90 min of re-incubation was least than 0 min, 30 min and 60 min (P<0.05) of re-incubation period suggesting maximum killing of bacteria by the murine peritoneal macrophages during that period (Fig. 1).

Clearance of *S. aureus* (ICH-757) from the spleen and blood of infected mice

Mice were infected (*in vivo*) with 90 min re-incubated intracellularly survived bacteria (ICH-757) and the bacterial burden in the spleen (Fig. 2) was increased significantly after 10 days (P<0.05) of infection than in the blood (Fig. 3) of infected mice. However, pretreatment of ATZ reduces (P<0.05) the

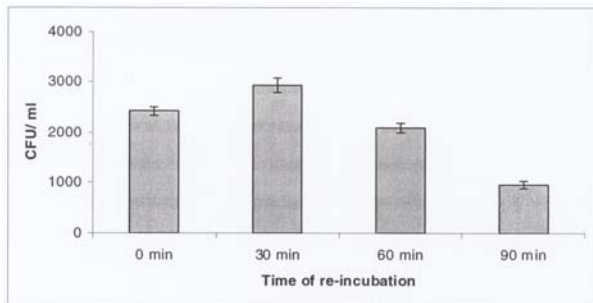


Fig. 1 : Number of intracellularly survived bacteria after one hour phagocytosis followed by time dependent re-incubation. *S. aureus* (10^7 cells/ml) were allowed to interact with murine peritoneal macrophages (10^7 cells/ml) initially for 1h and then were re-incubated for the time indicated. Survival of *S. aureus* (ICH-757) in murine peritoneal macrophages was determined using dilution plate counts. Numbers of surviving bacteria were represented as cfu/ml of macrophages. Results are shown as means \pm SD ($P<0.05$) of three independent experiments.

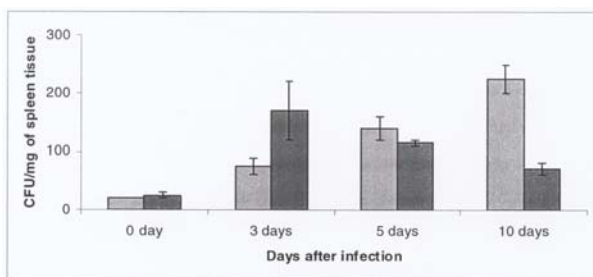


Fig. 2 : Bacterial density in spleen when normal and catalase deficient mice were infected (i.v.) with intracellularly survived *S. aureus* recovered after 90 min of re-incubation with macrophages. Normal and catalase deficient mice were infected with the 90 min re-incubated *S. aureus* as mentioned previously and the numbers of bacteria present in the spleen after 0, 3, 5 and 10 days of infection were estimated by colony counting method. Numbers of surviving bacteria were represented as cfu/mg of spleen. Results are shown as means \pm SD ($P<0.05$) of three independent experiments. ■ *S. aureus* infected, ■ ATZ treated and *S. aureus* infected.

Catalase activity in the spleen and liver

As ATZ pretreatment lowers tissue catalase level we are interested to see the catalase activity in the liver and spleen of *S. aureus* infected mice. The result showed decreased catalase content in the ATZ

bacterial burden in the spleen after 10 days of *S. aureus* infection than that in the blood (Figs. 2 and 3).

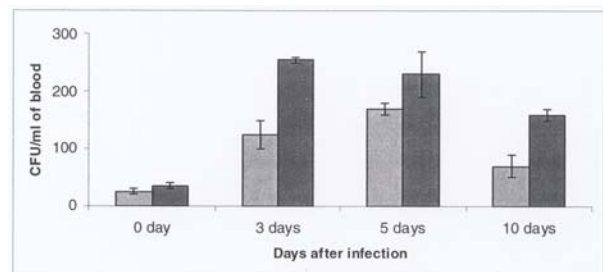
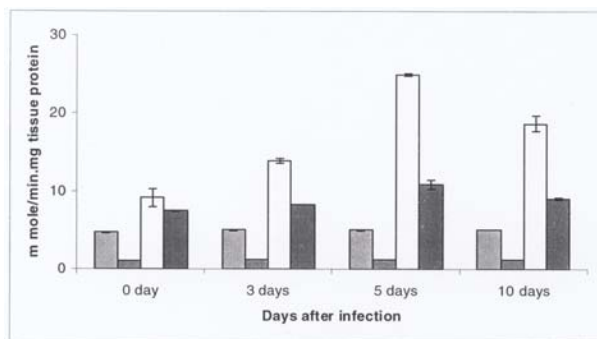


Fig. 3 : Bacterial density in blood when normal and catalase deficient mice were infected (i.v.) with intracellularly survived *S. aureus* recovered after 90 min of re-incubation with macrophages. Normal and catalase deficient mice were infected with the 90 min re-incubated intracellularly survived *S. aureus* as mentioned previously and the numbers of bacteria present in blood after zero, 3, 5 and 10 days of infection were estimated by colony counting method. Numbers of surviving bacteria were represented as cfu/ml of blood. Results are shown as means \pm SD ($P<0.05$) of three independent experiments, ■ *S. aureus* infected, ■ ATZ treated and *S. aureus* infected.

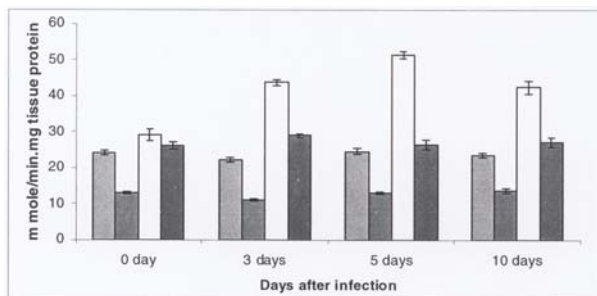
treated uninfected spleen than that of uninfected control. The catalase activity was highest in the spleen of both ATZ non-treated and ATZ-treated plus infected group after 5 days of infection ($P<0.05$) which decreased afterwards. However in the ATZ treated plus infected group the level of catalase activity was found lower ($P<0.05$) compared with the ATZ non-treated plus infected group (Fig. 4A). We have found an identical pattern of results in case of catalase activity of liver in all the four experimental groups (Fig. 4B).

SOD activity in the spleen and liver

SOD activity in the spleen was significantly ($P<0.05$) higher after 5 days of *S. aureus* infection than the uninfected control group. Pretreatment of mice with



4(A)



4(B)

Fig. 4 : Catalase activity in the spleen and liver. Catalase activity in the spleen (A) and liver (B) of all the four experimental groups were estimated as mentioned in the methodology. Catalase activity was expressed in terms of m.mole/min.mg of spleen and liver tissues respectively. Results are shown as means±SD (P<0.05) of three independent experiments. ■ Control, ■ ATZ treated, □ *S. aureus* infected, ■ ATZ treated and *S. aureus* infected.

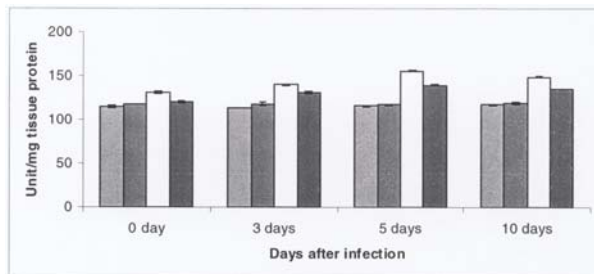
ATZ was unable to reduce the SOD content in the spleen. However, ATZ pretreatment followed by *S. aureus* infection reduced (P<0.05) SOD content in the spleen than the ATZ non-treated *S. aureus* infected mice (Fig. 5A)

SOD activity was significantly (P<0.05) higher in the liver of *S. aureus* infected mice than the uninfected control group. No significant alteration in the liver SOD level was found after ATZ treatment. However, ATZ pretreatment followed by *S. aureus* infection reduced (P<0.05) SOD content in

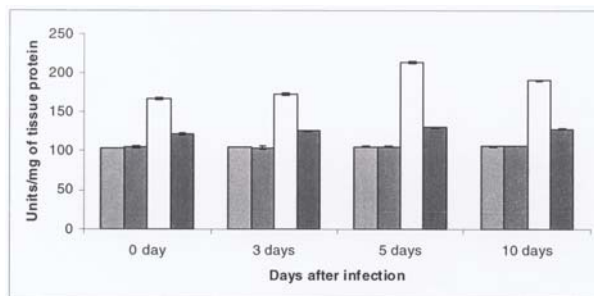
the liver than the ATZ non-treated *S. aureus* infected mice (Fig. 5B).

Catalase activity staining

Fig. 6 shows the native polyacrylamide gel electrophoretic (PAGE) analysis of catalase activities in extracts of ATZ treated and non treated *S. aureus* (ICH-757) infected spleen in 8% native polyacrylamide gels. A single band was observed in each of the four lanes of gel. A prominent yellow band with increased intensity on dark green background confers the presence of catalase in the lysates. Increased catalase expression in the spleen after 5 days of ICH-757



5(A)



5(B)

Fig. 5 : SOD activity in the spleen and liver. SOD activity in the spleen (A) and liver (B) of all the four experimental groups were estimated as mentioned in the methodology. SOD activity was expressed in terms of Unit of SOD/mg of spleen and liver tissues respectively. Results are shown as means±SD (P<0.05) of three independent experiments. ■ Control, ■ ATZ treated, □ *S. aureus* infected, ■ ATZ treated and *S. aureus* infected.

infection was observed in the zymographic analysis of their crude lysates (Lane 1) which was reduced after 10 days of infection (Lane 3). ATZ pretreatment reduced the catalase content in the spleen more after 10 days (Lane 4) compared to 5 days (Lane 2) of *S. aureus* infection (Fig. 6).

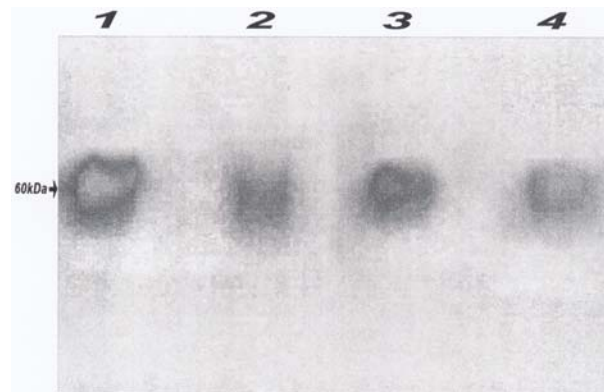


Fig. 6 : Zymographic analysis of catalase activity in the spleen of normal and catalase deficient mice. Lane 1, catalase activity in the spleen after 5 days of *S. aureus* infection in ATZ non-treated mice; Lane 2, catalase activity in the spleen after 5 days of *S. aureus* infection in ATZ treated mice; Lane 3, catalase activity in the spleen after 10 days of *S. aureus* infection in ATZ non-treated mice; and Lane 4, catalase activity in the spleen after 10 days of *S. aureus* infection in ATZ treated mice.

TABLE I: Serum uric acid concentration.

Increased serum uric acid concentration ($P < 0.05$) was found in *S. aureus* infected mice compared to uninfected control group. However, ATZ treatment could not reduce serum uric acid level significantly. Uric acid content in the serum of uninfected control, ATZ non-treated infected and ATZ treated infected group is expressed as mg/ml of serum. Results are shown as means \pm SD ($P < 0.05$) of three independent experiments.

Days after infection	Control	<i>S. aureus</i> infected	ATZ-treated and <i>S. aureus</i> infected
Zero	28.5 \pm 8.5	26.7 \pm 5.6	22.7 \pm 1.4
3	35.9 \pm 1.8	44.6 \pm 5.4	35.3 \pm 2.3
5	29.8 \pm 5.3	52.1 \pm 2.5	47.4 \pm 2.3
10	19.1 \pm 2.0	55.1 \pm 1.5	53.5 \pm 2.5

Values are shown as means \pm SD ($P < 0.05$).

TABLE II: Clinical evaluation of arthritis.

Arthritis was defined as visible joint swelling of at least one joint. The arthritic index was constructed by adding the scores from all 4 limbs for each animal. According to the score, 0: no swelling, 1: mild swelling, 2: moderate swelling, 3: marked swelling, 4: maximum swelling. The result showed that there was an increase in the swelling of joints in bacteria infected mice after 3 days of infection compared to the control group of mice which advanced progressively with the increase of post infection time. In case of ATZ pretreated *S. aureus* infected mice, there was marked swelling after 5 days and 10 days of infection compared to the ATZ non-treated infected mice.

Name of group	0 day		3 days		5 days		10 days	
	R	L	R	L	R	L	R	L
Control-ankle	0	1	1	0	0	1	1	1
Control-knee	0	0	1	1	0	1	1	1
Control-elbow	1	0	0	1	1	1	1	1
Control-wrist	0	0	1	1	1	1	0	1
<i>S. aureus</i> Infected-ankle	0	0	1	2	2	1	4	3
<i>S. aureus</i> Infected-knee	0	1	1	0	1	1	3	4
<i>S. aureus</i> Infected-elbow	0	0	2	3	2	4	4	3
<i>S. aureus</i> Infected-wrist	2	0	0	2	2	2	2	3
ATZ Pretreated <i>S. aureus</i> Infected-ankle	2	0	3	4	3	4	3	3
ATZ Pretreated <i>S. aureus</i> Infected-knee	1	0	1	1	1	1	3	4
ATZ Pretreated <i>S. aureus</i> Infected-elbow	0	0	2	3	2	2	3	4
ATZ Pretreated <i>S. aureus</i> Infected-wrist	0	1	1	0	2	3	4	4

R-Right; L-Left

Serum uric acid level

Uric acid level was significantly ($P < 0.05$) increased in the serum of *S. aureus* infected mice as compared to uninfected control group (Table I).

Assessment of arthritic index

The result showed that there was increased swelling of joints in *S. aureus* infected mice after 5 and 10 days of infection compared to uninfected control group (Table II). In case of ATZ pretreatment followed by *S. aureus* infection, there was also marked joint swelling after 5 and 10 days of infection compared to the ATZ non-treated infected group.

DISCUSSION

Staphylococcus aureus like other virulent bacteria sometimes manage to survive and multiply within the host cells by impairing the host's natural defense mechanism and these intracellularly survived bacteria may be more virulent and lead to pathogenic effects *in vivo*. In the present study we use those intracellularly survived bacteria for *in vivo* infection to catalase deficient mice.

Effective phagocytic activity has been observed for the murine peritoneal macrophages at 90 min re-incubation time. Least cfu count of the intracellularly survived *S. aureus* after 90 min of re-incubation suggests the maximum intracellular killing activities to have occurred by that time. However, we did not extend the re incubation time beyond 90 min. So the fate of the bacteria after 90 min re-incubation time cannot be visualized from this study. Thus the gradual decrease in the number of intracellularly survived bacteria after different re-incubation period may suggest that the phagocytic capacity of the murine peritoneal macrophages for ICH-757 increases with the increase of re-incubation time. The Gram staining of the intracellularly survived bacteria showed the identical features as that of the mother strains suggesting the presence of same

viable bacteria in the re incubated plates. But these survived bacteria may have the highest virulence property since they overcome the host's immune system for longer period.

We have reported that the intracellularly survived bacteria recovered after 90 min of re-incubation were found more virulent in the sense to nullify the toxic effects of H₂O₂ by expressing more catalase (14). So, we attempted to delineate whether these bacteria could potentiate inflammatory response in the *in vivo* condition when animals were made catalase deficient. We were also interested to know whether bacterial catalase plays any significant role in the *in vivo staphylococcal* pathogenesis ultimately leading to arthritis.

It is notable that *S. aureus* burden in the spleen was highest when recovered from catalase deficient mice 3 days after infection and it was decreased with time. However, the bacterial density in the spleen was increased gradually in ATZ non-treated mice. 10 days after infection, the *S. aureus* density in spleen of ATZ-non treated mice was severe, while in catalase deficient mice it was largely resolved after 10 days of infection.

Although the catalase deficient mice cannot effectively clear the bacterial density even after 10 days of infection, the bacteria were mostly cleared from the blood 10 days after infection in ATZ non-treated mice. Therefore, spleen of normal mice was more severely affected 10 days after infection than mice deficient in catalase. We assume that catalase in the ATZ non-treated infected mice kept the organism from killing themselves thus permitting elaboration of additional toxic substances.

ATZ effectively inhibits the catalase activity in solid tissues most prominently in spleen than in the liver as compared to uninfected control mice, which was also supported by previous study (17). We inhibited the tissue catalase activity by pre-treatment of mice with ATZ and subsequent *S. aureus* infection still increased the catalase activity in spleen and liver with the increase of time being highest after 5 days of infection. The level of catalase activity in the ATZ treated infected mice though somewhat lower compared to the ATZ non-treated infected group strongly suggested their bacterial origin.

A large number of studies have demonstrated the protective effect of SOD in various models of endotoxic shock; furthermore there is a large amount of evidence to show that production of reactive oxygen species (ROS) such as superoxide anion, H_2O_2 , and hydroxyl radical, occurs at the site of inflammation and contributes to tissue damage. To protect the tissues from these ROS, tissue SOD converts superoxide anion to H_2O_2 which is subsequently eliminated by tissue catalase. But as ATZ treatment inhibited the tissue catalase activity therefore, H_2O_2 generated during the tissue SOD activity started to be accumulated which inhibited further SOD release by the tissue after a certain time. Therefore, the enhanced tissue SOD activity in both liver and spleen after 5 days of *S. aureus* infection in catalase deficient mice strongly points to its bacterial origin.

From the catalase activity staining there is higher catalase expression in ATZ non-treated infected spleen after 5 and 10 days of infection which was reduced in case of ATZ pre-treated group as evident from the band intensity suggesting the catalase content are from bacterial origin. It also

suggests that *S. aureus* release catalase for survival in host cell.

Arthritis is a highly and rapidly destructive hematogenously spread joint disease in which there is accumulation of synovial fluid at the joint. After *S. aureus* infection, activated macrophages produce cytokine mediators that may trigger inflammation. Our results showed that marked joint swellings were evident in the *S. aureus* infected mice compared to the uninfected control mice after 3 days of infection which progressively increased with time. However, in ATZ treated mice significant joint swellings were very prominent after 5 days of bacterial infection compared to the ATZ non-treated infected mice which also was shown to increase with the increase of time. There is hyperuricemia after *S. aureus* infection with respect to uninfected control also corroborates with the enhanced joint swelling after 3 and 5 days of infection. However, treatment of ATZ was unable to reduce significantly the serum uric acid level after *S. aureus* infection. Joint infection caused by *S. aureus* has been reported (23). Therefore, impaired host defense mechanisms due to the inhibition of tissue catalase may cause severe joint infection that may be recognized as risk factor for bacterial arthritis. Previous studies have also supported that injection of virulent *S. aureus* may induce local inflammation due to elevated IL-6 and TNF- α which could also reach at its peak after 3 days of inoculation ultimately leading to severe joint inflammation (24).

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