Tranexamic acid, an inhibitor of Plasminogen activation aggravates stephylococcal septic arthritis

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Abstract

Staphylococcus aureus is common micro-organ in the society. The bacteria are carried on people's skins or in their noses. S. aureus can causes a wide range of minor infections like pimples, impetigo, boils, cellulitis, and other life threatening diseases like septic arthritis, sepsis, pneumonia, meningitis, endocarditis, toxic shock syndrome(TSS) and septicemia.

During the experiment the S. aureus was used to induce septic arthritis. The results showed that during the bacterial infection the sepsis response to infection causes inflammation. Due to inflammation factors like cytokines and leukocytes, the accumulation of the immune response cells will lead to synovitis and bone erosion in joints. This is due to inflammation in the joints.
According to the viable count of bacterial in the kidneys of the subject, it was shown that there was a higher accumulation of bacterial in the kidneys. Tranexamic acid (cyklokapron) was used to treat mice, which inhibits plasminogen activation to plasmin. The act of tranexamic acid showed clearly that it also aggravates staphylococcal sepsis and septic arthritis whereby this deteriorated effect is not due to down regulation of bacterial clearance. However the up-regulation of pro-inflammatory cytokines like IL-6 might be the explanation. There was indeed a tendency that IL-6 levels in the mice treated with cyklokapron were higher than in the mice in the control group. It has been shown that pro-inflammatory cytokines like TNF-alpha and IL-6 significantly contribute to mortality of LPS-endotoximic. Moreover fibrin deposition has been hypothesized to act as an inflammation initiator in autoimmune diseases. The degradation of fibrin by active plasmin maintains vascular potency whereas inhibition of fibrinolysis leads to multiple organ failure and death by causing microthrombosis in the system. This is called disseminated intravscular coagulation (DIC). Total inhibition of plasminogen activation by higher dose of cyklokapron in mice with staphylococcal sepsis leads to a higher mortality in comparison to control group.

Tranexamic acid treatment might also have some effect on the subject's weight loss because the data showed that there was a significant weight loss of about $p < 0.05$ between the mice treated with tranexamic acid compared to the mice in control group on day 6.

To summarise, the present study suggest that tranexamic acid should not be used for patients with staphylococcal sepsis.

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1. Introduction

1.1 *S. aureus*

Staphylococcus aureus (golden cluster seed or golden staph) is commonly found in human population. It's Gram-positive spherical bacterium (caccus), and it appears in pairs, short chains or bunched, grape-like clusters.

The bacteria are usually carried on the skin or in the nose. The golden staph is the common cause of staph infections. About 20-30% of the general population are carriers of staphylococcus aureus [1]. *S. aureus* can cause a wide range of infectious diseases from minor skin infections like pimples, impetigo (can also be caused by streptococcus progenes), boils, cellulitis, to life threatening diseases like pneumonia, meningitis, endocarditis, septic arthritis and septicemia. It is one of the most common causes of nosocomial infections, which often cause postsurgical wound infections. The people who are at risk for S. aureus are the patients who have low immune response like those who have undergone surgery, have burn injurys or diabetes, etc.

1-2. Staphylococcal Septic Arthritis

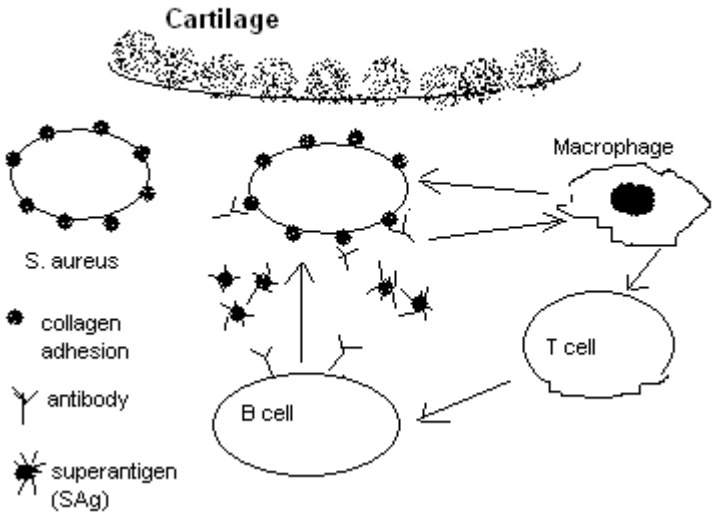

Figure 1 shows the interaction between the host defence and bacterium in sepsis arthritis

Septic arthritis (also called infectious arthritis) caused by a bacterial infection may rarely be caused by fungal or viral infection. This is an acute condition with severe joint pain, inflammation, redness, and in some cases fever and chills but it can also become chronic. Septic arthritis can affect any joint but it is most frequently found in the larger joints like knee, hip, shoulder, wrist, elbow. Commonly only one joint is affected but in some cases, more than one joint are infected. Bacterial arthritis causes acute medical emergency due to its destruction and mortality mostly in the elderly people and patients with pre-existing aseptic arthritis. It is estimented that the Gram-positive bacteria cause about 80% of all the joint infections. 60% of septic arthritis in adults is caused by *S. aureus* [2].

Septic arthritis occurs most often in patients who have had a recent traumatic injury to a joint, joint surgery or joint replacement and in patients who currently have had an infection in their blood. Micro-organisms can spread from original sites of infection into the blood, where they are able to be carried into the joints. Other additional risk factors of septic arthritis include age (>80years), immune-deficiency, or rheumatic diseases like gout or rheumatoid arthritis [3].

1-3. Staphylococcal Sepsis

Sepsis is common as a clinical syndrome, resulting from an overwhelming systemic host response to infection [6,7]. During staphylococcal infection the release of pro-inflammatory cytokines activate coagulation and down regulates anticoagulant systems and fibrinolysis. This process shifts hemostasis to pro-coagulation, which in turn deteriorates inflammation. Sepsis also causes hypovolemia and hypotension, which cause endothelial dysfunction [4,5]. During physiological condition, the endothelium provides a rich anticoagulant surface, which thus is impaired during sepsis. Sepsis is the most common cause of death among hospitalized patients in non-cardiac intensive care units [7,8]. Normally activation of coagulation and subsequent fibrin deposition are essential parts of the host defence against infectious agents due to attempt to constrain the invading microorganisms and the subsequent inflammatory response [6]. However an exaggerated response to a severe sepsis leads to a hypercoagulant situation, which in turn causes microvascular thrombosis and multiple organ dysfunctions, a syndrome called disseminated intravascular coagulation (DIC) [7].
When the inflammatory response is initiated a large number of host-derived mediators like cytokines, chemokines, and products of the complement system activate endothelial cells (ECs). The response of the endothelium to these mediators causes structural changes like cytoplasmic swelling, detachment, and functional changes like expression of adhesion molecules that will result in increased platelet adhesion and leukocyte trafficking. The common feature of endothelial dysfunction in sepsis is increased vascular permeability that results in redistribution of body fluid and edema. This fluid leakage from the intravascular space contributes to hypovolemia and hypotension which are major signs of the sepsis syndrome [9].

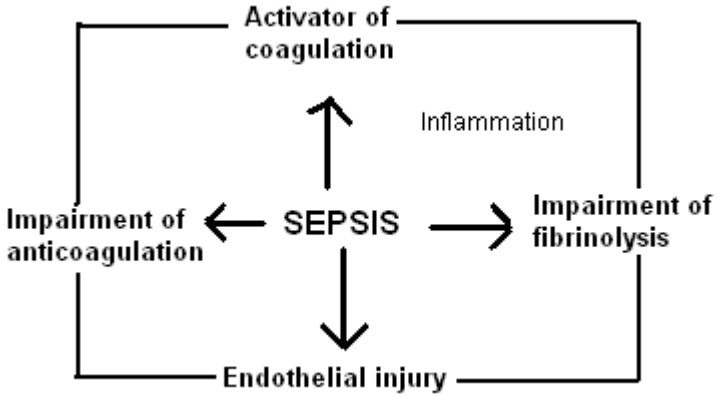

Figure 2, shows that the extensive crosstalk exists between coagulation and inflammation during sepsis. It is characterised by inflammation-induced activation of coagulation, impairment of anticoagulant systems and fibrinolysis, and dysfunction of endothelial.

1-4. Coagulation

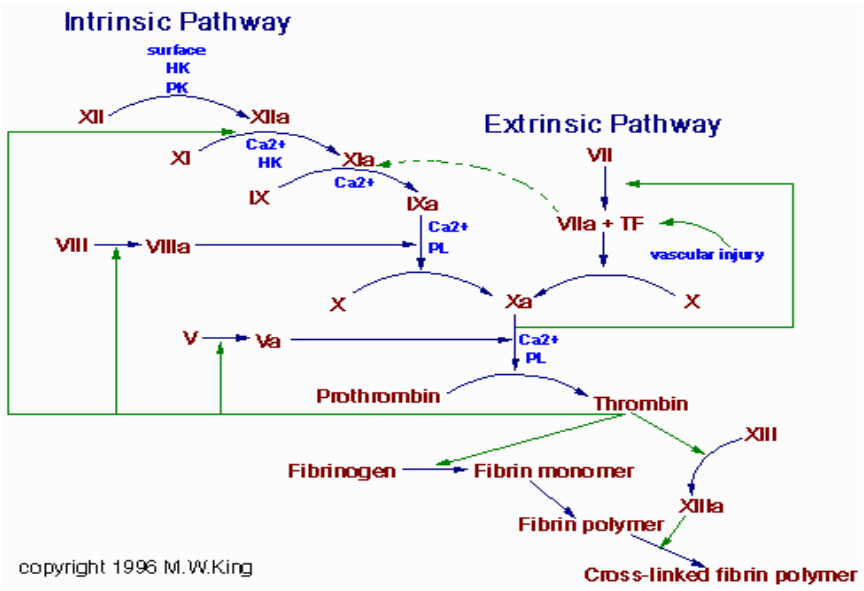

Figure 3, shows the coagulation cascades. The extrinsic pathway is initiated due to vascular injury, which leads to exposure of tissue factor (TF) (also identified as factor III), an endothelial cell-surface glycoprotein that binds phospholipid. The green dotted arrow represents a point of crossover between the extrinsic and intrinsic pathways. The two pathways converge at the activation of factor X to Xa. Factor Xa's role is to further activate factor VII toVIIa as shown with the green arrow. The active factor Xa hydrolyzes and activates pro-thrombin to thrombin. Thrombin can then activate factors XI, VIII and V furthering the cascade. Thrombin is role is to convert fribrinogen to fibrin and to activate factor XIII to XIIIa. Factor XIIIa (transglutamidase) cross-links fibrin polymers solidifying to clot. HK = high molecular weight kininogen. PK = prekallikrein. PL = phospholipid.

After injury to the vasculature, the haemostatic system act in order to prevent blood loss as shown in the figure above. Formation of a fibrin-rich clot at the site of vessel injury is a highly complex process that is orchestrated by the coagulation cascade. This cascade comprises an intricate system of serine proteases that, once activated, leads to the formation of thrombin, which subsequently cleaves fibrinogen into fibrin.
Platelets and vWF (Willebrand factor) are involved in the pathophysiology of sepsis, which is marked by the frequent occurrence of thrombocytopenia [11]. Platelets can be activated directly in sepsis by endotoxin or by pro-inflammatory cytokines [15].

1-5. Anticoagulant

The anticoagulant system is activated to prevent blood from clotting on the EC surface. The endothelium's role is to maintain the anticoagulant condition. The blood clotting is controlled by three major anticoagulant proteins, which are tissue factor pathway inhibitor (TFPI), antithrombin (AT), and activated protein C (APC) [7].

TFPI is a serine protease inhibitor that is secreted by ECs. TFPI inhibits the activation of FX to FXa by forming a TF-FVIIa complex, whereby TFPI protein binds with activate protein VIIa and inhibit the activation of protein Xa. TFPI is normally attached to the endothelium via proteoglycans (PGs), which are bound to GAGs (glycosaminoglycans) to exert its TF-FVIIa-FX – inhibiting properties on the endothelial surface [16]. During sepsis, the pro-inflammatory cytokines reduce the synthesis of GAGs on the endothelial surface, consequently affecting the TEPI function.

The AT inhibits factor Xa, thrombin, TF-FVIIa and factor IXa. AT has direct anti-inflammatory activity [12-13]. In sepsis, AT levels are decreased due to impaired synthesis and degradation by elastase secreted by neutrophiles.

The PC system controls the coagulation in association with APC, which inactivate the cofactors Va and VIIIa. The APC is generated by (trombomodulin) TM-bound thrombin, converts thrombin into an anticoagulant but it has also pro-coagulant properties [14]. TM inhibits coagulation when it generates the ant-coagulant (activated protein C) APC, which accelerates the inhibition of thrombin as well as preventing thrombin from exerting pro-coagulant properties on fibrinogen or platelets. When sepsis occurs, the protein C (PC) is impaired [17]. This will cause the increase of consumption of protein S (PS) and PC as well as decrease the production of PC by the liver due to less expression of TM on ECs [20].

1-6. Fibrinolysis is impaired in sepsis.

One of the key molecules in fibrinolytic system is plasmin that degrades the fibrin clots. Plasmin is converted from plasminogen by different proteases most commonly tissue type plasminogen activatior (tPA) and urokinase – type PA (uPA). PAI-1 is the main inhibitor of PAs and its mainly produced

by the endothelium and the liver. PAI-1 binds to active sites on tPA/uPA, thereby inhibiting PA activation.

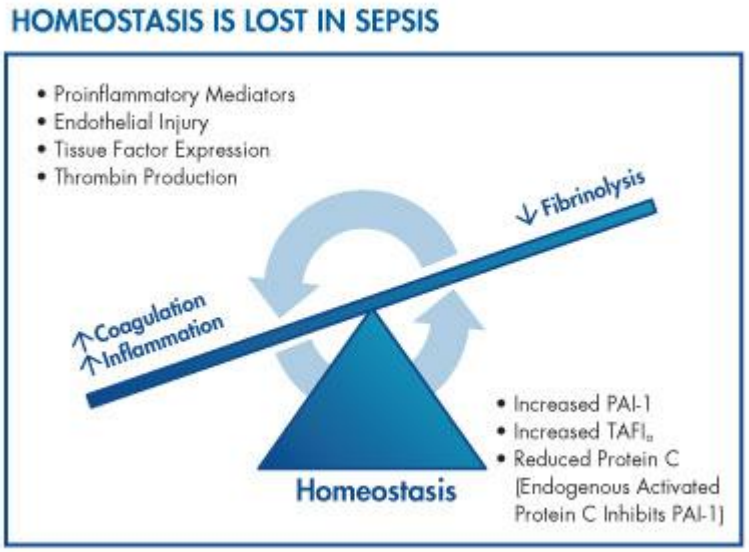

Figure 4, Homeostasis is lost in sepsis. The endothelial injury up-regulates the pro-inflammatory mediators and causes tissue factor expression, which leads to thrombin formation. The impaired fibrinolysis during the septic arthritis is due to increase of PAI-1, which decrease the plasmin activity. Plasmin activity is also reduced by thrombin-activatable fibrinolysis inhibitor (TAFI), which modifies fibrin to make a less potent cofactor for the t-PA mediated plasminogen

Plasminogen contains secondary structure motifs which bind to lysine and arginine residues on fibrin(ogen). When plasminogen is converted to plasmin it functions as a serine protease, cutting the C-terminal of these lysine and arginine residues. The fibrin monomers are polymerized to form proto-fibrils. These protofibrils have two strands normally but with a single strand, the fibrin monormers will covalently link through the actions of coagulation factor XIII. The plasmin will act on a clot and through further digestion it will lead to solubilization [16]. Tissue plasminogen activator (tPA) and urokinase are the agents that convert plasminogen to active plasmin hence allowing fibrinolysis to take place. The t-PA is released into the blood very slowly by the damaged endothelium of the blood vessels, so it will take several days to break down the clots. t-PA and urokinase are themselves inhibited by plasminogen activator inhibitor-1 (PAI-1) and action of coagulation factor XIII. Alpha 2-antiplasmin and alpha 2-macroglobulin inactivate plasmin. Plasmin activity is also reduced by thrombin-activatable fibrinolysis inhibitor (TAFI), which modifies fibrin to make a less potent cofactor for the t-PA mediated plasminogen [18-19].

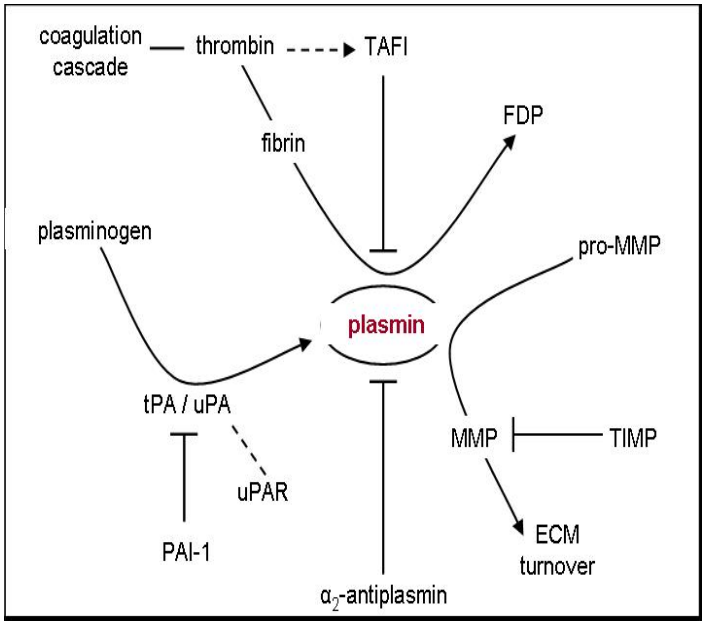

Figure 5. This is a schematic overview of the fibrinolytic system. The T-shaped lines indicate inhibition and the arrow line indicate activation. TATI prevents fibrin cleavage by plasmin. The tPA (tissue-type plasminogen activator) and uPA (urokinase plasminogen activator), activate the plasmin. Such activation is inhibited by plasminogen activator inhibitor type 1. The MMP (matrix metalloproteinases) are inhibited by TIMP (tissue inhibitors of matrix metalloproteinases) from activating the ECM (extracellular matrix) [18].

When the inflammation is initiated, fibrinolysis is inhibited by up-regulated PAI-1 levels in response to TNF-alpha and IL-1-beta [19]. The endothelium plays double roles in fibrinolysis during sepsis, by producing profibrinolytic factors as well as PAI-1.

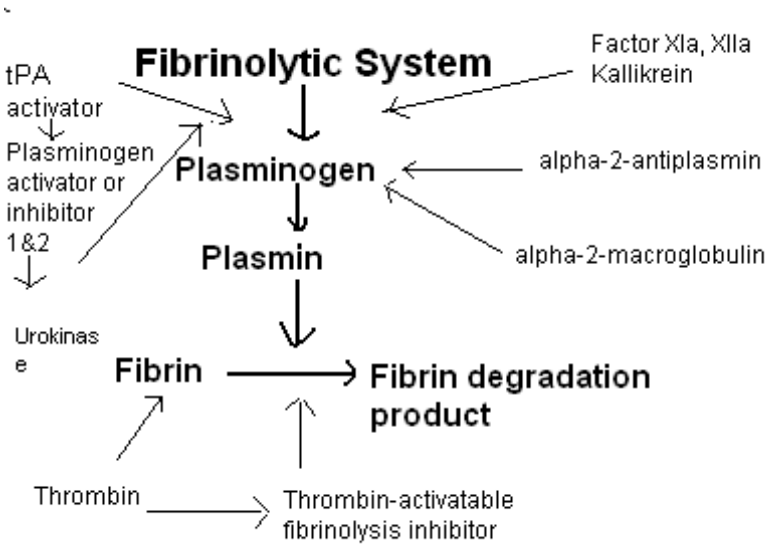

Figure 6. Fibrinolytic cascade under psysiological situation

1-7. Tranexamic acid (Cyklokapron)

Tranexamic acid is a synthetic derivative of the amino acid lysine and often used for treating excessive bleeding. It is an antifibrinolytic peptide that inhibits the activation of plasminogen to plasmin, which potentially degrades fibrin. Its antifibrinolytic effect is reversible and blocks lysine binding sites on plasminogen molecules. Prolonged treatment of tranexamic acid results in a high risk of an increased thrombotic tendency, like deep vein thrombosis. Clinically, tranexamic acid is used for treating dysfunctional uterine bleeding, heavy bleeding associated with uterine fibroids, haemophilia, angioedema, cardiac surgery and orthopedic surgery. Some studies show that patients treated with tranexamic acid are more likely to develop thrombosis and necrosis in their fibroids, resulting in pain and fever [10].

This study was to try and use tranexamic acid as a treatment for disseminated intravascular coagulation (DIC) in the patients with staphylococcal sepsis as well as analysing the role of pasminogen activation in staphylococcal septic arthritis

2. Material and Methods

2-1. *Staphylococcus aureus* strain

The staphylococcus aureus strain LS-1 was isolated from a spontaneous outbreak of murine S. aureus arthritis. Since the majority of the human septic arthritis have a haematogenic spread, so the LS-1 strain was administrated to the mice through the intravenous (I.V) route from the tail veins. Through this administration the bacteria are able to penetrate the endothelium and interact with synovium and cartilage.

2-2. Mice

The mice were females of NMRI mice bred from B&K Universal AB, Sollentuna, Sweden. They originate from the Swiss mice. The mice were from six to eight weeks old.

Thirty mice were used in this study and they were evenly divided into three groups (10 mice per group). The groups were one: control group: drinking water and a intra-peritoneous (i. p) 200ul of PBS injection twice a day; low dose of cyklokapron: tranexmic acid 5mg/ml in their drinking water and a 200ul (i. p) injection of PBS twice a day; and high dose of cyklokapron: 5 mg/ml of cyklokapron in drinking water as well as a 200ul of (i. p) injection of cykolapron 100mg/ml twice a day.

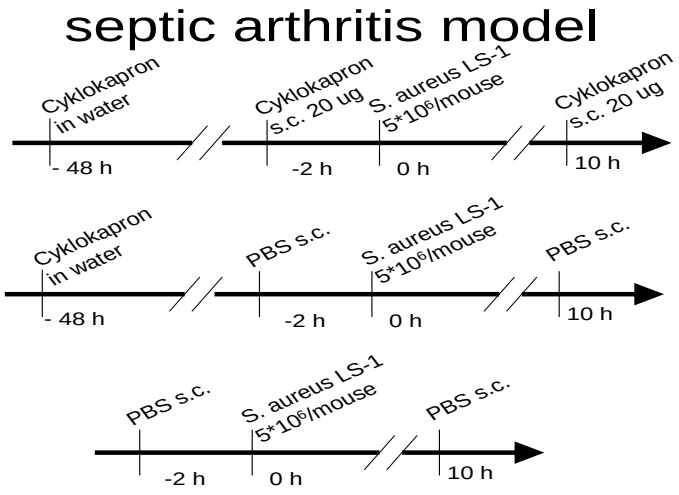

Figure 7, shows the experimental protocols of staphylococcal septic murine model.

2-3 Experimental protocols

Viable count:

Firstly the LS-1 bacteria are planted on the agar (horse blood) plate and incubated over night in the 37 ^{o}C. The next day one single colony of bacteria were transferred and planted on another new agar plate, which was then incubated in 37 ^{o}C also for overnight.
The freezing solution was prepared for the next day.

- 18 ml sterile PBS x 3
- 1000mg of bovine serum albumin (BSA; sigma, St. Louis) x 3 were added in the PBS
- 2 ml of dimethylsulfoxide (DMSO; Sigma Chemical Co.) were then added to complete the freezing solution.

The bacteria were then transferred from the agar plate and mixed in a sterilized PBS in a glass vial. The solution with the bacteria was then well vortexed. The solution was then centrifuged at 4000 rpm for 10 minutes. The supernatants were then discarded and the pellet left in the tube was mixed in 1ml of freezing solution. The aliquate was then divided into 28 plastic tubes and left in the freezer of – 20^{o}C for overnight.

The next day one vial was taken from the freezer for viable count. The bacteria solution was washed in 10 ml of PBS by centrifuging it at 4000 rpm for 10 minutes. The supernatant was discarded and 10 ml of PBS was added. Seven small tubes are used, and in each of them 900ul of PBS limbs of the mice. To fix the slides for histopathologic examination one followed a routine fixation. The limbs of the mice were first put in medium for preserving tissue (80-120 mM NaCl), the formalin. After 2-3 days the limbs were decalcificated in the liquid called “paraengy urkalkningslosing”, which softens the limbs and this process took about three days. The limbs were added.
The bacteria solution was diluted:

- By adding 100ul of bacteria solution in the first of the seven tubes and vortexed and 100ul was taken from the first small tube into the next small tube.
- The second tube was vortexed and 100ul taken from it and added to the next tube.
- This process continued until to the last of the seven tubes.

100ul of bacteria solution was taken from the last two tubes (tube 6 and 7) and spread on an agar (horse blood) plate. The plates were then incubated for overnight in 37^{o}C.

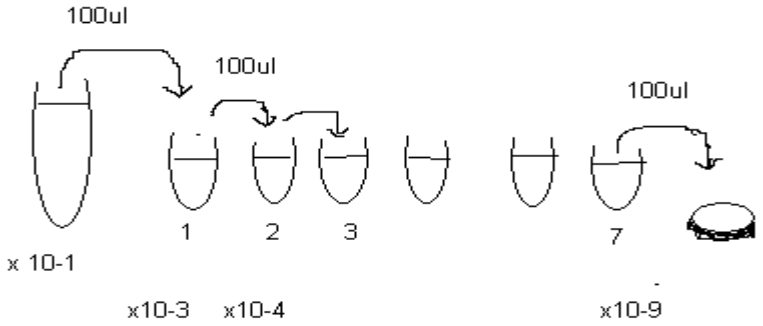

Figure 8, show the protocol of viable count of the S. aureus strain LS-1 solution.

The next day the bacterial colonies were counted from the agar plates and average number of the colonies was obtained by:

	LS –1	$X10^{-7}$	$X10^{-8}$
Plate 1	1	401	47
	2	324	42
Plate 2	1	392	38
	2	328	39

The two groups' value of $x10^{-7}$ and that of $x10^{-8}$ were totalled up and each group four values were totalled and the average was got.

x10-7 (401 + 324 +392 + 328 = 1445)/4 = 366.2

x10-8 (47 + 42 + 38 + 39)/4 = 41.5

The volume of arthritogenic dose of LS-1 was about 4×10^{-6} per mouse. This dose of LS-1 strain gave the mice profound septic arthritis after 3 days inoculation bacteria. After the bacterial pellet was washed, 10ml of PBS was added and vortexed. It was then diluted once again by taking 1ml of bacterial solution and adding it into 15ml PBS to get the total of 16ml.
30 mice were weighted and then given the bacteria through intravenous route. After i.v injection, viable counts were used to check the amount of bacteria injected. This bacterial solution is farther diluted into five small tubes. In each was 900ul of PBS and 100ul of bacterial solution was added to the first tube. The dilution continues by taking 100ul from the first small tube to the next and the next until one reaches the last tube. Then one takes two ager plates for each concentration of the last two tubes and spread 100ul on each plate with the same concentration. The plates are then marked with

the concentration and incubated in the 37^{o}C for overnight. The next day the bacterial colonies are counted.
Weight loss, arthritis development and mortality were monitored at regular intervals. All the mice were numbered from number one to number ten in each group.

Clinical examination of infected animals

There were three cages and each cage contained 10 mice of the same group. All the mice were observed individually. The weight, arthritis index, and the mortality were recorded at the regular interval (3 days, 5 days and 7 days post injection). The arthritis was defined as visible erythema and the swelling of at least one joint. The evaluation of the arthritis graded with a clinical scoring system of 0 to 3 points for each limb e.g 1, was a mild swelling or erythema, 2, was a moderate swelling and erythema while 3 was a marked swelling and erythema. Then the total arthritis index was constructed by adding up the scores from all the four limbs of each animal. The score could range from 0 to 12 depending on severity of arthritis. The clinical examination usually corresponds with histopathologic examination.

Bacterial load in kidney

Bacterial load in kidneys was used for demonstrating bacterial clearance in mice. The kidneys for each mouse were mechanically crushed and diluted in PBS 1:10. 100ul of the diluted kidney homogeneneity was put in the first small tube. The dilution continued until to the last firth tube. This dilution of the kidney solution was done for each of the 20 mice. Two agar plates were used for value x 10^{-4} and x 10^{-5} for each mouse and were incubated for overnight in the 37^{o}C. The next day the viable count was counted.
Histopathology

Histopathologic examination was done by examining sliced were then embedded in the paraffin. This process made it easier to slice the limbs. The tissue sections that were most important were from the upper extremities are the elbow, wrist, carpal bones and the fingers and in the lower extremities were the knee, ankle, tarsal bones and toes. The sections were stained with hematoxylin and eosin.
Staining procedure:

- The slides were deparaffinize and hydrated to water
- They were then put in iodine and then cleared with sodium thiosulphate (hypo)
- They were then put in Mayer's hematoxylin for 15 minutes.
- The washing was run for 20 minutes in H_2O
- The eosin staining was programmed for 2 minutes
- The slides were then dehydrated in 95% and absoluted in alcohols, two changes of 2 minutes each until the eosin was removed.
- They were then cleared in xylene, two changes of 2 minutes each and later they were mounted in Permount.

The joints were then analysed under a microscope under the supervision of the expert supervisor. The joint were analysed for synovial hypertrophy, erosion of the bone structure and for accumulation of immune cells in a given section of the limb. The histological scoring was given according to the synovial hypertrophy and the erosion of the bone structure. The scores were grade from 1 point for mild, 2 points for moderate and 3 points for severe synovial hypertrophy and erosion of the joint.

IL-6 bioassay

The collected blood was used for the analysis of the concentration of the IL-6 during the sepsis. The bioassay method was used to detect the serum biological activity of IL-6. The measurement of interleukin-6 (IL-6) was done by using a murine hydridoma cell line (B9) for their dependant on the IL-6 for proliferation. During the experiment the B9 cells were harvested from tissue culture flasks, and later seed into 96-well microplates (Nunc, Roskilde, Denmark) at a concentration of 5,000 cells per well. They were then cultured in complete medium (Iscove's medium supplemented with 5 x 10^{-5} M 2-mercaptoethanol, 5% fetal calf serum (Seralab, Sussex, United Kingdom), penicillin (100U/ml) and streptomycin (100ug/ml)), and serum samples were added. The dilution was done as follows:
In the ratio orders of 1:250, 1:1000 and 1:5000. To do the dilution one needed to dilute beginning with the ratio of 1:50. The dilution is as follows:

- In tube number one was 1:50, tube 2 1:250, tube 3 1:1000, and tube 4 1:5000.
- Tube 1, 10ul sample of serum was added in 490ul of complete medium found in the first tube.
- 100ul was taken from the first tube into the second tube, which contains 400ul of complete medium.
- 150ul was taken from the second tube to the third tube, which contains 450ul of complete medium.
- 100ul was then taken from the third tube into the fourth tube, which contains 400ul of complete medium.
- The standard curve medium is also prepared by using IL-6 standard and diluting it from 64 pg/ml, 32 pg/ml, 16 pg/ml, 8 pg/ml, 2 pg/ml, 1 pg/ml and 0 pg/ml.
- The dilution was as follows:
- Tube 1 for 64 pg/ml contained 800ul of complete medium, whereby 200ul of IL-6 was add to give 1000ul altogether.
- 400ul was taken from the first tube to the next for 32 pg/ml, which contained 400ul of complete medium.
- 400ul was taken from the second to the third tube for 16 pg/ml with 400ul complete medium
- 400ul was taken from the tube of 16 pg/ml and added to the fourth tube with 400ul complete medium
- This process continued until to the last tube which will contain 1 pg/ml
- The tube that contained 0 pg/ml had only complete medium.
- 100ul of diluted samples and the standard were put in the wells.
- The B9 cells were washed in 10 ml complete medium. They were then adjusted to cell concentration of 5 x 10^{4}/ml and the end concentration

was diluted to 2.5 x 10^4/ml. The adjusting of the cell concentration was done according to the concentration of the cells. The cell-suspension was then added into the wells of the standard and the diluted samples by putting 100ul per well. The samples are then incubated for 72h in the 37^{o}C under and environment of 5% CO_2. After the incubation the [^{3}H] – thymidin(Radiochemical Centre, Amersham, United Kingdom) was added into the wells of the samples and then incubated for 4 hours. The dilution of the [^{3}H] – thymidin had a ratio of 1:10 in the complete medium. This gave a dilution of 300ul [^{3}H] – thymidin added in 2.7 ml of complete medium. 10ul was added in each well.

- After incubating for 4 hours the cells were harvested. By using microbeta harvester. A filtermat was used in the microbeta harvester. The samples were sucked out through the under side of the filtermat and out into the waste. The cells were then attached on the under side of the filtermat. The filtermats were washed according to the protocol and left to dry overnight.
- The next day a scintillator sheet was melted on the filtermats on the side of the harvested cells. The filtermats were then sealed in the bags and made ready for reading in the microbeta computer.
- The results for IL-6 bioassay were read by following the protocol of the microbeta.

3. **Results**

Clinical examination of infected animals

3-1 Mortality

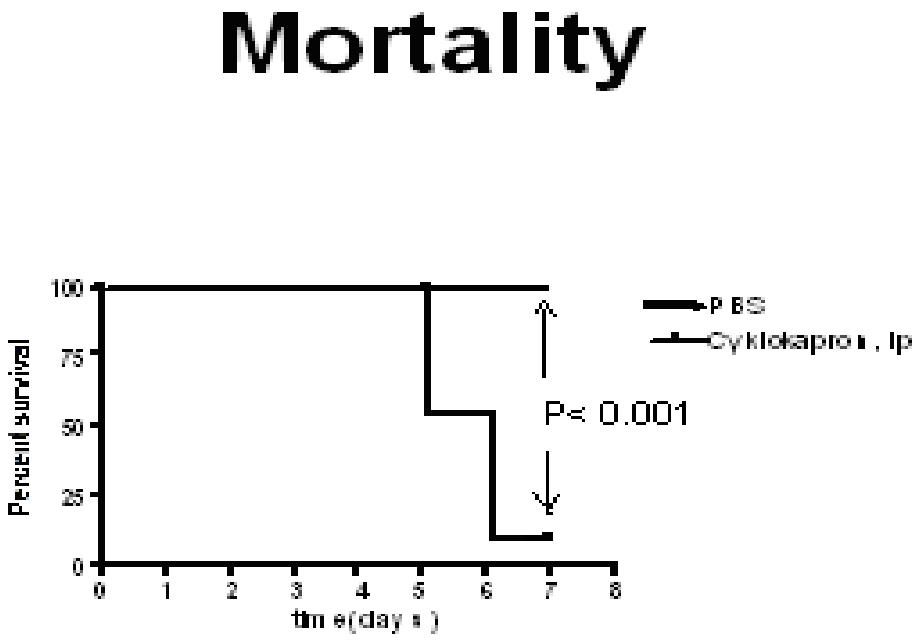

Figure 9 Cyklokapron treatment increase mortality in stephylococcal sepsis. There is a significant increase in the mortality of septic mice treated with high dose of cyklokapron. At day 5, half of the cyklokapron i.p mice died and none from the PBS control group. On day 7 there was only one mouse in the cyklokapron i.p that survived while all those in the PBS control group were still alive.

3-2. Septic arthritis

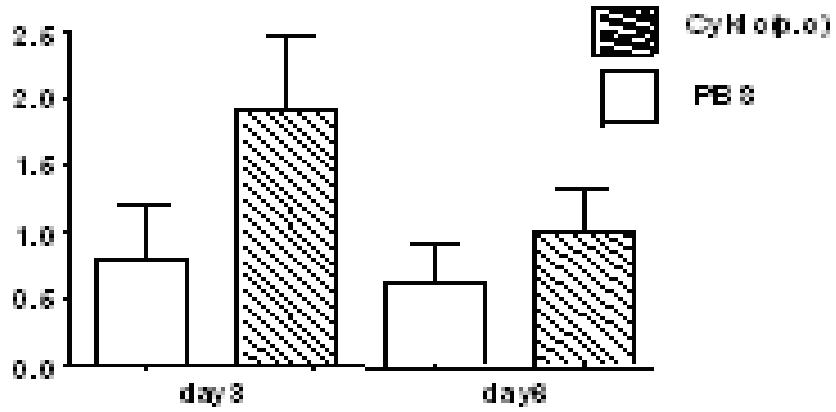

Figure 10 shows on day 3 the mean arthritis index for low dose cyklokapron group was 1.9 and the standard error of mean was 0.57 while the mean for PBS control was 0.8 and the standard error of mean was 0.42. On day 6 the mean for low dose cyklokapron was 1.0, while the mean for PBS control group was 0.6, suggesting that cyklokapron treatment might aggravate staphylococcal arthritis. Here one has to point out that there is no significant difference between groups.

3-3 Weight loss

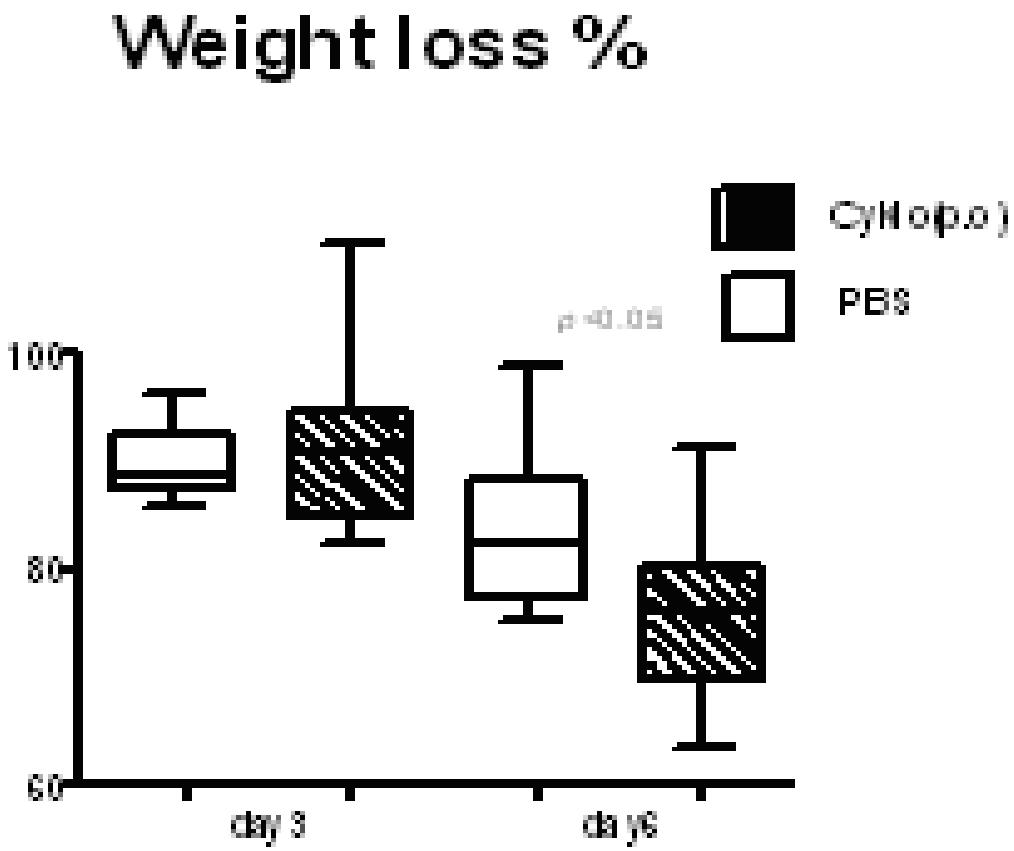

Figure 11, show significant difference of weight loss between the PBS control and the cyklokapron (p. o) group. After 3 days inoculation mice in both treatment and control groups lost around 10% body weight due to sepsis (no significant). However, after day 6 inoculation, the mice receiving cyklokapron lost around 25% body weight. In contrast, control group lost only 17% body weight ($p<0.05$), suggesting that cykokapron aggravates stephylococcal sepsis.

3-4. Viable count

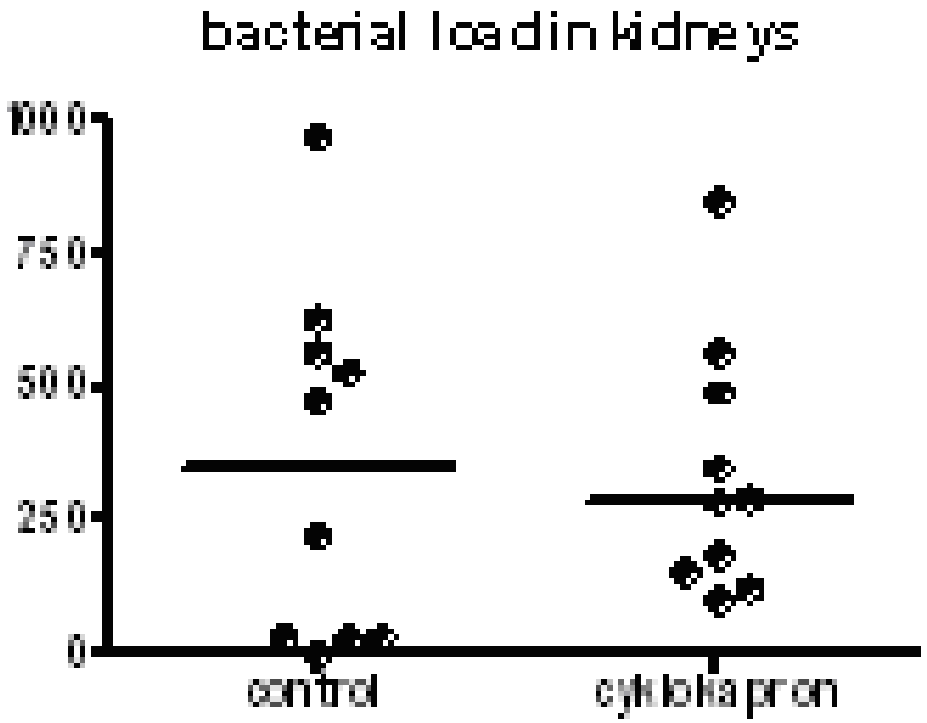

Figure 12 shows the bacterial load in the kidneys. The bacterial load in the kidney in cyklokapron group is around 3.4 x 10^6 cfu/mouse, and is similar in PBS control group 3.5 x 10^6 cfu/mouse. There is no significant difference between the PBS control group and the low dose cykokapron (p. o) group.

3-5. Histopathology

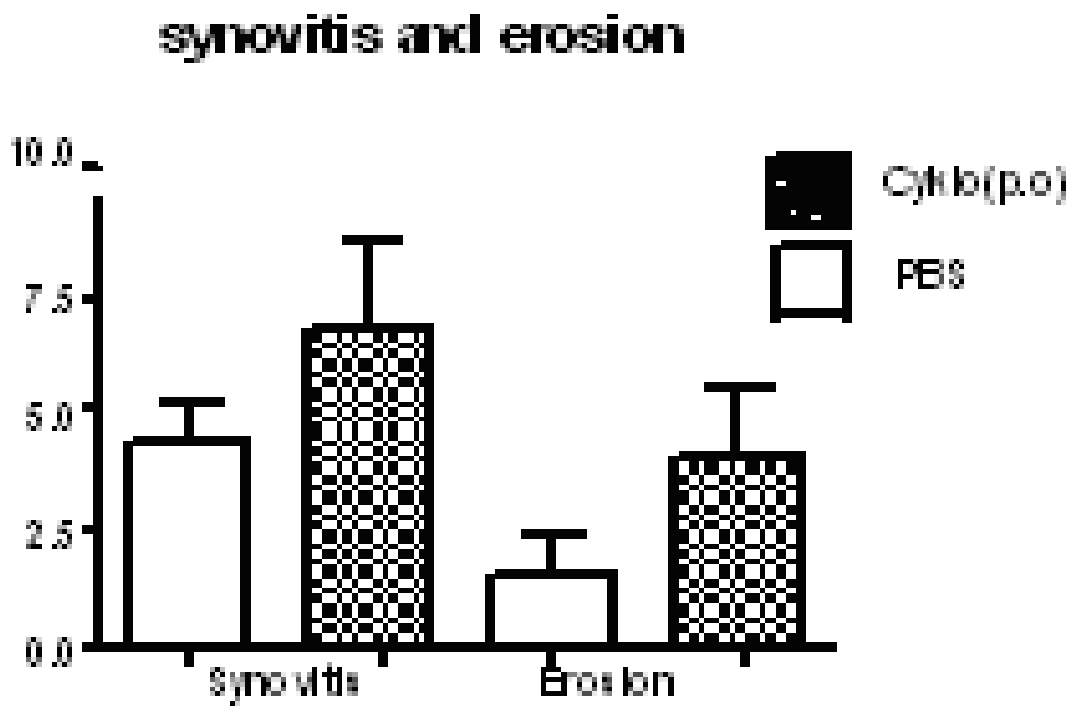

Figure 13, show the grade of synovitis and erosion between the PBS control and the cyklokapron (o. p) group. No significant difference was found regarding synovitis and erosion between the two groups. The mean of synovitis for mice in control group was 4.4 while the mean synovitis for low dose cyklokapron group was 6.8. The mean of erosion for mice in control group was around 1.6, while the mean erosion for cyklokapron group was around 4.2. Histopathological finding corresponds with the clinical evaluation.

3-6. IL-6 level

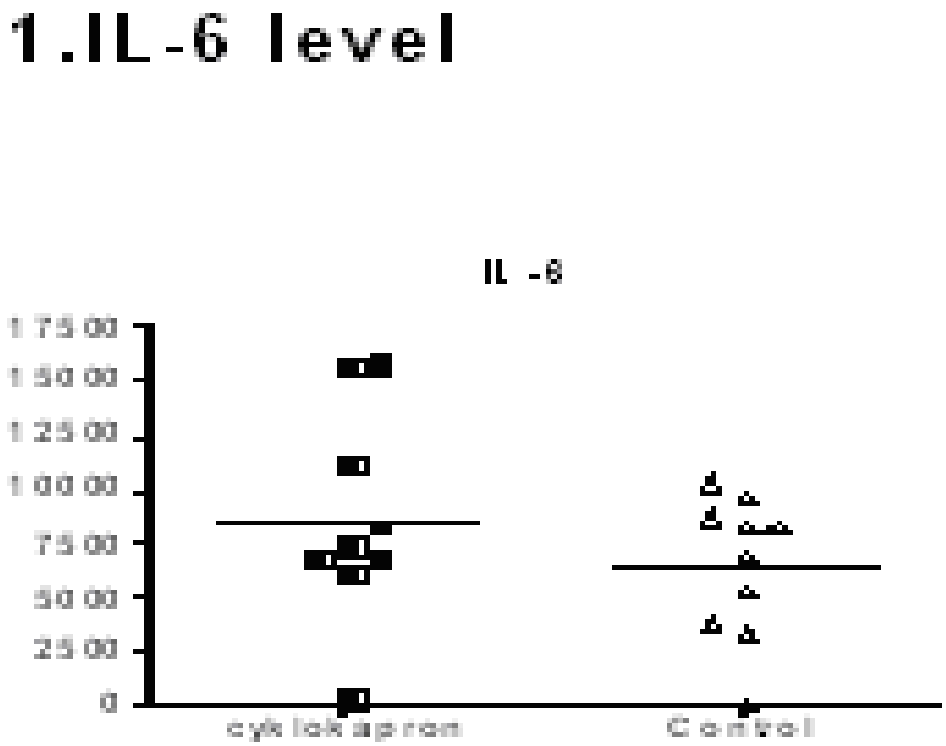

Figure 14, show no significant difference between the PBS control group and the cyklokapron (o. p) group. There is a tendency that cyklokapron group has higher IL-6 in blood than control group. Mean level of IL-6 for control group was 6434 pg/ml, and the mean for low dose cyklokapron was 8660 pg/ml.

4. Discussion

S. aureus strain LS-1 was used in this staphylococcal arthritis model. The LS-1 strain was given to the mice intravenously (i.v). This is because the overwhelming majority of cases of human infectious arthritis have a haematogenic spread. This animal model makes it possible to study bacterial survival in the bloodstream and how they penetrate the endothelium and interact with the synovium as well as the cartilage. During staphylococcal infections the immune cell and pro-inflammatory cytokines up-regulation are host defence mechanisms. In severe infection systemic inflammation leads to profound pro-coagulation and impairment of fibrinolysis giving rises to uncontrolled hemostasis, which in turn contributes to septic arthritis disease. During septic arthritis pro-inflammatory cytokines like IL-6 is up-regulation, which directly or indirectly up-regulates host immune responses, and contributes to development of synovitis and bone erosion.

Cyklokapron (tranexamic acid) is used to treat women who are bleeding heavily during their menstrual periods, and to treat serious bleeding after surgery or nasal bleeding. Tranexamic acid is an antifibrinolytic that competitively inhibits the activation of plasminogen to plasmin. In the present study we wanted to investigate the effect of tranexamic acid on the DIC in the patient of staphylococcal sepsis. We found that total blockage of plasminogen activation by higher dose of cyklokapron in mice with staphylococcal sepsis leads to a higher mortality in comparison the control group. The weight loss between the PBS control group and the cyklokapron (o. p) group shows a clear difference between the two groups. More than this, there was a tendency that cyklokapron gave rise to more severe arthritis than the control group. We also wanted to know if cyklokapron upregulate pro-inflammatory cytokines such as IL-6, during staphylococcal infections? Measuring the level of IL-6, we found that there was indeed a tendency that IL-6 levels in the mice treated with cyklokapron were higher than the mice in the control group. It has been shown that pro-inflammatory cytokines like TNF-alpha and IL-6 significantly contribute to mortality of LPS-endotoximia. Moreover, fibrin deposition has been hypothesized to act as an inflammation initiator in autoimmune diseases. Depletion of fibrinogen clearly down-regulates local inflammation in SLE nephritis and rhematoid arthritis. We hypothesize that in our setting, inactivation of plasminogen by cyklokapron gave rise to increased fibrin deposition in multiple organs, which in turn leads to a systemic up-regulation of pro-inflammatory cytokines, for example IL-6, that induces pro-coagulation status.

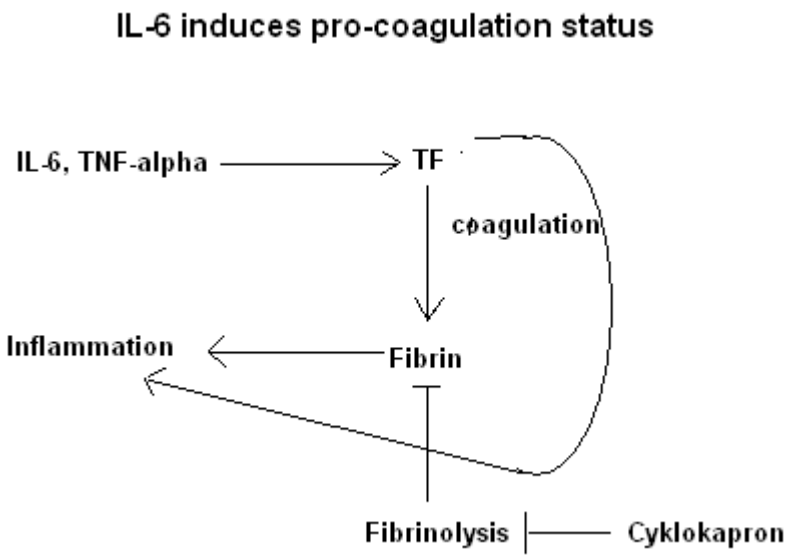

Figure 15 Show a systemic up-regulation of pro-inflammatory cytokines.

Such kind of positive feed back circle continues and in most severe situation, micro-thrombosis forms in multiple organs, DIC and death.

In conclusion, inhibition of plasminogen activation by tranexamic acid clearly aggravates staphylococcal sepsis and septic arthritis. This deterioratable effect is not due to down regulation of bacterial clearance. However, up-regulation of pro-inflammatory cytokines – IL-6 might be the explanation. Our data suggest that the use of tranexamic acid for patients with staphylococcal sepsis should not be encouraged.

5. Conclusion

1. Staphylococcal aureus induces septic arthritis and septic shock on mice
2. Cyklokapron, an inhibitor of plasminogen activator will aggravate septic arthritis and septic shock.
3. Deterioative effect of cyklokapron might be mediated by up-regulation of pro-inflammatory cytokines like IL-6.

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