



Original Contribution

STUDIES ON THE THERAPEUTIC EFFECT OF FLUCLOXACILLIN IN DOGS WITH EXPERIMENTAL STAPHYLOCOCCAL INFECTION IN THE SKIN AND SOFT TISSUES

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ABSTRACT

The therapeutic effect of flucloxacillin sodium was studied on experimentally provoked infection in the skin and soft tissues with field strain *Staphylococcus aureus* (broth culture with thickness 1×10^8 colony forming cells /ml). Twelve sexually mature dog, 3-4 years old, weighing 14-18 kg from both genders (6 male and 6 female), were used of which two groups were formed. One of the groups was treated with flucloxacillin-sodium and the other, which we used as a control group, was injected with tylosin tartrate. We evaluated some changes of clinical parameters (body temperature, heart and respiratory rates, general condition) and some laboratory indices of the blood (hematocrit, hemoglobin, red blood cell (RBC) counts, erythrocyte sedimentation rate (ESR), total and differential white blood cell (WBC) counts, index of phagocytic activity, phagocytic number, total protein and protein fractions, fibrinogen, urea, creatinine) after the experimental staphylococcal infection and treatment with the antibiotics. The *i.m.* administration of flucloxacillin-sodium at a dose 34 mg/kg of body mass 7 days at 8-hour intervals, appeared to be the suitable regimen for the treatment of soft tissue and skin infections caused by *Staphylococcus aureus*.

Key words: Dogs, Experimental infection, Flucloxacillin, *Staphylococcus aureus*

INTRODUCTION

The wide spread of resistant strains of microorganisms from human and veterinary origin and the resulting lowered effectiveness of the existing antibacterial agents conditions the necessity of constant synthesis and manufacture of new chemotherapeutics effective against the antibiotic resistant forms of pathogenic microorganisms. At the same time there are insufficient experimental data about a large number of already synthesized chemotherapeutics allowing their usage in the veterinary practice. There is lacking or scarce data about the clinical therapy of soft tissue infections in domestic animals caused by staphylococci or streptococci.

Flucloxacillin, which is a representative of the isoxazolympenicillin group, is known to be effective against a number of pathogens causing infections of the skin and the soft

tissues (*Staphylococcus aureus*, *S. epidermidis* and *S. pyogenes*).

The data concerning the efficacy of flucloxacillin shows that over 93.4 % of the treated patients with dermal or vulnarary infections were completely healed after the intramuscular (*i. m.*) or internal application of the antibiotic (1, 2).

The data about its effectiveness in the treatment of pyoderma in horses, dogs, and cats are too spare (3-6). It has also to be emphasized that the fore-mentioned facts concern spontaneous diseases. There is a lack of information about the therapeutic effect of flucloxacillin in experimental infection of the soft tissues in dogs. The aim of this study was to determine the healing effect of flucloxacillin sodium salt (Fluisopen-vials) injected in dogs with experimental staphylococcal infection.

MATERIALS AND METHODS

Animals

The study was performed on 12 mix-breed, sexually mature dogs of both genders (6 male and 6 female), weighing 14-18 kg, at 3-4

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years of age. They were divided into two groups (3 male and 3 female in each). One of the groups was treated with flucloxacillin sodium salt (Fluisopen-vials 500 mg and 1 g for *i.v.* and *i.m.* usage; Balkanpharma - Razgrad Co., Razgrad, Bulgaria), and the other (control) was treated with tylosin tartarate (Pharmasin 50 - vials 50 ml for *i.m.* usage; Balkanpharma - Razgrad Co., Razgrad, Bulgaria).

The dogs were housed in individual metal cages, at light regime, room temperature and air humidity corresponding to the conditions appropriate for this animal species. They were fed twice a day (morning and evening) with commercial food for dogs Lubimetz® (Bulgaria). The access to drinking water in all cages was *ad libitum*. The experiments with animals were in conformity with the Act and Code of Practice for the Housing and Care of Animals used in Scientific Procedures (1986; 1989).

In the week prior to the experiment the animals were dewormed with a combination of praziquantel and abamectin (Prazimec D - tabletes, Biovet Co., Peshtera, Bulgaria) at a dose rate of 1 tablet per 10 kg of body mass. The combination of permethrin and carbaryl was given against ectoparasites (Tapilan B, Dorvet, Israel).

Drug and treatment

Drugs used in the experiments were from commercial batches and were administered prior to the expiry dates: flucloxacillin sodium salt (Fluisopen - vials 500 mg, Balkanpharma-Razgrad Co., Bulgaria) (test drug) and tylosin tartrate (Pharmasin 50 - vials 50 ml, Balkanpharma-Razgrad Co., Bulgaria) (control drug).

The two drugs were administered *i.m.* in the right hind limb. Preliminary dosage regimen of the flucloxacillin sodium in dogs was determined in our previous studies (7) according to the equation:

$$D_m = (C_p^\infty \times Cl_B \times \tau) / F, \quad (8)$$

where: C_p^∞ was the average stationary concentration equal to the minimum inhibiting concentration against β -lactamase *S. aureus* strains; Cl_B - total body clearance of the antibiotic; F - bioavailability of the flucloxacillin in *i.m.* administration of the antibiotic and τ - dosage interval. The calculated dose was 34 mg/kg body mass, applied at 8 hour intervals (*t.i.d.*). To avoid the pain from the injection, flucloxacillin sodium was dissolved *ex tempore* in 0,5% solution of procaine hydrochloride (Procainum hydrochloridum - ampullae 0,5%,

Sopharma Co., Sofia, Bulgaria). The dose of tylosin tartarate was 10 mg/kg of body mass - at 12 h intervals (*d.i.d.*).

Experimental infection

After the examination including the clinical and paraclinical status of each dog, the animals were contaminated via *s.c.* inoculation of 5 ml 24-hour broth culture of *S. aureus* - field strain with thickness of 10^8 colony forming units (cfu) per ml, in the left hind limb.

The sensitivity of the strain to flucloxacillin and tylosin was tested *in vitro* on Müller-Hinton agar with 5 % blood. The morphology and the biochemical properties of the isolated strain were evaluated in the Department of Microbiology and Virology, Faculty of Medicine, Trakia University - Stara Zagora. The identification and determination of the biochemical properties were performed with the Sceptor system (Becton Dickinson Diagnostic).

Experimental design

Dogs were randomly assigned to two (experimental and control) groups of six animals each (3 males and 3 females).

The treatment with the antibiotics (flucloxacillin sodium - experimental group and tylosin tartarate - control group) was initiated 48 hours after the infection of the dogs and went on for 7 days. Physical examination, hematological, immunological and biochemical analyses were carried out before and after the treatment. The blood samples were obtained via venflon cannula from *v. cephalica antebrachii* at the following times: pre-inoculation (as an autocontrol, baseline) and after inoculation at days 1 (24 h before initiation of treatment), 2, 3 and 8.

Examinations and assays characteristics

The following clinical parameters were evaluated for each dog - body temperature, heart rate, respiratory rate, locomotor activity, changes in the colour of conjunctivas, the presence of oedema, tenderness, painfulness, alopecia, discharges or impaired skin entity, the appetite and the general condition prior to the infection, at the time of infection (at hours 24 and 48) and during the whole period of the treatment with the tested and the control antibiotic. The intervals of assays are indicated in Figure 1.

The following hematological parameters were determined: hematocrit (l/l) by a microHematocrit method using a hematocrit centrifuge (15000 rpm, 10 min); hemoglobin (g/l) - by an acid-base analyser (ABL-3, Radiometer, Denmark); red blood cells (RBC) counts and total white blood cell (WBC)

counts with the Bürker's chamber; the erythrocyte sedimentation rate (mm/h) - according to Westergreen; the differential WBC counts - via counting on a blood smear stained according to Pappenheim.

The parameters of innate immunity (phagocytic index and number) were determined by the method of Samnaliev et al. (9) via fluorescein-isothiocyanate (FITC) conjugated staphylococci.

The biochemical parameters determination of the total protein (by the Biuret's reaction) and the protein fractions (albumin, α_1 , α_2 , β and γ -globulins) - by agar gel electrophoresis; fibrinogen (g/l) - with a commercial kit (Hemo Stat Fibrinogen Test Set, GmbH, Germany), urea (mmol/l) and creatinine ($\mu\text{mol/l}$) - with commercial kits (Bioagrogen, France).

The results were processed statistically using one-way ANOVA (computer programme StatMost for Windows, 1994). Comparisons were made to baseline values and differences were considered significant at the $p < 0.05$ level.

RESULTS

The *in vitro* tests for determination of the sensitivity of the field *S. aureus* strain ascertained that it was sensitive to flucloxacillin with an inhibition zone of 37 mm. The identification and the study of its biochemical properties, revealed that the field isolated was *S. aureus*, producing a yellow pigment on blood agar with a complete β -hemolysis. The morphology of colonies was characteristic of *S. aureus*. The strain was catalase-positive, oxidase-negative, plasmocoagulase-producing, DNA-ase producing.

The staphylococcus infection was accompanied by painfulness, expansiveness and oedema of the soft tissues on the place where the broth culture was injected at the 4th hour after the infection. An intraction in the dog's movement was present which was followed by an increase of the inguinal lymph nodes and oedema of the scrotum at the 24th hour. On the second or third day after the contamination a hair loss around the place of injection (of staphylococcal broth culture) was present. The place was around 20 cm in diameter with many abscesses and a crater like erosion 5-8 cm in diameter. A lack of appetite and polydipsia were observed.

The changes of the internal body temperature are given in Figure 1. It becomes clear that the Staphylococcus infection was accompanied by a statistically significant increase in the body temperature ($39.7 \pm 0.15^\circ\text{C}$ for the flucloxacillin group and $39.5 \pm 0.08^\circ\text{C}$ for the tylosin group, $p < 0.001$). After the beginning of the therapy with the two antibiotics (the tested and the control one) the body temperature decreased towards the values measured at the 24th hour (before the treatment). At the 8th day it was $38.8 \pm 0.09^\circ\text{C}$ for the flucloxacillin and $38.7 \pm 0.01^\circ\text{C}$ for the tylosin group with the presence of statistical significance in the detected differences ($p < 0.001$).

The rise of the body temperature in both groups was accompanied by a significant acceleration of the heart rate compared to that before the infection of the dogs (Table 1). During the time of the treatment with flucloxacillin sodium or tylosin tartarate a significant decrease to the initial value of this clinical parameter was determined in the flucloxacillin group.

The respiratory rate at post infection day 1 was maximally accelerated in both groups infected with *S. aureus* versus the baseline values of 49.50 ± 2.07 for the flucloxacillin group and 48.8 ± 3.90 respiratory movements/min for the control antibiotic ($p < 0.001$).

After administration of the flucloxacillin and tylosin the values became lower, more pronounced in the tylosin group. At the end of the treatment respiratory rate was equal or lower compared to that of the baseline. After the infection the amount of hemoglobin decreased versus the baseline in both groups with a presence of statistical significance in the values of the dogs injected afterwards with flucloxacillin ($107.0 \pm 9.20\text{ g/l}$, $p < 0.05$) (Table 2).

Hematocrite also decreased significantly in the tylosin group. At the end of treatment Hemoglobin concentration in both group, although increased, but did not reach its baseline values, whereas Hematocrit was nearly restored.

The staphylococcal infection caused a statistically significant decrease in the count of the RBC in both groups. At the end of the experimental period (the 8th day), the values of this hematological parameter of the group treated with tylosin remained significantly lower versus the baseline.

Table 1. Some clinical parameters in dogs with experimental *S. aureus* infection, treated with flucloxacillin sodium or tylosin tartarate (mean±SEM).

Parameter	Prior to infection (Baseline) 0 h	Experimental infection			
		Prior to the treatment day 1 *	Treatment with antibiotics		
			day 2 *	day 3 *	day 8 *
I group	– treated	with flucloxacillin			
Heart rate (HR/min)	82.5±5.04	96.83±1.45 ^a	76.33±7.24 ^{b,f}	73.33±0.54 ^h	80.61±5.20 ^h
Respiratory rate (RR/min)	27.33±1.40	49.50±2.07 ^d	47.50±1.34 ^d	38.7±0.54 ^d	26.1±0.52 ^h
II group	- treated	with tylosin			
Heart rate (HR/min)	74.0±2.65	84.0±2.92 ^a	80.0±2.78	77.2±5.9	74.8±4.80
Respiratory rate (RR/min)	31.0±2.15	48.8±3.90 ^c	33.0±0.95 ^g	31.0±0.58 ^g	26.1±0.52 ^g

* day after the beginning of experimental infection;

Significance versus baseline (0 h): a) at $p < 0.05$; b) at $p < 0.02$; c) at $p < 0.01$;

Significance versus day 1 (prior to the treatment: d) at $p < 0.001$; f) at $p < 0.02$; g) at $p < 0.01$; h) at $p < 0.001$.

Table 2. Hematological parameters in dogs with experimental *S. aureus* infection, treated with flucloxacillin sodium or tylosin tartarate (mean ± SEM).

Parameter	Prior to infection (Baseline) 0 h	Experimental infection			
		Prior to the treatment day 1 *	Treatment with antibiotics		
			day 2 *	day 3 *	day 8 *
I group	– treated	with flucloxacillin			
Hematocrit (l/l)	0.34±0.03	0.27±0.03	0.35±0.01 ^e	0.36±0.01 ^f	0.34±0.1
Hemoglobin (g/l)	123.0±1.40	107.0±9.20 ^a	112.0±3.20 ^b	115.0±3.20 ^a	113.0±6.6
RBC (10^{12} /l)	5.52±0.19	3.77±0.16 ^d	4.52±0.10 ^{c,g}	4.76±0.21 ^{a,g}	5.87±0.12 ^h
Erythrocyte sediment. rate (mm/h)	4.17±0.83	11.50±3.11 ^a	8.17±3.59	5.0±0.96	5.0±1.5
WBC (10^9 /l)	9.70±0.48	18.14±0.54 ^d	16.02±0.76	12.04±0.9 ^{a,h}	8.2±0.76 ^h
Differential white blood cell counts :					
Lymphocytes (%)	32.0±0.58	15.46±0.74 ^d	19.33±1.86 ^d	17.46±0.54 ^d	20.33±1.24 ^{d,g}
Monocytes (%)	3.00±1.73	4.92±1.34	5.67±2.60	3.56±0.92	1.67±0.84
Band neutrophils(%)	3.00±1.99	11.40±1.24 ^c	12.0±4.04 ^c	10.34±0.87 ^c	7.00±1.11 ^e
Segm. neutrophils (%)	61.0±1.73	65.24±1.67	58.33±1.20 ^g	57.92±1.43 ^g	69.0±5.21
Eosinophils (%)	1.0±0.53	3.24±0.10 ^c	4.67±2.66	3.24±1.10	2.0±0.36 ^g
II group	- treated	with tylosin			
Hematocrit (l/l)	0.33±0.01	0.27±0.01 ^c	0.29±0.009 ^b	0.30±0.01	0.31±0.08
Hemoglobin (g/l)	136.0±11.2	112.0±6.7	102.0±8.1 ^a	112.0±8.9	118.0±8.6
Red blood cells(10^{12} /l)	5.7±0.12	4.7±0.24 ^c	4.52±0.10 ^d	4.83±0.21 ^c	4.96±0.29 ^a
Erythrocyte sedimentation rate (mm/h)	5.6±4.48	19.0±6.57	18.63±5.1	15.00±5.1	9.17±6.17
WBC (10^9 /l)	9.6±0.76	15.6±1.47 ^c	11.7±1.23	11.6±1.47	10.5±0.79 ^f
Differential white blood cell counts :					
Lymphocytes (%)	35.70±2.90	17.70±4.41 ^c	12.30±4.98 ^d	19.20±1.84 ^c	23.90±1.76 ^c
Monocytes (%)	4.30±1.86	1.30±0.88	6.70±2.67 ^g	5.40±0.67	5.00±2.08
Band neutrophils (%)	4.00±1.00	14.00±1.53	15.60±1.86 ^d	14.00±1.00 ^d	9.30±1.76 ^a
Segm. neutrophils (%)	51.3±2.96	65.3±5.17 ^a	56.30±4.70	51.00±2.96 ^e	51.00±4.36
Eosinophils (%)	4.67±1.86	4.30±0.67	2.70±1.20	3.00±1.60	3.60±1.20

* day after the beginning of experimental infection;

Significance versus baseline (0 h): a) at $p < 0.05$; b) at $p < 0.02$; c) at $p < 0.01$;

Significance versus day 1 (prior to the treatment: d) at $p < 0.001$; f) at $p < 0.02$; g) at $p < 0.01$; h) at $p < 0.001$.

The erythrocyte sedimentation rate (ESR) accelerated at the first day after inoculation 11.50 ± 3.11 mm/h for the dogs of the flucloxacillin group ($p < 0.05$) and 19.0 ± 6.57 mm/h for the control group, respectively. After initiation of the treatment with the two antibiotics the values of the ESR began to decrease but without reaching to the baseline. At the end of the period they varied around the baseline.

WBC counts in both groups increased to $18.14 \pm 0.54 \times 10^9/l$ ($p < 0.001$) and $15.6 \pm 1.47 \times 10^9/l$ ($p < 0.01$), respectively. Values persisted till day 3, but decreased to the baseline at day 8 in both groups. The percentage of the lymphocytes fell statistically significantly in both groups as on the first day after the infection and amounted to 15.46 % and $17.70 \pm 4.41\%$ respectively. The treatment with both antibiotics led to significant increase in the values to a similar extent in both groups, but without a return to the initial values.

The staphylococcus infection was also accompanied by a nuclear left shift expressed in the band neutrophil percentage increase compared to the baseline (up to 11.40 ± 1.24 % for the flucloxacillin group and up to 14.00 ± 1.53 % for the tylosin group). The antibiotic treatment favoured the decrease of their neutrophil number more pronounced in the flucloxacillin group but without full return at day 8.

The percentage of monocytes increased in both groups to 4.91 ± 1.34 % (on day 1) and 6.70 ± 2.67 (on day 2), respectively. Administration of tylosin did not lead to decrease at day 8, while in the flucloxacillin group at the end of the follow-up period values were below the baseline. The percentage of the eosinophils in both groups varied during the whole course of treatment around the baseline.

The results of the phagocytic parameters (index of phagocytic activity and phagocytic number) are given in Table 3. In the presence of staphylococcal infection the values of the index of phagocytic activity were statistically significantly higher in dogs of the first group (up to 29.33 ± 0.42 %, $p < 0.01$). The values of the control group showed only a tendency to rise (31.1 ± 1.46 %) compared to the baseline. The values of phagocytic number after the infection with *S. aureus* for the two groups were significantly higher (2.0 ± 0.003 for the first group and 3.35 ± 0.2 for the tylosin group) compared to the initial ones. During the whole course of treatment the

values of the phagocytic number were significantly higher towards the baseline (Table 3).

The changes of the biochemical parameters in dogs with experimental infection are given in Table 4. The total protein concentration at day 1 was decreased in both groups. Statistical significance was detected in the control group. During the treatment with flucloxacillin total protein values slightly rose and varied around those of the baseline. The administration of tylosin led to same increase but the values remained significantly lower (compared to baseline) till the end of the period.

The albumin fraction in both flucloxacillin and tylosin groups tended to decrease. At day 1 post inoculation it was 11.58 ± 2.32 g/l and 14.53 ± 1.83 g/l compared to baseline (13.91 ± 2.45 g/l and 21.45 ± 4.76 g/l, respectively). The values remained lower, till the 8th day.

There were some changes/ fluctuations in α_1 -globulins, α_2 -globulins and β -globulins, however significant differences versus baseline or versus day 1 post infection were not found. The γ -globulins tended to be increased during flucloxacillin treatment whilst in the tylosin group an opposite tendency was observed.

Staphylococcal infection caused a significant increase of fibrinogen in both groups. In spite of its decrease, the values at the end of the experimental period remained higher both versus baseline and versus day 1 post infection.

The values of urea and creatinine were also increased after the inoculation. At post infection day 8 both parameters returned near to baseline in the flucloxacillin group whilst in the tylosin group urea and creatinine were with higher values, statistically significant versus baseline only for creatinine.

The outcome of the experimental disease at the end of testing period (the 8th day) in the tylosin (control) group was positive for 4 animals (66.6 %) which were clinically healed (conducted physical examination revealed lack of oedema, tenderness and pain, regained appetite and normal loading of the treated limb). In spite of the conducted therapy in two of the dogs from the control group a healing effect wasn't achieved. In these dogs the lameness persisted. At the site of the injection of the staphylococcal culture an oedema affecting the skin and the underlying muscles, was developing and later a fistula with purulent discharge appeared. In the flucloxacillin group 6 animal (100 %) were clinically healed.

DISCUSSION

Our experiment showed that during an experimental staphylococcal infection, in the dogs' organism a large number of local and systematic changes occurred. The haematological changes, manifested by increased total WBC count and the occurrence of a left shift, confirmed the presence of a focal infection.

The rise of the internal body temperature accompanied by an accelerated heart and respiratory rate could be explained by an activation of the monocytemacrophagal system and release of soluble mediators, such as interleukin-1 (IL-1) and prostaglandin E₂ (PGE₂). According to Jampel et al. (10), Kluger et al. (11) the fore-mentioned mediators are primary endogenic factors participating in the mechanisms of body temperature increase.

It is known that the increase in the body temperature is associated with activation of the systemic immunobiological reactivity - increase in the blood bactericidal activity and activation of lysozyme, complement and phagocytosis. Our data concerning the natural immunity - the index of phagocytic activity and the phagocytic number, both showing an increase in experimental infection, demonstrated that these interactions between body temperature and phagocytosis have been observed by us, too. The ability of canine neutrophils to phagocyte particles of dead Staphylococci has been studied in the last twenty years by some authors (12, 13).

According to their findings the staphylococcal antigen is capable to stimulate an *in vitro* neutrophil burst. Staphylococci can produce a number of components, interfering with the process of opsonization - an important prerequisite for the performance of phagocytosis. Furthermore, in dogs with pyoderma, Shearer (13) observed an elevation in serum levels of staphylococci - specific IgG antibodies that represent specific opsonins. It could be accepted that the changes in the differential WBC count and particularly the increase in monocytes and band neutrophil percentages (Table 2) relate to the discussed events. The conducted treatment with an antibiotic from the group of isoxazolympenicillins (flucloxacillin) exerted a

stimulating effect upon the fore-mentioned parameter of the natural immunity - the phagocytosis (Table 3).

The analysis of our blood biochemistry data (Table 4) showed decreased total protein concentrations in staphylococcal infection. A similar decrease, although not statistically significant, was observed in albumin concentrations, as well. Taking into account, that the latter determines the oncotic blood pressure, it could be accepted that its decrease is a mechanism for triggering oedema, that was clinically observed in soft tissues of the treated limb and the scrotum of male dogs. The changes in albumin could also be interpreted as a possible disorder in liver synthesis. The changes in urea levels, as an important product of protein metabolism and synthesized in the liver, could probably be due to impaired renal function. A confirmation of this is the elevated creatinine level.

The current results allow us to assume that the application of flucloxacillin similarly to tylosin in experimental staphylococcal infection restores the pathological deviations in body temperature, heart and respiratory rates, in ESR and partly in WBC count and some blood biochemical parameters. Along with this the tested isoxazolympenicillin has a positive influence on the changes in the blood immunological parameters concerning the rise of the immunological reactivity of the infected dogs.

After comparing the results of the conducted treatment with flucloxacillin and the control antibiotic on dogs with experimental staphylococcal infection, we can summarize that after the application of flucloxacillin we achieved 100% effect of treatment. After the application of tylosin the percentage was 66.6 (only 4 dogs were clinically healed).

At the end of the experimental period (the 8th day) the conducted inspection revealed the lack of oedema tenderness and pain. The dogs regained their appetite and began to load normally their treated limbs. The issue of the experimental disease showed that both administered antibiotics exerted a healing effect, which in this case was better expressed for flucloxacillin.

Table 3. Changes in index of phagocytic activity (IPA) and phagocytic number in dogs with experimental *S. aureus* infection, treated with flucloxacillin sodium or tylosin tartarate (mean±SEM).

Parameter	Prior to infection (Baseline) 0 h	Experimental infection			
		Prior to the treatment day 1 *	Treatment with antibiotics		
			day 2 *	day 3 *	day 8 *
I group	– treated	with flucloxacillin			
Index of phagocytic activity (%)	27.00±0.52	29.33±0.42 ^c	27.00±0.43 ^g	26.33±0.80 ^g	26.17±0.79 ^g
Phagocytic number	1.21±0.03	2.00±0.03 ^d	1.82±0.03 ^{d;g}	1.58±0.05 ^{d;h}	1.85±0.05 ^{d;e}
II group	- treated	with tylosin			
Index of phagocytic activity (%)	27.2±1.40	31.0±1.46	27.8±0.83	26.0±1.65 ^e	24.2±0.54 ^g
Phagocytic number	2.25±0.06	3.35±0.21 ^d	2.64±0.12 ^{b;f}	2.10±0.14 ^h	2.43±0.07 ^g

* day after the beginning of experimental infection;

Significance versus baseline (0 h) : a) at $p < 0.05$; b) at $p < 0.02$; c) at $p < 0.01$;

Significance versus day 1 (prior to the treatment: d) at $p < 0.001$; f) at $p < 0.02$; g) at $p < 0.01$; h) at $p < 0.001$.

Table 4. Biochemical parameters in dogs with experimental *S. aureus* infection, treated with flucloxacillin sodium or tylosin tartarate (mean ± SEM).

Parameter	Prior to infection (Baseline)	Experimental infection			
		Prior to the treatment day 1 *	Treatment with antibiotics		
	0 h			day 2 *	day 3 *
I group	– treated	with flucloxacillin			
Total protein(g/l)	65.00±3.5	60.30±4.4	68.83±3.9	64.12±3.4	62.00±2.71
Protein fractions :					
Albumin (g/l)	13.91±2.45	11.58±2.32	11.91±1.35	11.91±3.12	11.90±2.95
α ₁ -globulins (g/l)	7.88±0.77	7.61±1.00	8.00±1.00	7.61±0.75	7.09±0.14
α ₂ -globulins (g/L)	7.40±1.19	10.18±1.19	12.40±1.80	10.87±2.14	8.85±0.68
β-globulins (g/l)	18.05±3.12	18.05±4.14	18.53±4.14	17.34±2.34	15.18±2.84
γ-globulins (g/l)	15.76±1.76	15.76±0.93	17.83±3.65	17.45±1.23	18.99±0.93
Fibrinogen (g/l)	2.45±0.01	3.40±0.03 ^d	3.20±0.03 ^{d,h}	2.95±0.04 ^{d,h}	2.72±0.01 ^{d,h}
Urea (mmol/l)	4.52±0.48	6.32±0.39 ^b	3.91±0.10 ^h	3.34±0.16 ^{a,h}	3.43±0.16 ^h
Creatinine (μmol/l)	54.83±5.93	67.67±4.10	66.33±7.30	60.48±1.20	55.33±1.80 ^e
II group	- treated	with tylosin			
Total protein (g/l)	66.17±3.10	52.0±2.59 ^c	55.20±1.60 ^b	52.0±2.59 ^c	54.50±2.80 ^b
Protein fractions :					
Albumin (g/l)	21.45±4.76	14.53±1.83	9.93±0.40	12.36±0.39	11.34±0.54
α ₁ globulins (g/l)	7.02±2.05	6.14±0.88	4.25±0.37	5.25±0.24	4.88±0.77
α ₂ globulins (g/l)	8.09±1.34	7.05±0.98	8.58±1.35	8.09±0.50	8.46±0.62
β globulins (g/l)	14.31±1.19	12.05±2.08	16.00±0.90	14.35±0.61	11.81±1.32
γ globulins (g/l)	15.45±6.90	10.55±3.93	10.58±0.94	11.94±1.72	10.52±1.55
Fibrinogen (g/l)	2.40±0.04	4.10±0.01 ^d	3.10±0.02 ^{d,h}	2.62±0.02 ^{d,h}	2.60±0.01 ^{d,h}
Urea (mmol/l)	3.20±0.44	4.30±0.21a	4.20±0.28	3.90±0.11	3.70±0.16 ^e
Creatinine (μmol/l)	66.80±1.64	112.0±7.50 ^d	98.80±3.5 ^{d,h}	101.0±5.5 ^{d,h}	87.0±2.10 ^{d,h}

* day after the beginning of experimental infection

Significance versus baseline (0 h): a) at $p < 0.05$; b) at $p < 0.02$; c) at $p < 0.01$;

Significance versus day 1 (prior to the treatment: d) at $p < 0.001$; f) at $p < 0.02$; g) at $p < 0.01$; h) at $p < 0.001$.

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