

Effect of the antibiotic Cloxacillin sodium on the level of haemoglobin and packed cell volume in experimental Septicemia caused by Staphylococcus aureus in rabbits

تأثير المضاد الحيوي Cloxacillinsodium

على مستوى خضاب الدم وحجم الخلايا المرصوصة في علاج حالات

تعفن الدم التجريبي بالمكورات العنقودية Staphylococcus

aureus الذهبية في الأرانب

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Abstract

The study includes the side effects resulted after the use of the antibiotic Cloxacillin sodium in the treatment of experimental *Staphylococcus aureus* septicemia in rabbits, the effect of this antibiotic was studied on Haemoglobin level and packed cell volume.

The study revealed that antibiotic in the two doses (50 and 100) mg Cloxacillin sodium /Kg body weight causes a significant decrease in the Hb concentration and the percentage of PCV.

Introduction :

Septicemia: is a clinical term used to describe bacteremia with the clinical manifestations of severe infection.

Septicemia is still a serious condition and continues to be an important cause of morbidity and mortality in the neonatal period despite advances in antimicrobial therapy and supportive care (Polin and Jwill, 1992).

Predominant organisms vary between geographical areas and time of onset of septicemia. Currently, *Staphylococcus aureus* was found to be the most common isolate in neonatal septicemia (35%). The next most predominant organism was *Klebsiella pneumoniae* (18.4%), followed by *E. coli* (10.9%), *Acinetobacter species* (10.4%), *Pseudomonas aeruginosa* (8.7%), *Enterobacter species* (7.8%), *Enterococcus faecalis* (5.1%) and *Proteus species*, Coagulase Negative Staphylococci, *Streptococcus pyogenes* (3.7%), (Agnihotri et al., 2004).

Antibiotics was used in the treatment of many infections including septicemia, Angyo et al., (2001) indicated that (96% , 84% and 81%) of *Salmonella species*, *Staphylococcus aureus* and *Klebsiella species* isolated were sensitive to the antibiotic gentamicin respectively. (14%) of

Staphylococcus aureus isolated were resistant to cloxacillin. Two (1.7%) of the *Staphylococcus aureus* isolated were resistant to all the antibiotics tested. With the exception of *Pseudomonas species* (80%) sensitive only to ceftazidime, sensitivity of most of the organisms to the third generation cephalosporins was generally excellent. It is concluded that gentamicin remains an effective drug in childhood septicemia.

Al-Zwaini (2002) study showed that *Staphylococcus aureus*, *Klebsiella pneumonia* and *E. coli* isolates showed *in vitro* susceptibility to cefotaxime, chloramphenicol and gentamicin, but resistance to more commonly used antibiotics such as ampicillin and cloxacillin. Al-Talib study (2002) showed that some bacterial isolates from early and late onset neonatal sepsis in premature and newborn infants revealed sensitivity to the higher concentrations of cefotaxime, ceftriaxone, piperacillin and to the lower concentrations of amikacin. Roy and co-workers (2002) showed that more than (89%) of *Staphylococcus aureus* isolated were resistant to penicillin. None were resistant to vancomycin or teicoplanin. More than (95%) of enterobacteria were resistant to anti gram negative penicillin group of drugs and more than (40%) were resistant to extended spectrum cephalosporins.

Aim of Study :

To show the side effects resulted after the use of the antibiotic cloxacillin sodium in the treatment of experimental septicemia caused by *Staphylococcus aureus* in rabbits on :

1. Concentration of Haemoglobin (Hb).
2. Percentage of Packed Cell Volume (PCV).

Materials and Methods :

Preparation of bacterial suspension used in experimental animalinfection :

1. Five ml of nutrient broth was inoculated with pure culture of *Staphylococcus aureus* isolated from clinical cases and incubated at 37°C for (18-24) hrs.
2. Bacterial cells were isolated by centrifugation of inoculated nutrient broth at (2000 cycle / min) for 10 minutes. The cells were washed with normal saline by the addition of (2) ml of normal saline to the precipitate resuspension and centrifugation. This process was repeated for (3) times. At last (5) ml of normal saline was added to the precipitate.
3. Dilutions were prepared till the dilution (10^{-4}).
4. Bacterial number was fixed at (5×10^6 - 1×10^7) cfu/ml by using the viable plate count method (Yao et al., 1997).

Laboratory animals :

Male albino rabbits weighed between (1000-1600) gram and aged (6) months were used, (5) animals for each group.

Experimental infection :

Rabbits were injected with (1) ml of the bacterial suspension at the dilution (10^{-4}) intravenously. After (24) hr., blood was withdrawn by venipuncture and blood culture was done to indicate infection with septicemia.

Blood culture test :

Blood taken from infected rabbits were cultured on Brain Heart Infusion Broth (BHIB), (1) ml of blood for each (10) ml of BHIB and incubated at 37°C for (24-48) hrs., turbidity of the broth was regarded as indicator of infection of rabbits with septicemia (Vandepitte et al., 1991). One drop of BHIB

was cultured on blood agar plate (to ensure the presence of infection), this test was done every (48) hr. from the first day of the infection till the 9th or 11th day were the rabbits recovered.

Preparation of the antibiotic cloxacillin sodium and study its effect on laboratory animals :

The antibiotic cloxacillin sodium was prepared according to (Al Hadidi, 2006), the laboratory animals were injected by the two doses (50, 100) mg cloxacillin sodium / kg body weight.

Effect of cloxacillin sodium on laboratory animals :

Experimental animals were divided into (4) groups. The first group was the control negative group including healthy rabbits injected with normal saline. The second group was the control positive group including rabbits experimentally infected with septicemia and injected with normal saline. The third group includes rabbits experimentally infected with septicemia and treated with the two doses of cloxacillin sodium while the fourth group includes healthy rabbits injected with the two doses of the same antibiotic.

Laboratory Tests :

Three ml of blood were collected by venipuncture from rabbits in the four mentioned groups, (1) ml of blood samples (from the second and the third groups) was used for blood culture while the remaining (2) ml was collected in EDTA tubes for the following blood tests, depending on (Dacie and Lewis, 1970) :

1. Haemoglobin test : the test was done by the cyanmethaemoglobin method.

2. Determination of Packed Cell Volume : the test was done by the Micro-Method using the capillary tubes.

Statistical analysis :

Statistical analysis was done by using (the One Way Anova) and (Duncan test) in comparing between the days and the groups to find the coefficient and the tests was regarded as significant at $p \leq 0.05$ (Al-Rawi and Khalafalla, 1980).

Results :

The results showed a significant decrease in the level of Hb and PCV in both groups of rabbits experimentally infected and treated with cloxacillin sodium at a dose of 50 mg/kg body weight. Tables (1,2) showed that the concentration of Hb and the percentage of PCV were (115.50) g/L and (36) % respectively at starting treatment and decrease stepwise to reach (74.50) g/L and (23) % respectively at the end of treatment and when compared with the group experimentally infected and injected with normal saline as a control positive group showed a significant decrease in their levels too, Hb and PCV decrease from (98) g/L and (30.50) % respectively at the beginning of infection to (64.50) g/L and (20) % respectively after 5 days of infection, while in the healthy groups injected with the same dose of cloxacillin sodium, the decrease was significant also in both Hb and PCV, (107.30) g/L and (33.33) % respectively at the start of injection to (79) g/L and (24.50) % respectively at its end, and their levels didn't decrease significantly in the healthy rabbits injected with normal saline as a control negative group, which indicates the effect of cloxacillin sodium at a dose of 50 mg/kg body weight on the level of Hb and on the rate of PCV in addition to the effect of bacterial infection.

Tables (1,2) also showed a significant decrease in Hb and PCV as a result of using cloxacillin sodium at a dose of 100 mg/kg body weight, Hb and PCV in both rabbit groups experimentally infected and treated with cloxacillin sodium were (90.30) g/L and (30) % respectively at the beginning of

experiment and decrease stepwise till they reach (73.30) g/L and (22.67) % respectively at its end, while their levels decrease from (110.70) g/L and (34.33) % respectively at the beginning of injection to (80.70) g/L and (25) % respectively at the end of injection in both healthy rabbit groups injected with the same dose of cloxacillin, which indicates that cloxacillin sodium at this dose affect the level of Hb and PCV.

Table (1): The effect of antibiotic Cloxacillin sodium at a dose of (50,100) mg / kg body weight on Hemoglobin concentration (Hb) in g/L

Groups	Mean ± Standard Error				
	1 th day	3 th day	5 th day	7 th day	9 th day
Healthy rabbits + normal saline	109.00± 9.40 abc	102.40± 8.80 a-f	101.00± 5.10 b-f	94.00± 7.40 d-i	95.70± 4.40 c-h
Rabbits infected with septicemia + normal saline	98.00 ±2.20 b-g	93.00 ±0.00 e-j	64.50 ±5.60 p	*	*
Rabbits infected with septicemia + 50 mg Cloxacillin sodium / kg body weight	115.50± 8.00 a	106.50± 5.50 a-e	93.50 ±5.50 d-i	83.50 ±3.80 h-o	74.50 ±3.80 m-p
Healthy rabbits + 50 mg Cloxacillin sodium / kg body weight	107.30± 3.00 a-d	91.30 ±3.00 f-k	76.30 ±7.90 l-p	77.00 ±0.00 l-p	79.00 ±4.60 j-o
Rabbits infected with septicemia + 100 mg Cloxacillin sodium / kg body weight	90.30 ±2.40 f-l	95.70 ±0.80 c-h	86.00 ±0.60 g-n	77.70 ±2.10 k-p	73.30 ±2.50 nop
Healthy rabbits + 100 mg Cloxacillin sodium / kg body weight	110.70± 2.50 ab	90.30 ±3.10 f-l	71.00 ±1.80 op	76.00 ±0.60 l-p	80.70 ±3.10 i-o

Different letters vertically and horizontally indicates the presence of significant difference at a level of significance $p \leq 0.05$

* Dead rabbits

Table(2): The effect of antibiotic Cloxacillin sodium at a dose of (50,100) mg / kg body weight on Packed Cell Volume (PCV) in %

Groups	Mean \pm Standard Error				
	1 th day	3 th day	5 th day	7 th day	9 th day
Healthy rabbits + normal saline	33.80 ± 2.92 abc	31.80 ± 2.75 a-f	31.40 ± 1.57 a-g	29.20 ± 2.31 c-i	29.71 ± 1.77 b-h
Rabbits infected with septicemia + normal saline	30.50 ± 0.87 b-g	29.00 ± 0.00 c-i	20.00 ± 2.31 l	*	*
Rabbits infected with septicemia + 50 mg Cloxacillin sodium / kg body weight	36.00 ± 1.15 a	33.00 ± 1.73 a-d	29.00 ± 1.73 c-i	26.00 ± 1.15 g-k	23.00 ± 1.15 kl
Healthy rabbits + 50 mg Cloxacillin sodium / kg body weight	33.33 ± 0.92 abc	28.33 ± 0.92 b-j	23.67 ± 2.43 jkl	24.00 ± 0.00 jkl	24.50 ± 1.44 i-l
Rabbits infected with septicemia + 100 mg Cloxacillin sodium / kg body weight	30.00 ± 0.73 b-g	29.00 ± 0.73 c-i	26.67 ± 0.21 f-k	24.00 ± 0.63 jkl	22.67 ± 0.76 kl
Healthy rabbits + 100 mg Cloxacillin sodium / kg body weight	34.33 ± 0.76 ab	28.00 ± 0.97 e-j	22.00 ± 0.37 kl	23.67 ± 0.21 jkl	25.00 ± 0.97 h-k

Different letters vertically and horizontally indicates the presence of significant difference at a level of significance $p \leq 0.05$

* Dead rabbits

Discussion :

The results showed that cloxacillin sodium at a dose of (50,100) mg/kg body weight causes a significant decrease in the level of Hb and in the rate of PCV. This result agree with Nissen (2002) and Karch (2003).

Lubran (1989), Chaturvedi and Nair (2005) showed that the penicillins (like cloxacillin sodium) may act as a hapten or may affect the immune system leading to the production of antidrug antibodies and sometimes autoantibodies. Haemolytic anemia may result.

The results also showed that the infection with *Staphylococcus aureus* causes a significant decrease in Hb and PCV which confirm the results of Austin and Fackrell (1984) that the rate of influx of $SO_4(2-)$ in rabbit erythrocytes exposed to Staphylococcal alpha-hemolysin was twice that of the untreated cells which causes the lysis of RBCs, and also the results of Kato and co-workers (1975) as they pointed to the hemolytic action of staphylococcal alpha-toxin on rabbit erythrocytes, probably by its interaction with the alpha-toxin binding sites on the cell membrane, and also the study of Marshall and co-workers (2000) which investigates the hemolytic activity of staphylococcal beta-toxin on RBCs

of various species. One nanogram of beta-toxin lysed 115,000 sheep erythrocytes and 82,000 human erythrocytes.

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