Response of *Pseudomonas aerugionsa* to Sub Minimal Inhibitory Concentrations of Ciprofloxacin Using Mouse Model of Infection

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Abstract

In vivo response of non mucoid pilated native isolate of *Pseudomonas aeruginosa* (SLS – 2) to sub minimal inhibitory concentrations of ciprofloxacin (8 ug/ml) was tested using mouse model of infection. The results indicated that ¾ MIC of ciprofloxacin was effective in reducing number of *Pseudomonas aeruginosa* in the mouse lungs . It also caused a reduction in infection capability of SLS- 2 and progress of the disease.

Introduction

Antibiotics have been used to prevent colonization and development of diseases by inhibition of growth through mechanism of biological actions on particular target in the microbial cell [1]. Accordingly , bacterostatic dose of antibiotic here categorized as minimum inhibitory concentration (MIC). On the other hand, some suggested sub-bacterostatic doses (Sub MICs) for attenuation of bacterial adhesion to the target cells[2,3], through abolishment of some components on bacterial surfaces called adhesions which are either pili or alginate [4]. Ciprofloxacin, cefotaxime, gentamycin and piperacillin were used for this purpose and found to be effective at sub MICs levels[4-9].

The purpose of this research paper is to *In vivo* test the response of *P. aeruginosa* to ciprofloxacin at sub minimal inhibitory concentrations

Materials and Methods
Culture conditions

Pseudomonas aeruginosa isolate (SLS–2) piliated non mucoid isolated from medical specimen of Iraqi patients[4,5] was used in this study.

Bacterial suspension was prepared by inoculating Muller–Hinton broth medium with bacterial growth. The culture was incubated at 37°C overnight to (O.D$_{600}$ about 0.4) giving 1×10^9 CFU/ml .

Antibiotic sensitivity test

Minimum inhibitory concentration of ciprofloxacin for strain (SLS – 2) was determined using agar dilution method [7]. Accordingly, ¾ and ¼ MIC were calculated and used for performance of infection experiment.

Mouse Model of Infection

An experiment was conducted to study the *in vivo* effect of ciprofloxacin at ¾ and ¼ MIC levels on adherence capability and pathogenesis of *P. aeruginosa* strain SLS – 2 using twenty five of seven day old mice housed in cages into room free known pathogens.

Infection Procedure

Each animal was weighed then inoculated with 2μl aliquots of bacterial suspension inserted directly into the nares, this process was repeated until the animal received entire 10 μl of inoculums containing 10^8 CFU / ml (previously cultured on nutrient broth containing sub minimal inhibitory concentrations ¾ of ciprofloxacin and incubated overnight at 37°C . The entire process required approximately 1 ml per animal. The mice were returned to their mothers following the inoculation . At 4, 24, 48 hrs. postinoculaiton , the animals were sacrificed and weighed. The chest was opened using sterile technique ; the 2 lungs were weighed and homogenized in 400 μl of sterile PBS until the consistency were smooth. 100 μl protein was placed onto king A agar then incubation 37°C for 24 hr. After incubation, bacterial colonies were counted and considered as CFU/ml of suspension and drown as numbers verses exposure time.

Results and Discussion

Pseudomonas aeruginosa SLS–2 , a native isolate obtained from medical specimen of Iraqi patients[4,5], was selected as a non mucoid piliated isolate[4]. Sensitivity of strain SLS – 2 as well as minimum inhibitory concentrations of piperacillin, gentamicin, ciprofloxacin and cefotaxime were estimated as 1042, 128, 8 and 512 ug/ml respectively[5]. It is worth to mention that previously, we demonstrated the contribution of pili in adhesion of isolate SLS-2 to epithelial cells and polysaccharide was found not the mechanism of adherence [4].

Accordingly this isolate was considered as non mucoid piliated isolate. In vitro effect of sub-inhibitory concentrations of antibiotic on adherence was also tested and found that ¾ MIC of ciprofloxacin was the most effective concentration for attenuation of adherence capability [4,6].

The in vivo response of *Pseudomonas aeruginosa* SLS – 2 to sub MIC (¾ and ½) of ciprofloxacin was tested using mouse model of infection.

An inoculum of 10^8 CFU of *Pseudomonas aeruginosa* isolate SLS-2 was evident through comparison the results with that of sacrified control having no detectable count of *Pseudomonas aeruginosa* in the lungs and acquisition of *Pseudomonas aeruginosa* did not occur from environment or from transmition from infected mice and inoculated on at zero time that did not reach for the lungs of the animals. Moreover, all others have pulmonary infections after 4 hrs. Post inoculation as demonstrated by appearance of disease symptoms and bacterial counts which increased to 10^5 CFU[8,9].

Moreover, bacterial counts increased from 10^5 to 10^7 CFU/100 µl of tissue after 24 hrs. of infection as shown in Figure (1). Then decreased from 10^7 to 10^4 CFU/100 µl of tissue after 48 hrs. of inoculation ; such result agree with that of tang *et al.*, 1995 whom also found an acute pulmonary infections by *Pseudomonas aeruginosa* as results of adherence capability caused by the presence of pili on the bacterial cell surfaces.
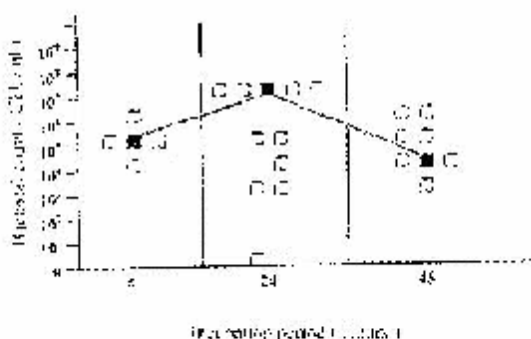

Figure (1): Time course of *Pseudomonas aeruginosa* in neonatal mice treated without treatments of antibiotics. All values are listed in CFU of *Pseudomonas aeruginosa* recovered per 100µl of homogenized tissue from animals dead or sacrificed at a given time Point.☐ , lung cultures from individual animals; ■ , Median CFU of all lung cultures

Growth kinetics of *P. aeruginosa* follows the same trend as that belongs to the behavior of the animals Maximum responses were found to be after 24 hrs. of pulmonary infections.

Treatment with at ¾ MIC of ciprofloxacin cause alteration in the behavior of bacteria, strains and hence progress of pulmonary infection as shown in Figure (2). Bacterial count was altered at that level of sub MIC. However, exposure of cells to ½ sub MICs of ciprofloxacin showed no clear effect neither on infections and progress of disease nor on bacterial counts as indicated by data shown in Figure (3).
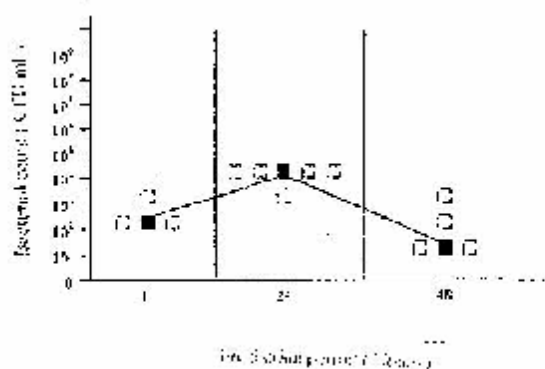

Figure (2): Time course of *Pseudomonas aeruginosa* in neonatal mice treated with ¾ MIC of ciprofloxacin. All values are listed in CFU of *Pseudomonas aeruginosa* recovered per 100µl of homogenized tissue from animals dead or sacrificed at a given time Point. ☐ , lung cultures from individual animals; ■ , Median CFU of all lung cultures.

Figure (3): Time course of *Pseudomonas aeruginosa* in neonatal mice treated with ½ MIC of ciprofloxacin. All values are listed in CFU of *Pseudomonas aeruginosa* recovered per 100µl of homogenized tissue from animals dead or sacrificed at a given time Point. ☐ , lung cultures from individual animals; ■ , Median CFU of all lung cultures.
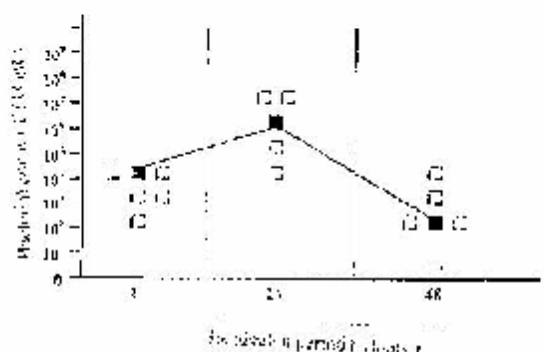
The results of *in vivo* infections followed the same trend as that described previously in the *in vitro* study of the effect of antibiotics on adherence of bacterial isolate [3,6]. This experiment was considered as a concrete evidence for the effect of some antibiotics sub MICs levels on adherence throuy their effect on protein synthesis.

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الخلاصة

درست استجابة عزلة محلية لبكتريا *Pseudomonas aeruginosa* (وصفت بانها مهدبة غير لزجة) لتراكيز تحت المثبط الدنيا من مضاد السبروفلاكسين باستخدام نظام اصابة الفئران. اظهرت النتائج بان ¾ التركيز دون المثبط الادنى البالغ (8 μg/ml) من مضاد السبروفلاكسين كان فعالا في خفض اعداد بكتريا *P. aeruginosa* في رئات الفئران تحت التجربة . وادى هذا التركيز الى خفض قدرة البكتريا على احداث الخمــج وتقدم المرض.