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

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Abstract

In vivo response of non mucoid piliated 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 ¼ 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 targets in the microbial cell [10]. Accordingly, bacteriostatic dose of antibiotic here categorized as minimum inhibitory concentration (MIC). On the other hand, some suggested sub-bacteriostatic doses (Sub MIC) for attenuation of bacterial adhesion to the target cells [11], through abolition of some components on bacterial surfaces called adhesions which are exenter pil or alginate [12]. Ciprofloxacin, enoxacin, gentamicin and piperacillin were used for this purpose and found to be effective at sub MICs level [12].

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) pilated non mucoid isolated from medical specimen of Iraqi patients [45], was used in this study. Bacterial suspension, was prepared by inoculating Mueller-Hinton broth medium with bacterial growth. The culture was incubated at 37°C overnight (0.4-0.8) giving 1×10^8 CFU/ml.

Antibiotic sensitivity test

Minimum inhibitory concentration of ciprofloxacin for strain (SLS-2) was determined using agar dilution method [7]. Accordingly, ¼ and ½ 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 ¼ and ½ 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 2.41 aliquots of bacterial suspension inserted directly into the nasae, 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 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 postinoculation, the animals were sacrificed and weighed. The cell was opened using sterile technique; the 2 lungs were weighed and homogenized in 400 µl of sterile PBS until the consistency was smooth. 100 µl protein was plated 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 crown as numbers versus exposure time.

Results and Discussion

*Pseudomonas aeruginosa* SLS-2, a native isolate obtained from medical specimen of Iraqi patients [21], was selected as a non mucoid pilated isolate [46]. Sensitivity of strain SLS-2 as well as minimum inhibitory concentrations of ciprofloxacin, gentamicin, ciprofloxacin and piperacillin were estimated as 1042, 128, 8 and 5.2 ug/ml respectively [46]. It is worth to mention that previously, we determined the contribution of pil in adhesion of isolate SLS-2 to epithelial cells and poly saccharide was found not the mechanism of adherence [46].

Accordingly this isolate was considered as non mucoid pilated isolate. In *in vivo* effect of sub-inhibitory concentrations of antibiotic on adherence was also tested and found that ¼ MIC of ciprofloxacin was the most effective concentration for attenuation of adherence capability [46].
The in vivo response of *Pseudomonas aeruginosa* SLS-2 to sub MIC (¼ and ½) of ciprofloxacin was tested using a mouse model of infection.

An inoculum of 10^6 CFU of *Pseudomonas aeruginosa* isolate SLS-2 was evident through comparison the results with that of sacrificed control having no detectable count of *Pseudomonas aeruginosa* in the lungs and acquisition of *Pseudomonas aeruginosa* did not occur from environment or from transmission 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 10^8 CFU/10^2.

Moreover, bacterial counts increased from 10^5 to 10^7 CFU/10^2 µl of tissue after 24 hrs of infecting as shown in Figure (1). Then decreased from 10^2 to 10^2 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 a result 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 with ¼ 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 1, lung cultures from individual animals; m, Median CFU of all lung cultures](image1)

![Figure (2): 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 1, lung cultures from individual animals; m, Median CFU of all lung cultures](image2)

![Figure (3): Time course of *Pseudomonas aeruginosa* in neonatal mice treated with 100µl of homogenized tissue from animals dead or sacrificed at a given time Point 1, lung cultures from individual animals; m, Median CFU of all lung cultures](image3)

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 [5]. This experiment was considered as a concrete evidence for the effect of some antibiotics sub MICs levels on adherence through their effect on protein synthesis.

References


الخلاصة

Pseudomonas درست استجابة عزلة مختبرية لبكترييا P. aeruginosa ووصفها بفترة غير قيمة تحت التركيز تحت الفاصل الذي بدأ من مصابين السيرفوكلينين باستخدام نظام إمساك الفراغ. أظهرت النتيجة أن 74% التركيز في الفاصل العاشر من مصاب السيرفوكلينين كان عالياً في خفض P. aeruginosa عدد البكتيريا في نبات الفراغ تحت التجربة. وادي هذا التركيز في تقليل عدد البكتيريا على أعداد المجموع وتقدم المرض.