J. Hyg., Camb. (1983), **91**, 421–427
Printed in Great Britain

A cephalosporin active *in vivo* against *Nocardia*: efficacy of cefotaxime in murine model of acute pulmonary nocardiosis

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SUMMARY

Cefotaxime, a cephalosporin drug, has been shown to be active *in vitro* against nocardiae, a finding confirmed in this study. Pharmacokinetic studies were performed in mice to define regimens which provided peak serum levels comparable to that achieved in man with currently used doses. These regimens were shown to be effective with only short courses of therapy of rapidly progressive and highly lethal *N. asteroides* infection, produced by pulmonary challenge of mice. This suggests the possible utility of this drug in human nocardiosis.

INTRODUCTION

Sulphonamides have been the standard for treatment of nocardiosis. However, other agents such as trimethoprim-sulphamethoxazole, ampicillin, erythromycin, minocycline, and amikacin have been shown to be active *in vitro* or in isolated case reports (Bach, Gold & Finland, 1973; Bach, Sabath & Finland, 1973; Epstein, 1974; Finland *et al.* 1974; Maderazo & Quintiliani, 1974; Carroll, Brown & Haley, 1977; Cook & Farrar, 1978; Wren, Savage & Alford, 1979; Smith *et al.* 1980; Yogev *et al.* 1980; Wallace *et al.* 1977, 1979, 1982), though treatment failures have also been recorded. Recently, several groups have studied the *in vitro* activity of newer β-lactam antibiotics against *Nocardia* species (Cynamon & Palmer, 1981; Martin-Luengo, Lopez-Azorin & Bernal, 1981; Garcia-Rodriguez *et al.* 1982; Gombert, 1982). These drugs are of particular interest because of their high therapeutic:toxic ratio. While these reports suggest that cefotaxime has useful *in vitro* activity against up to 80% of *N. asteroides* isolates, *in vivo* data are currently not available to verify *in vitro* activity. This paper is the first to present evidence for *in vivo* activity of a cephalosporin against nocardiae. We used an acute pulmonary model of nocardiosis in mice. This model has the same portal of entry as the human disease, i.e. respiratory, and mimics human pulmonary disease histopathologically and clinically (Beaman *et al.* 1978). The results clearly demonstrate a protective effect in this experimental model.

MATERIALS AND METHODS

Mice. Pathogen-free female BALB/cByJIMR (Institute for Medical Research, San Jose, California) mice, 3½ weeks old, were used in these studies. Mice were housed ten per cage, and fed acidified water and Wayne Sterilizable Lab-Blox (Wayne Feed Division, Chicago, Illinois) *ad lib.*

Organism. A recent clinical isolate of *N. asteroides* was used. The organism was maintained in 20% glycerol at −70 °C.

Preparation of inoculum. A modification of previously described methods (Beaman, 1975; Beaman *et al.* 1980) was used. Nine days prior to infecting the mice, the frozen Nocardia stock was thawed and approximately 0·1 ml was inoculated into 50 ml brain-heart-infusion (BHI) broth in a 250 ml Erlenmeyer flask. This represented the starter culture. After 1 week of incubation on a rotary shaker (250 rev/min) at 35 °C, 0·5 ml was inoculated into 200 ml fresh BHI in a 1000 ml Erlenmeyer flask which contained a magnetic stirring bar. The culture was incubated at 35 °C with constant stirring until harvest, 48 h post-inoculation, during the late logarithmic phase of growth.

Five or six 50 ml conical tubes were filled to capacity with the 48 h culture. An initial centrifugation at 100 ***g*** for 5 min removed any large clumps of organisms that were present. The supernatant was then centrifuged at 500 ***g*** for 15 min. The resulting supernatant was then carefully decanted until 5–10 ml remained above the pellet. The contents of the conical tubes were then combined and a final centrifugation at 1000 ***g*** for 15 min was performed. This 3-step centrifugation procedure enabled the preparation of a consistent inoculum free of large clumps of organisms.

Infection of mice. Mice were infected intranasally with one or two 30 μl suspensions of 1×10^7 c.f.u. of *N. asteroides* in BHI, for the high and low dose challenge, respectively, using ether anesthesia to suppress sneeze and cough reflexes.

Drug. Cefotaxime (lot number W301/180231) was obtained from Hoechst-Roussel, Somerville, New Jersey, as a sterile powder. Appropriate dilutions were made with sterile physiologic saline.

Pharmacokinetics. A bioassay of cefotaxime concentrations in mouse sera, obtained by bleeding from the orbital plexus, was performed. *Escherichia coli* ATCC 25922, the indicator organism, was grown overnight in BHI at 35 °C on a rotary shaker (250 rev/min), and a sample was adjusted to an optical density of 0·22 at 540 nm. Two ml of this sample was streaked onto a 43 × 43 cm glass plate which contained 500 ml of Mueller–Hinton agar. Wells (5 mm in diameter) were cut, 35 mm apart. Cefotaxime standards were diluted in mouse serum. Standards and unknown samples were then placed randomly in the wells. After overnight incubation of the plates at 35 °C, zone sizes were measured with calipers.

In vitro *susceptibility testing.* Minimal inhibitory concentrations (MICs) were determined as previously described (Galgiani & Stevens, 1976) except for the use of BHI broth and an inoculum of 4×10^3 c.f.u./ml. The minimal bactericidal concentration (MBC) was defined as that concentration which killed ≥ 98% of the original inoculum at the time of the MIC reading, as determined by subculture from tubes to blood agar plates.

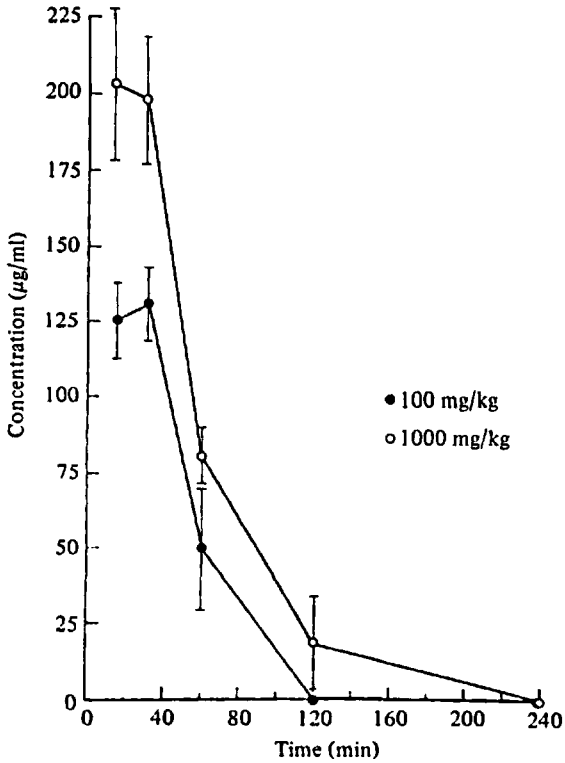

Fig. 1. Mice were given a subcutaneous injection of cefotaxime 1000 or 100 mg/kg and bled at the indicated times. Each data point is the mean ± S.D. serum concentration of two mice.

Treatment regimen. Mice were treated subcutaneously (dorsal site) with their drug dose or saline placebo in 0·5 ml three times daily: 7.00 a.m., noon and 4.30 p.m. for 7 days. Following discontinuation of therapy, mice were observed for 30 days.

Statistics. Significance of survival of the treatment groups was determined by the Wilcoxon rank-sum test.

RESULTS

In vitro *susceptibility of* N. asteroides. By a tube dilution method, the MIC of cefotaxime for the isolate used to infect the mice was 12·5 μg/ml. Six other clinical isolates were tested. MIC's for three were ⩽ 3·9 μg/ml, another was determined to be 1·95 μg/ml. One isolate had an MIC > 62·5 μg/ml, and the other was determined to be 250 μg/ml. These latter two isolates were termed resistant, based on achievable serum concentrations in man (Dalovisio & Pankey, 1982).

The MBC of the isolate used for challenge was also 12·5 μg/ml.

Pharmacokinetics of cefotaxime in mice. Single dose pharmacokinetics after subcutaneous administration were determined using high (1000 mg/kg) and low (100 mg/kg) doses of cefotaxime. As is shown in Fig. 1, peak levels of active drug were achieved within 30 min following injection. One hundred and twenty minutes after the low dose and 240 min after the high dose, no biologically active drug could be detected. The peak levels achieved following these two doses of cefotaxime were

https://doi.org/10.1017/S0022172400060459 Published online by Cambridge University Press

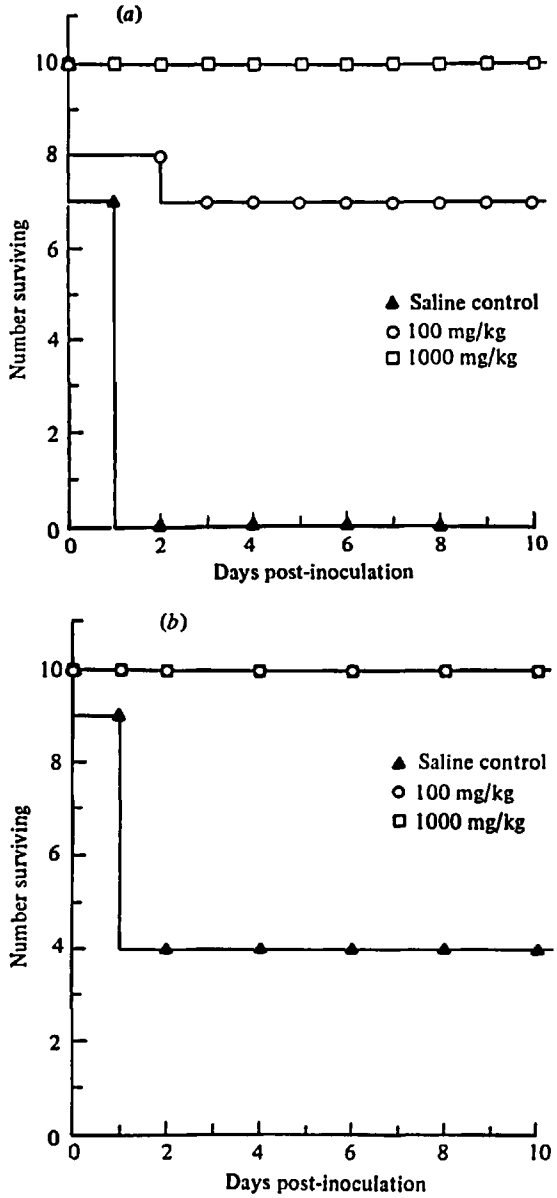

Fig. 2. Survival of mice following inoculation with (*a*) high dose (2×10^7 c.f.u.) or (*b*) low dose (1×10^7 c.f.u.) *N. asteroides*. In both (*a*) and (*b*), saline treated *v.* either cefotaxime (1000 or 100 mg/kg) group, $P < 0{\cdot}01$.

comparable to those seen in man after the doses usually prescribed (Delovisio & Pankey, 1982).

Protection of mice from death from lethal challenge with N. asteroides. The data from one experiment are shown in Fig. 2. Two different inocula were given to mice, achieving an LD100 (2×10^7 c.f.u./mouse) and an LD60 (1×10^7 c.f.u./mouse). That the differences in mortality are due to numbers of c.f.u. delivered to the lungs in each mouse and not volume alone is suggested by the following: (*a*) the extra

https://doi.org/10.1017/S0022172400060459 Published online by Cambridge University Press

30 μl volume in the high inoculum group was not detrimental to survival since cefotaxime-treated mice survived, and cefotaxime would not be expected to favourably influence volume overload in the lungs; (*b*) other experiments in our laboratory with more dilute inocula and up to four administrations (30 μl/mouse) did not show an increase in mortality with these larger volumes.

A dose-response effect with cefotaxime is seen when one compares the high and low inocula and the high and low dose cefotaxime therapy. With the high nocardia inoculum, mortality was 100% in the saline control group, 30% in the low dose cefotaxime group and 0% in the high dose cefotaxime group. Both treatment groups show statistically significant protection when compared to the control group ($P < 0{\cdot}01$). With the low nocardia inoculum, mortality in the saline control group was 60, and 0% in both low dose and high dose cefotaxime groups. These differences are also statistically significant ($P < 0{\cdot}01$).

A similar experiment with an LD40 showed 100% protection with both doses of cefotaxime (data not shown).

DISCUSSION

This study demonstrates the beneficial effect of parenteral cefotaxime in treating acute pulmonary nocardiosis in mice. These results are even more impressive when one considers that within 2 days of challenge with *N. asteroides*, all of the mice in the high inoculum challenge group were dead, and that all the mortality in the low dose challenge group occurred by day 3 after inoculation. It was because of the rapidly fatal infection in this model that we decided to begin treatment 1 h following inoculation of the mice with this pathogen. Experience in this laboratory (unpublished observations) and that of others (Beaman *et al.* 1980) documents that progressive pulmonary infection is the cause of death and that death occurs early; in our experiments no mice have died after 4 days following inoculation with 3 different strains of *N. asteroides*.

It should be noted that, of seven isolates tested *in vitro*, only two were resistant to cefotaxime. Our results suggest at least that infection produced by a nocardia isolate susceptible *in vitro* can be treated successfully *in vivo*. Whether this would apply to an isolate resistant *in vitro* cannot be answered at this time. Further studies should expand these observations with other strains of nocardiae.

As the pharmacokinetic data show, we used doses that provided peak serum concentrations comparable to those achieved in humans (Dalovisio & Pankey, 1982). It is interesting that negligible levels of active drug were present in serum 3 h following injection, yet both low dose (100 mg/kg) and high dose (1000 mg/kg) regimens afforded significant protection.

Cefotaxime, like other third generation cephalosporins, has broad-spectrum activity against most gram-positive and gram-negative bacteria, including aerobes and anaerobes (Jones & Thornsberry, 1982). Therefore, these drugs have been under study (Karakusis *et al.* 1982) as initial therapy in febrile immune-impaired hosts, where prompt broad-spectrum therapeutic activity is critical, until the etiologic agent(s) can be defined. Nocardial infections are particularly a problem of immune-impaired hosts (Young *et al.* 1971). The present study suggests that the clinician starting cefotaxime for broad-spectrum therapy in the setting of a febrile

illness in an immune-impaired host would be thus including coverage for the possibility of nocardial infection. This would also be desirable in that it would contribute to narrowing the number of drugs needed to cover the etiologic possibilities, thus averting problems of additional toxicity and undesirable drug interactions, and the use of fewer drugs could lessen the impact on the normal flora. Moreover, often in the immune-impaired host the etiology of an infectious episode is ultimately determined to be more than one pathogen. In that situation, i.e. choice of long-term therapy after diagnosis, a single agent with activity against other pathogens and nocardiae would again be advantageous, for the reasons stated above. In other settings, cost, narrowness of spectrum and route of administration (oral *v.* parenteral for cefotaxime) will be pertinent considerations in choice of therapy.

The authors thank Elmer Brummer, Suzanne Smith and Phuoc T. Vo for assistance.

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