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Effect of Antibiotics on Pathophysiological Responses Induced in Mice Infected with *Bordetella pertussis*

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Abstract: This study was undertaken to evaluate and understand the effect of Augmentin, Erythromycin and Ofloxacin against *Bordetella pertussis* (*B. pertussis*) in murine model. Mice (BALB/C) were challenged with highly infective dose (2.2×10^9 CFU mL⁻¹) of *B. pertussis* and relatedness of pathophysiological responses, its intensity and the course was studied by monitoring the infection with mouse weight gain, temperature, induction of leukocytosis and lung score. Treatment was started giving first dose orally after an hour of infection, therapy was continued for six days. Antibiotics appeared to have provided benefit in decreasing the infection. Conclusively, Augmentin was found more effective than Erythromycin and Ofloxacin and indicated more persistent and potent efficacy *in vivo*.

Key words: Pathophysiological responses, *Bordetella pertussis*, antibiotics

INTRODUCTION

Whooping cough or pertussis is a non-invasive, highly communicable acute infection of ciliated cells of upper respiratory tract caused by the bacterium *Bordetella pertussis*, which mainly affects young children and occasionally infants die from this disease. Symptoms of pertussis include having a cough lasting 14 or more days accompanied by a gasping sound or 'whoop' while coughing. Children vomit or choke, have difficulty in breathing during a coughing spell.

An effective vaccine for pertussis was developed in the 1940s^[1]. It has been estimated that the vast majority of the world's unvaccinated children acquire the infection by the age of five years and life-threatening cases occur in very young children under six months^[2]. However, persons of all ages are susceptible, and although outbreaks in adults are uncommon, there is increasing evidence that adults serve as an important reservoir of infection. The global burden of pertussis is approximately 45 million cases and 0.409 million deaths per year, with highest incidence rates and major risk of deaths and complications occurring in developing countries^[3]. In United States, 17 babies died of whooping cough in year 2000^[4]. In England and Wales, around 80% of the bed-days and 90% of the deaths occur due to pertussis in those too young to be immunized (<3 months of age)^[5].

Transmission is usually due to droplet infection from infected carriers and the attack rate may be high, when

they are exposed with in a closed environment^[6]. Pertussis is a frequent and significant illness in adults. In a recent study by Heining *et al.*^[7] compared serologic responses in adults after infection with serologic responses after vaccination, titers of antibody to pertussis toxin decreased after reaching peak levels and the antibody decay patterns were similar in both groups proving that the immunity wanes after few years of infection and vaccination both.

Keeping in view that pertussis, although preventable by vaccine, is still prevalent due to waning nature of the immunity, search for targeted antibiotic treatment should be taken in account in order to combat with this serious infection. Investigators must feel that the question remains unanswered about the efficacy of antimicrobial therapy against such a common disease after so many years. The prophylactic administration of the antibiotics during the incubation period remains controversial^[7,8]. Several antibiotics reached in the market did not fulfill the gap including β -lactamase inhibitors, cephalosporins, monobactams, and quinolones; but most of them with a very broad antibacterial spectrum^[9]. However, so far the information on the activity of these compounds against *B. pertussis* is concerned, not many studies have been performed on this subject and the reports are widely scattered in the literature.

Knowledge on the pathogenesis and immunity in pertussis has resulted from studies of the mouse respiratory infection models. *B. pertussis* is not a natural

pathogen for mice, but the species has provided basic information about the pathogenesis of human pertussis. According to Pittman^[10], the period of pulmonary infection in mice is of similar duration to that of in humans. There are pathophysiological changes such as histamine sensitization, hypoglycemia, hyperinsulinemia, cough and marked leukocytosis that persist after the bacteria disappear^[11]. Therefore, respiratory infection of mice with *B. pertussis*, introducing intranasally is an extremely useful and appropriate for studies of pathophysiology in several stages such as infection route, localization of infective bacteria and duration of clinical symptoms i.e. leukocytosis,^[12]. Thus, the model possesses considerable value for evaluating protective activity of *B. pertussis* antigens, their antibodies and dose of drugs.

Present study was carried out to evaluate and compile the activity of antibiotics *in vivo* in murine model, as due to the difference in pharmacokinetics properties, good *in vitro* activity of antibiotic does not necessarily imply good treatment results *in vivo*^[13]. The aim of the study was to assess the potential usefulness of antibiotics for treatment of whooping cough by analyzing the effect on growth, phenotype and pathogenesis of *B. pertussis in vivo* which would be beneficial in preventing the severity of infection in susceptible individuals and should contribute in the reduction in spread of the disease.

MATERIALS AND METHODS

Bacterial strains: *B. pertussis* strain BP 18-323 (ATCC 9797) was obtained from the University of Glasgow, Scotland UK. Strain was stored as freeze-dried suspension and recovered by growing for 48 h at 37°C on Bordet-Gengue (BG); (Difco laboratories) containing 10% (V/V) defibrinated sheep blood (NIH Islamabad).

Preparation of inoculum/Infective dose: The *B. pertussis* from 48 h growth on BG agar plates was dispensed in 1% (W/V) casamino acids (Difco Laboratories) and serially diluted to yield a challenge suspension of 2.2×10^9 CFU mL⁻¹ (estimated by viable count).

Infection procedure: Three independent experiments were performed. Mice (BALB/C) were anaesthetized with ether and infected intranasally with challenge suspension in a volume of 100 μ L. After one hour of infection, the first dose of test antibiotics in a volume of 50 μ L was given orally. Antibiotic administration was continued once a day for a period of six days. To the control group, equal volume of distilled water was given.

Post-infection observations: The mice were weighed on the day zero and every two days thereafter. The pathophysiological response was monitored by recording of deaths, mouse weight gain, hypothermia (through rectum with Natsume electronic MCA-111 2A 33/thermal sensor, Shibaura electronics), leukocytosis and lung score.

On day 7, Mice were sacrificed by over anesthesia. Leukocytosis was measured by taking blood through heart puncture with sterile disposable syringe containing 0.0125 mg mL⁻¹ EDTA (BDH).

Leukocyte count was performed with improved Neubauer counting chamber. Lung score was estimated by excising lungs aseptically, homogenized in 2 mL sterile saline and 50 μ L of this suspension was plated on BG plates. After incubation at 37°C for 48 h, lung score was counted according to graded scale of 0-4, which represented no growth to confluent growth^[10].

RESULTS

An infective dose of *B. pertussis* containing 2.2×10^9 CFU mL⁻¹ significantly reduced the body weight gain as compared to the control mice. Recovery in the weight gain observed in antibiotic treated mice was dose-dependent (Fig. 1). Comparatively, Augmentin was found more effective than Ofloxacin and Erythromycin. Hypothermia was observed due to infection and there was marked and constant difference of -1°C (below the normal). Hypothermia was controlled by antibiotic treatment (Fig. 2). Each concentration of antibiotic controlled the temperature in similar way and no variation in effect was observed, where antibiotic itself did not affect the normal temperature of control mice. The infection highly elevated the levels of leukocytosis. In the case of Erythromycin, counts were comparatively higher than the other antibiotic tested (Fig. 3). Spleen/body weight ratio was found interesting. The spleen weight was increased in all antibiotic treated mice where the weight was low in infected untreated mice (Fig. 4). Effect of all antibiotics was dose-dependent. Augmentin brought the infection towards normal at 10 fold lower concentration of the other two antibiotics where Erythromycin was found effective at human prescribed dose. In this sense, the efficacy of Augmentin was found higher than Erythromycin and Ofloxacin. In antibiotic control mice, leukocyte count was slightly increased than normal but the change was insignificant. After performing lung score, bacterial cells were recovered from infected lungs on day seven but the lung score was not so high. Antibiotics did not eliminate

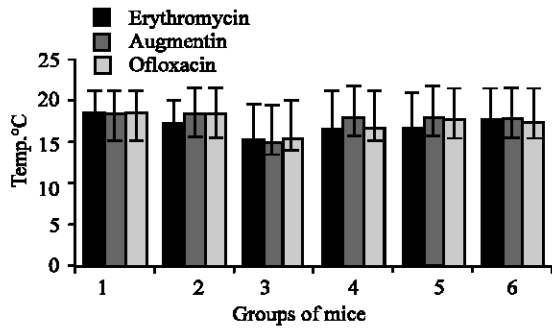


Fig. 1: Effect of antibiotics on weight gain (grams) of mice infected with *B. pertussis*.

Effect of antibiotics i.e erythromycin, augmentin and ofloxacin on mice (BALB/c) weight due to infection with *B. pertussis* was determined. Mice infected intranasally with high challenge dose (2.2×10^9 cfu mL⁻¹) were treated orally with first dose of antibiotic after an hour of infection and treatment was continued for six days. Data is given as the mean of the number of observation (n=6) on 7th day of infection.

1: Normal uninfected mice, 2: Uninfected mice treated with ten time's higher dose of human prescribed dose of antibiotics, 3: Untreated infected mice, 4: Infected mice treated with ten times lower human prescribed dose, 5: Infected mice treated with human prescribed dose, 6: Infected mice treated with ten times higher human prescribed dose.

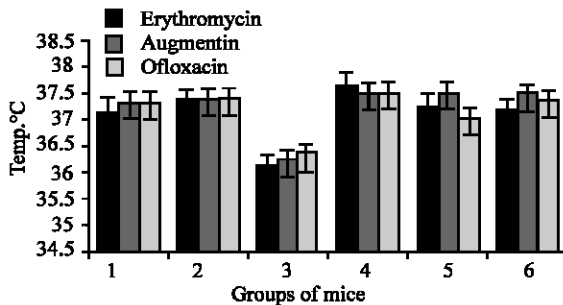


Fig. 2: Effect of antibiotics on body temperature (°C) of mice infected with *B. Pertussis*

Effect of antibiotics i.e erythromycin, augmentin and ofloxacin on mice temperature due to infection with *B. pertussis* was determined. Mice (BALB/c) infected intranasally with high challenge dose (2.2×10^9 cfu mL⁻¹) were treated orally with first dose of antibiotic after an hour of infection and treatment was continued for six days. Data is given as the mean of the number of observation (n=6) on 7th day of infection

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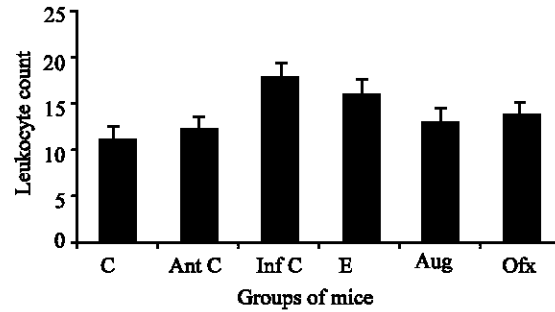


Fig. 3: Effect of antibiotics on leukocyte count (x1000/cmm) of Mice infected with *B. pertussis*

Effect of antibiotics i.e erythromycin, augmentin and ofloxacin on mice (BALB/c) leukocyte count due to infection with *B. pertussis* was determined. Mice infected intranasally with high challenge dose (2.2×10^9 cfu mL⁻¹) were treated orally with first dose of antibiotic after an hour of infection and treatment was continued for six days. Data is given as the mean of the number of observation (n=6) on 7th day of infection.

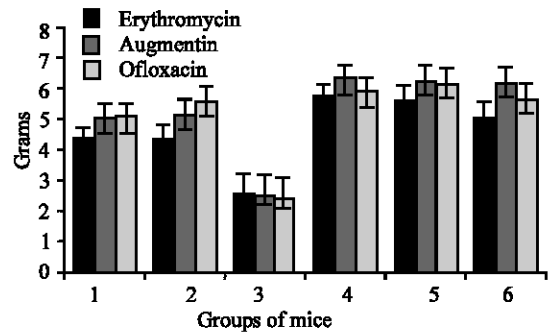


Fig. 4: Effect of antibiotics on spleen/body weight ratio (grams) of mice.

Effect of antibiotics i.e erythromycin, augmentin and ofloxacin on spleen/body weight ratio due to infection with *B. pertussis* was determined. Mice (BALB/c) infected intranasally with high challenge dose (2.2×10^9 cfu mL⁻¹) were treated orally with first dose of antibiotic after an hour of infection and treatment was continued for six days. For observation on 7th day, mice were weighed individually, spleen was excised and spleen/ body weight ratio was calculated. Data is given as the mean of the number of observation (n=6).

1: Normal uninfected mice, 2: Uninfected mice treated with ten time's higher dose of human prescribed dose of antibiotics, 3: Untreated infected mice, 4: Infected mice treated with ten times lower human prescribed dose, 5: Infected mice treated with human prescribed, 6: Infected mice treated with ten times higher human prescribed dose.

B. pertussis completely from the infected lungs however, it reduced the recovery in a dose-dependent manner.

Absence of *B. pertussis* from lungs of normal and antibiotic control mice confirms that the infection cannot be transferred from mice to mice.

DISCUSSION

Treatment of pertussis with antibiotics has not received importance in clinical field. It has been reported that antibiotic treatment has negligible effect of preventing the progression, clinical course or the establishment of disease^[14]. However, Erythromycin may reduce infectivity^[15]. Effect of antibiotics *in vivo* in experimental animals and humans has expanded the field of virulence factors. The efficacy of antibiotics tested in present study clearly demonstrates the usefulness of antibiotic treatment. In a similar study on *E. coli* infection, using mice as model. Comber^[16] showed that 50% of the mice were protected from infection when concentration of ¼ of MIC of amoxycillin was maintained in the blood. Zak and Kradolfer^[17] reported that amoxycillin at ¼ MIC in the blood and peritoneal fluid did not protect mice. By antibiotic sensitivity, placebo-controlled trial have been applied in this study for antibiotics (Augmentin, Erythromycin and Ofloxacin) appears to provide benefit in decreasing the infection. Effect of all antibiotics was dose-dependent; conclusively augmentin was more effective than Ofloxacin and Erythromycin. The result also indicates the more potent and persistent efficacy of Augmentin *in vivo*. The increased potency of Augmentin could be due to its formulation as it is a combination of amoxycillin, a broad- spectrum antibiotic and potassium clavulanate, a potent inhibitor of β-lactamase enzyme. This acts at the enzyme site by variety of mechanisms including comparative inhibition of hydrolysis and other more complex reactions and is mainly active against plasmid-mediated penicillinases^[18].

Although in humans it has been reported that antibiotic treatment has negligible effect on clinical course, progression and establishment of whooping cough^[14] and antibiotic can act only as prophylactic measure^[19,20] but in our study the results clearly demonstrate that antibiotic treatment in early course of infection significantly reduced the pathophysiological symptoms and appeared to be effective in murine model.

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