The Treatment of Macrolide-Resistant *Mycoplasma Pneumoniae* Pneumonia in Children

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Abstract

Objective: To evaluate the efficacy and safety of levofloxacin in children with macrolide-resistant *Mycoplasma pneumoniae* pneumonia.

Methods: We retrospectively enrolled six confirmed cases of refractory *Mycoplasma pneumoniae* pneumonia (n=6) who were admitted in the pediatric respiratory ward of Shandong provincial hospital Affiliated to Shandong first Medical University between January 1st, 2020 and February 29th, 2020. Levofloxacin was given to the patients through the intravenous or oral route as per the following dosages :< 5 years, 8-10 mg/kg q12hours; > 5 years, 8-10 mg/kg, qd for ten days. The clinical data were collected and analyzed.

Results: The average age of the enrolled cases was six years and nine months (range, four years, and seven months to eleven years and seven months). All cases were found to be drug-resistant and were treated with azithromycin combined with antibacterial drugs. Levofloxacin was used in the patient's refractory to macrolide antibiotics. The temperature of five cases returned to normal 1 to 2 days after treatment with levofloxacin, and the imaging of the four cases showed expected improvements. The gastrointestinal symptoms, neurological manifestations, joint symptoms, blood parameters, liver and kidney functions, and exercise conditions of the children were closely monitored. The follow-up time of the patients ranged from one week to five months. No drug-related adverse reactions were observed in patients during treatment or during follow up.

Conclusions: The clinical symptoms and imaging significantly improved after treatment with levofloxacin, and no drug-related adverse reactions were observed. Levofloxacin proved to be an effective and safe drug in the treatment of children with macrolide-resistant mycoplasma pneumonia.

1. Introduction

Mycoplasma pneumoniae (MP) is a common pathogen that causes community-acquired pneumonia in children. In recent years, the resistance of MP to macrolide antibiotics has increased significantly. At the same time, the number of children with refractory *Mycoplasma pneumoniae* pneumonia (MPP) is increasing year after year. The health systems are facing significant challenges in carrying out the diagnosis and treatment of MPP in children. As a third-generation fluoroquinolone, levofloxacin is a broad-spectrum antibiotic and has a wide distribution in the tissues of the body. Levofloxacin is suitable for treating infectious diseases in various systems. However, the use of fluoroquinolones is limited in children due to arthropathy issues in weight-bearing joints. At present, few studies have investigated the use of this drug in the pediatric population in China. To provide a reference for evaluating the efficacy and safety of levofloxacin in the pediatric population, we collected the clinical data of six children who were diagnosed with macrolide-resistant MPP and treated with levofloxacin in the department of Provincial hospital affiliated to Shandong first medical university.

2. Materials And Methods

2.1 General information

A total of six Cases (five males, one female) were admitted in the pediatric respiratory ward of Provincial hospital affiliated to Shandong first medical university from January 1st, 2020 to February 29th, 2020. These children had a mean age of six years and nine months and an age range of four years and seven months to eleven years and seven months. On admission, all the children tested positive for MP RNA and DNA. The mutational sites (2063A > G or 2064A > G) associated with drug resistance to macrolides antibiotics were present. According to the "Guide for the management of community-acquired pneumonia 2013 Edition" [1], if the clinical signs aggravate, fever persists, and pulmonary imaging findings aggravate even after treating the patients with macrolide antibiotics for seven days or more, the patients are diagnosed

with refractory MPP. In our study, all six children were diagnosed with refractory MPP. Further, one child had pulmonary embolism; one child had a streptococcal infection, and two children had bronchial asthma. One child underwent ventilator-assisted respiratory therapy due to severe hypoxemia. Despite having pleural effusion, the five children demonstrated good recovery (Table 1).

2.2 Etiological examination

MP RNA detection, bacterial culture, and antimicrobial susceptibility tests were carried out before treatment. MP RNA was detected using Simultaneous Amplification and Testing (SAT), and MP DNA was detected using Quantitative Real-time Polymerase Chain Reaction (qRT-PCR). All specimens were aseptically collected. A total of nine respiratory tract specimens (deep sputum, alveolar lavage fluid) were collected, and four specimens were collected from blood culture.

2.3 Levofloxacin treatment

In these children, the course of infection was 21–46 days before levofloxacin treatment, with an average of 31 days. They were given conventional azithromycin treatment for five days. After three days of discontinuation, either their body temperature was still unstable, or they had enlarged pulmonary consolidation and (or) atelectasis; Further, the children were given azithromycin for an extended course (7 days), with overall treatment time from 3 weeks to 4 weeks. Other antibiotics, immunoglobulin, methylprednisolone, and other drugs were also used.

Three children were administered fiberoptic bronchoscopy. The remaining children did not undergo fiberoptic bronchoscopy due to the 2019 novel corona virus situation. All the children were given a sufficient course of anti-infective treatment. The family members of the patients agreed and signed the informed consent forms. The application was then submitted to the Department of Pharmacy of the hospital.

The levofloxacin treatment modes were as follows: two children were given intravenous drip; two children were initially given intravenous drip but later changed to oral administration after signs of improvement while another two patients were given oral administration. Levofloxacin was produced by first three pharmaceutical (Beijing) Co. Intravenous dosage was 500 mg / bag, while the oral dosage was 500 mg /tablet. According to the expert consensus for the children's application in accordance with fluoroquinolone antibiotic drugs: children < 5 years were administered with 8–10 mg/kg/dose q12h (up to 750 mg/d) while the children > 5 years were administered with 8–10 mg/kg/dose qd, either intravenously or orally. The total course of treatment was ten days. During the levofloxacin treatment, other antibiotics, including azithromycin, were discontinued.

Table 1
Basic information and treatment characteristics

Case	Infection duration (days)	Hot Peak (°C)	Sputum culture	Complications	Drugs used	Azithromycin application time(weeks)	Course of levofloxacin	Side effects
1	46	38	Negative	Pulmonary embolism	cefoperazone, meropenem, immunoglobulin, methylprednisolone	3 weeks	10 days(oral)	none
2	29	38.5	Negative	Allergic rhinitis	cefoperazone, imipenem and Cilastatin, Immunoglobulin, methylprednisolone	3weeks	7 days (intravenous) 3 days(oral)	none
3	21	38.4	Negative	Bronchial asthma, liver damage, otitis media	Ceftriaxone, immunoglobulin, imipenem and Cilastatin methylprednisolone	3 weeks	10 days (intravenous)	none
4	22	38.8	Negative	none	cefoperazone, linezolid, methylprednisolone immunoglobulin	3 weeks	10 days (intravenous)	none
5	36	37.6	Negative	Streptococcus	Amoxicillin, linezolid, methylprednisolone	3 weeks	7 days (intravenous) 3 days(oral)	none
6	40	37.2	Negative	Liver damage, bronchial asthma	Ceftazidime, cefoperazone methylprednisolone	4 weeks	10 days(oral)	none

3. Results

3.1 Observation of curative effect after treatment with levofloxacin

Before levofloxacin treatment, the body temperature of five children (Case 1–5) was unstable. The highest body temperature fluctuated between 37.6–38.8 °C. After levofloxacin treatment, the body temperature became normal in 1–2 days. The fever did not recur during the hospitalization. The chest radiographs of four children showed significant post-medication improvements. However, the case 6 was critical with severe hypoxemia at the beginning of the illness and had undergone invasive ventilator-assisted breathing. This child took multiple fiberoptic bronchoscopies. The endoscopic mucosal erosion was severe. Ulcers appeared on carina and the walls of right main bronchus and other tubes Many plastic

phlegm plugs were seen blocking the orifice. The medial and lateral branches of the right middle lobe were almost occluded. The imaging improvements were not satisfactory. After ten days of treatment with levofloxacin, improvements were observed in the chest radiograph as compared with the one before treatment (Figure 1). Bronchoscopy findings showed that the mucosal ulcers recovered well. The medical condition of these six children improved, and the children were subsequently discharged.

3.2 Investigation side effects of levofloxacin

The adverse reactions of levofloxacin include abnormalities of the cardiovascular system such as prolonged QT interval, ventricular arrhythmia; central nervous system abnormalities such as dizziness, anxiety, insomnia, and seizures; peripheral neuropathy; skeletal muscle diseases such as arthralgia, myalgia, muscle weakness, and tendon rupture, and other abnormalities such as liver toxicity, digestive tract discomfort, allergic reactions, leucopenia, and granulocytopenia. During the course of treatment, we closely monitored signs and symptoms of the gastrointestinal tract and nervous system and changes in the skin, joints, blood parameters, and liver and kidney functions. The six children treated with levofloxacin did not experience any symptoms of nausea, vomiting, dizziness, or other discomforts. Further, no skin rashes, mental disorders, or convulsions were observed. During five months of follow-up, the children did not complain of any discomfort, including joint pain. Although two children had liver function abnormalities before medication, their liver function returned to normal after receiving hepatica. The rest of the children did not experience any abnormal liver or kidney function or a significant decrease in cell count.

4. Discussion

As one of the common community-acquired pneumonia pathogens, MP is one of the smallest microorganisms among bacteria and viruses. MPP accounts for 10–40% of community-acquired pneumonia cases [2]. Since MP lacks a cell wall, MP is resistant to cell wall targeting antibiotics [3]. Macrolides, tetracyclines, and quinolones are effective drugs for pediatric patients with MPP. In China, macrolide antibiotics are currently the first choice for the treatment of children with MPP. Fluoroquinolones, including levofloxacin, have not

been used extensively to treat children because of their safety concerns in the pediatric population. Therefore, levofloxacin is not approved for use in children.

Macrolide-resistant MPP was first reported by a Japanese scholar in 2001. The problem of macrolide resistance in MP is a global concern. The rate of MP resistance in macrolide antibiotics is 3.5%~13.2% in the US [4] [5] and 90% or more in china [6].

Macrolide antibiotics target MP mainly by binding to the central ring of the 23S rRNA V region of the ribosomal 50S large subunit and inhibiting protein synthesis. The mechanism underlying macrolide resistance in MP involves a single base mutation in the 23S rRNA gene, which changes the structure of the main binding site of the macrolide antibiotics and reduces the binding affinity resulting in resistance. For the 14 and 15 -membered ring macrolides, A-G point mutation of 2063 and A-G point mutations of 2064 cause high-level resistance. A-G point mutation of 2067 and C-G or A point mutation of 2617 cause a low-level resistance [7] [8]. Cao B et al. [9] analyzed more than 20 reviews of MP resistance from 2000 to 2015 and summarized the order of point mutation frequency: A2063G, A2064G, A2063T, A2063C, A1290G, C2617A, and A2067G. Out of these mutations, A2063G and A2064G account for about 80%-90% of the cases.

As compared with patients infected with macrolide-sensitive MP, patients infected with macrolide-resistant MP are reported to have a longer duration of symptoms and longer course of antibiotic treatment. [10] But macrolide-resistant MP does not necessarily cause more severe illness as compared with macrolide-sensitive MP. Some children who were infected with macrolide-resistant MP also demonstrated satisfactory results when treated with conventional therapy. These observations may be due to the following factors: the anti-inflammatory effects of macrolides, the self-limiting nature of MP infection,

and the different degrees of resistance to different macrolides. Zhuang Yuan^[11] reported that due to immune, conditioning, phagocytosis, and other body functions, the minimum inhibitory concentration (MIC) value in the laboratory does not perfectly correlate with the MIC value in the body. The pH of body has a greater impact on macrolide drugs. So, the laboratory drug sensitivity results are not completely consistent with drug resistance results in clinical studies. The coincidence rate between the two is about 70%. This may also explain the curative effect of conventional azithromycin treatment in children with macrolide resistant MP.

Some children with positive macrolide resistance mutations show poor results in conventional macrolide treatment and their condition even progresses to refractory MPP or severe MPP. Refractory MPP manifests as long fever and rapid clinical progression. Some patients even have alveolar consolidation, atelectasis, and necrotizing pneumonia. Complications also occur in the extra pulmonary system, including skin and mucous membranes, blood system, and central nervous system. Therefore, it is very important to timely select appropriate drugs to treat children infected with macrolide-resistant MP.

Morozumi et al.^[12] gave tetracycline and quinolones to children with MP infection who were not effective in prolonging the application of macrolide antibiotics and achieved satisfactory results. Diana et al.^[13] suggested that quinolones should be used to treat MPP in children who are refractory to macrolide antibiotics.

The helicase and topoisomerase IV of MP DNA. Were the target enzymes of quinolones. The quinolones inhibit MP by targeting protein synthesis. Levofloxacin is the third generation of quinolones. As compared with the first and second generation of quinolones, levofloxacin has the advantage of being a wide spectrum antibiotic with strong antibacterial activity, good tissue permeability, low toxicity, and less adverse effects. However, levofloxacin is reported to cause articular cartilage abnormalities, leading to limb dysfunction in some juvenile animals^[14]. These observations suggest that children might experience the same toxicity; therefore, quinolones are not recommended in the pediatric population. In China, systemic quinolones are not recommended for children under 18 years. Although animal and cell experiments have shown that a certain concentration of quinolones can cause chondrocyte damage, the cartilage toxicity of quinolones in animal experiments is largely related to the species of experimental animals. Besides, the degree of damage positively correlates with the dose of the drug. However, the doses of the drugs in animal experiments are 10 to 30 times higher than the conventional doses for children.

Binz^[15] reviewed and analyzed the clinical studies and concluded that there is no clear correlation between the application of fluoroquinolones and musculoskeletal adverse events. The latest five-year follow-up data showed that the incidence of musculoskeletal adverse events is low, and the events reverse after discontinuing the drug. Rosavonaet al.^[16] conducted a systematic review and meta-analysis that involved eight studies and 23166 patients. The findings demonstrated that fluoroquinolones do not cause musculoskeletal diseases in minors, and these drugs should not be banned for children suffering from specific infections. LIU et al.^[17] analyzed five randomized controlled trials (RCTs) involving 1968 patients in the levofloxacin group and 1640 patients in the control group. Out of these five studies, two studies showed that osteoarticular event rates were not statistically significant between the levofloxacin group and the control group. As per the other three studies, adverse events of bones and joints were not observed in both the groups during treatment and follow-up. These observations suggest that the incidence of levofloxacin-induced adverse events in bones and joints in children is low, and most of these adverse events can be attended during follow-up.

Pharmaceutical experts created the "Expert consensus on the application of fluoroquinolone antibacterial drugs in children"^[18] to standardize the application of these drugs in pediatrics. The "Expert consensus on diagnosis and treatment of mycoplasma pneumoniae pneumonia in children (2015)" recommends that children infected with macrolide-sensitive MP should be treated with macrolide antibiotics, and other antibiotics should be considered for MP resistant to macrolide antibiotics. The 6 cases in the present study were diagnosed with refractory MPP, and the mutations associated with drug resistance were present. After the conventional course of azithromycin treatment, the patients were still febrile. The course of treatment was extended to seven days. Three children were assisted by fiberoptic bronchoscopy. Although

methylprednisolone and immunoglobulin were also administered simultaneously, the six children still had a recurrent fever and/or progressed imaging. Among them, one child was complicated with pulmonary embolism, and five cases had massive consolidation hydrothorax of the lung. The clinicians adjusted the therapy to levofloxacin, and comprehensively evaluated the condition of the children. After treating the children with levofloxacin, the body temperature returned to normal, and the imaging also improved to varying degrees. The follow-up time of the patients after levofloxacin treatment ranged from one week to five months. There were no drug-related adverse reactions during the course of treatment or follow-up. This study is limited in terms of the small number of included cases and short follow-up time.

The pharmacokinetic analysis of azithromycin by Zheng et al. ^[19] ^[20] highlighted that the first 24–48 hours are crucial for the success of antimicrobial treatment and that the effective drug concentration is critical since it can lead to treatment failure or increased toxicity. The reduction in C-reactive protein (CRP) and neutrophil count in children with an azithromycin trough concentration of >0.25 mg/L was significantly higher than that in children with an azithromycin trough concentration of <0.25 mg/L. The azithromycin loading dose of 15 mg/kg reaches the effective target concentration within 24–48 hours. If this dose is followed by a maintenance dose of 10 mg/kg, the area under concentration-time curve over 24 h (fAUC) to the MIC_{90} (fAUC/MIC) can be maintained above 50%. In this study, the mean clearance of azithromycin in children aged two to twelve years was 1.288 liters/h/kg. This value is in accordance with a previous pharmacokinetic study of intravenous azithromycin that reported average clearance (CL) values of 1.062 liters/h/kg in 7 children aged 2 to 6 years and 0.960 liters/h/kg in 8 children aged 6 to 12 years. The related risks of overdose for the proposed dosing regimens were 5.8% and 3.8%, respectively, for pediatric patients with normal and impaired liver function. In case the 15% dose reduction is not performed in children with ALT of 40, the probability of overdose increases from 3.8–6.3%. At present, the conventional dose of azithromycin is 10 mg/kg. The hypothesis that whether some children suffer from insufficient effective drug concentration that leads to poor therapeutic effect still needs further confirmatory studies.

5. Conclusions

The clinical symptoms and imaging significantly improved after treatment with levofloxacin, Levofloxacin proved to be an effective and safe drug in the treatment of children with macrolide-resistant mycoplasma pneumonia. Levofloxacin in children is an off-label drug, and the child's condition and treatment risk should be fully evaluated before considering levofloxacin as a potential treatment option. Before the administration of levofloxacin, clinicians should communicate with the family members of patients; evaluate the risks and benefits of the drug, and choose the most beneficial option for the patient.

Abbreviations

abbreviation	Full name
MP	Mycoplasma pneumoniae
CRP	C-reaction protein
MPP	Mycoplasma pneumoniae pneumonia
DNA	Deoxyribonucleic acid
RNA	Ribonucleic Acid
SAT	Simultaneous Amplification and Testing
qRT-PCR	Quantitative Real-time Polymerase Chain Reaction
MIC	Minimum inhibitory concentration
fAUC	the area under concentration-time curve over 24 h
fAUC/MIC	the area under concentration-time curve over 24 h to the MIC ₉₀
CL	clearance

Declarations

1. Ethics approval and consent to participate

2. Consent for publication:

Not applicable

3. Availability of data and material

The data sets during and/or analyzed during the current study available from the corresponding author on reasonable request.

4. Competing interests

The authors declare that they have no competing interests

5. Funding

Not applicable

6. Authors' contributions

Fang-fang DAI: The first author and the corresponding author

design of the work; the acquisition; analysis and interpretation of data; Writing articles

Xing CHEN: guide the writing, verifying that all data, figures, materials (including reagents), and code, even those developed or provided by other authors

Feng-qin LIU; Juan YANG; Ke WANG and Chun-yan GUO: the acquisition, analysis and interpretation of data

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21. Acronyms.

Figures

Figure 1

Imaging changes in case 2 A) 7 days before levofloxacin B) 7 days after levofloxacin C) 1 month after levofloxacin