Concentrations of Azithromycin and Amoxicillin-Clavulanic acid in patients undergoing tonsillectomy

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Abstract

Background: In treating microbial infections, it is important to choose an antibiotic with appropriate spectrum of activity and one that achieves adequate concentration for a sufficient period of time at the site of infection. This concept becomes necessary when antibiotics fail to cure infection, along with increasing emergence of antimicrobial resistance.

Aim: The aim of the study is to assess the antibacterial activity of two antimicrobial agents indicated in the treatment of tonsillitis; azithromycin and amoxicillin-clavulanic acid.

Methods: A single blind comparative study was conducted on 43 patients with recurrent tonsillitis with mean age of 5.46±2.38 years who were scheduled for tonsillectomy in ENT department, Rizgary Hospital. The patients were allocated randomly into 2 groups. Group 1 patients (n=20) were given azithromycin and group 2 patients (n=23) received amoxicillin-clavulanic acid at the recommended dose for each antibiotic. Bacterial isolation and identification were performed and minimum inhibitory concentrations (MIC) of isolated bacteria were determined. Blood and tonsillar tissue samples were taken from each patient before, and 2 hours after, drug administration. The plasma and tonsillar tissue concentration of each antibiotic were determined.

Results: Staphylococcus aureus was the most predominant organism isolated from the patients. Azithromycin and amoxicillin-clavulanic acid attained mean plasma concentration of 0.27±0.04µg/ml and 5.49±0.33µg/ml respectively and the mean azithromycin concentration in tonsils tissues was 13.97± 2.75µg/g whereas no detectable concentrations of amoxicillin-clavulanic acid were determined in the tonsils tissue of the patients.

Conclusion: Azithromycin achieved higher tissue concentration than amoxicillin-clavulanic acid in tonsils tissues making this antibiotic a good choice for recurrent tonsillitis.

Key words: azithromycin, amoxicillin-clavulanic acid, tonsillitis, pharyngitis, resistance.

Introduction

There are continuous reports relating to failure of antimicrobial therapy to emergence of bacterial resistance (1, 2). This resistance problem markedly encouraged reassessment of antibacterial effectiveness of microbial infections with resistant organisms (3, 4).

Although, Streptococci group A beta-hemolytic (GABHS) is the main cause of pharyngo-tonsillitis (5), other bacteria such as S. aureus, S. pneumoniae, H. influenzae are also isolated (6,7). . Penicillin V is considered the drug of choice for the treatment of GABHS pharyngitis however it is not effective when the infection is caused by beta-lactamase-producing bacteria (8). Amoxicillin is an amino-penicillin with extended spectrum of activity combined with clavulanic acid (Amoxiclav)® to broaden its activity against resistant organisms (8). The semi-synthetic macrolide, azithromycin is effective against a wide variety of bacteria including those causing pharyngo-tonsilitis and is usually reserved for patients who are allergic to Penicillins (8, 9).

Although these antibiotics possess broad spectrum activity that cover most pathogens causing pharyngitis, they are still unsuccessful in preventing recurrences of these infections and 7%-37% of children treated with an appropriate antibiotic are considered bacteriologic failures (9, 10). This problem could be related to either infection with resistant bacteria or failure of drugs to achieve adequate antimicrobial concentrations in the site of infection (2). Therefore, this study was designed to compare the effectiveness of two commercially available antimicrobial agents indicated in the treatment of tonsillitis; azithromycin and amoxicillin/clavulanate by estimating their concentrations in plasma and tonsils tissue of children undergoing tonsillectomy and relate these levels with the minimal inhibitory concentrations (MIC) of the bacteria isolated from the patient's tonsils.

Patients and Methods

The study design was a single blind comparative study that included forty three children aged between 2-14 years of both gender with recurrent tonsillitis who were scheduled for tonsillectomy with no history of allergy to beta-lactams or macrolides antibiotics. Patients with preexisting medical condition that might affect drug pharmacokinetics or requiring perioperative antibiotics (i.e., endocarditis), or with history of antibiotic use within 2 weeks prior to tonsillectomy or with history of significant hematological, renal and hepatic disease, were excluded from the study.

The study was conducted with the approval of the Ethical Committee of the College of Medicine, Hawler Medical University and informed consent was taken from parents of each patient after explaining the study protocol in keeping with the Ethical Committee policy.

The patients were allocated randomly into 2 groups. The children were given the drug suspension by a calibrated syringe so that the volume of suspension given is measured precisely. Group 1 patients (n=23) were given amoxicillin-clavulanic acid (Julmentin®; Julphar, UAE) and azithromycin (Zomzx®; Hikma, Jordan) was given to group 2 patients (n=20). The drugs were given orally a day before and approximately 2 hours before the scheduled time of surgery at the recommended dose of 10 mg/kg for azithromycin and 156mg/5ml (24.96 mg/kg/day) for amoxicillin-clavulanic acid. Before starting medications, sterile swabs were taken from the core of the tonsil of each patient for microbiological isolation of bacteria (11) and thereafter bacteria were identified to the species level by VITEK 2 colorimetric identification card (12). The minimum inhibitory concentration (MIC) of each isolate was determined by broth dilution method according to the National Committee for Clinical Laboratory Standards (13).

Samples from venous blood were taken from each patient before drug administration and at time of operation corresponding to 2 hours after drug administration. The blood samples were collected in heparinized tubes and plasma was obtained by centrifugation of blood samples for 10 minutes. Tonsils were taken at the time of operation at the surgical theatre at times relevant to timing of the blood samples, weighed, wiped gently with dry sterile gauze. Plasma and tonsils samples were immediately stored in deep freeze (-40^{o} C) until analyzed by the microbiological assay method using standard S. aureus ATCC (6538P) sensitive to azithromycin and amoxicillin-clavulanic acid according to (14, 15) respectively.

For the determination of drugs concentrations, drug-free plasma and tonsil samples were spiked with different concentrations of each drug separately. The standard concentrations were analyzed in triplicate along with the samples by the microbiological assay method mentioned above and a standard curve was generated relating the diameter of zone of inhibition (mm) with different concentrations of either drug. Calculations of azithromycin and amoxicillin-clavulanic acid concentrations in plasma and tonsils samples were determined according to (16). The limit of detection for azithromycin and amoxicillin - clavulanic acid in plasma and tonsils were 0.01 µ g/ml and (0.05 µg/g respectively.

SPSS version 19 was used to analyze the differences between different concentrations of the drugs in plasma and tonsils samples. A $P \leq 0.05$ was considered statistically significant difference.

Results

The mean age, weight and distribution of gender of patients enrolled in the study in both treatment groups are shown in Table 1.

Table 1: Demographic characteristics of patients

Patient Characteristic	Amoxicillin-clavulanic acid group (n= 23)	Azithromycin group (n= 20)	Total
Male	15	11	26/43
Female	8	9	17/43
Mean age ± S.D. (years)	5.25 ± 2.13	5.7 ± 2.68	5.46±2.38
Mean body weight ±S.D.(kg)	20.087 ± 4.65	20.8± 6.42	20.42±5.49

The mean age of children was 5.25±2.13 years and 5.7 ± 2.68 year for amoxicillin-clavulanic acid and azithromycin group respectively (Table 1). The children had a mean weight of 20.42 ±5.49 kg and the ratio of distribution of male: female was 1.53:1 (Table 1).

Different microorganisms were isolated from the tonsils taken from the patients and S. aureus was isolated from the majority of the patients (Table 2).

Table 2: Microorganisms isolated from the tonsils of patients in different treatment groups

Microorganism isolated	No. isolated		%
	Amoxicillin-Clavulanic acid group	Azithromycin Group	
Staphylococcus aureus	7	4	27.27
Streptococcus pyogenes	1	1	4.55
Streptococcus mitis	2	3	11.6
Staphylococcus epidermidis	2	3	11.6
Streptococcus sanginus	2	1	7
Streptococcus agalactiae	1	1	4.7
Streptococcus salivarius	2	1	7
Streptococcus intermedius	2	1	7
Staphylococcus lentus	1	1	4.7
Staphylococcus haemolyticus	1	1	4.7
Pseudomonas aurogenosa	1	1	4.7
Proteous mirabilis	1	1	4.7
Granulicatella elegans	1	1	4.7

S. pyogenes isolated from patients was resistant to both amoxicillin-clavulanic acid and azithromycin antimicrobial agents whereas *S. agalactiae* was sensitive to amoxicillin-clavulanic acid but resistant to azithromycin. Four *S. aureus* isolates were sensitive, 2 were intermediately sensitive and only one was resistant to amoxicillin-Clavulanic acid while all four isolates of S. aureus were resistant to azithromycin antimicrobial agent as shown in Table (3).

Table 3: Susceptibilities of different micro-organisms isolated from tonsils of the patients to amoxicillin-clavulanic acid and azithromycin

Microorganism	Amoxicillin- Clavulanic acid No. cases / MIC (µg/ml)			Azithromycin No. cases/ MIC (µg/ml)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
S. pyogenes	-	-	1(0.5)	-	-	1 (16)
S. agalactiae	1 (0.125)	-	-	-	-	1 (4)
S. aureus	4 (2)	2 (4)	1 (8)	-	-	4 (4)

The mean concentrations of amoxicillin-clavulanic acid and azithromycin in plasma were 5.49± 0.33µg/ml and 0.27 ± 0.04µg/ml respectively. In the tonsils, the mean concentrations of azithromycin was 13.97 ± 2.75 µg/g whereas no detectable concentrations were determined for amoxicillin-clavulanic acid in tonsils according to the limit of detection of the assay (Table 4).

Table 4: The mean concentration of Amoxicillin-clavulanic group and Azithromycin in plasma and tonsils tissue

Amoxicillin-clavulanic group		Azithromycin group	
Plasma Concentration (µg/ml)	Tonsillar Concentration (µg/g)	Plasma Concentration (µg/ml)	Tonsillar Concentration (µg/g)
5.49 ± 0.33	0.0 ± 0.0	0.27 ± 0.04	13.97 ±2.75

Discussion

To obtain an effective clinical response to antimicrobial therapy, the drug should reach effective concentration at the site of infection greater than the MIC of the causative organisms (3, 4).

S. aureus was the most predominant organism (27.27%) isolated from the patients in the present study and has also been reported in other studies as the most predominant pathogen isolated from children with recurrent tonsillitis (6, 11). *S. pyogenes* was only isolated from 2 patients (4.55%) in the present study, which is also in accordance to other findings (11, 17). Another study stated that among a total of 294 children with acute tonsillitis, Group A streptococci was isolated only from three children (18).

The mean plasma amoxicillin concentration (5.49±0.33 µg/ml) estimated in the plasma of the patients, indicates that amoxicillin is well absorbed into systemic circulation. Indeed amoxicillin has high oral bioavailability (70-90%) with peak plasma levels occurring within 1 to 2 hours (19-21). This mean plasma levels (5.49 µ g/ml) is close to those determined (4.7 µ g/ml) in children (22) although higher peak concentration of 7.32 µg/ml and 10.8 µg/ml was detected 2 hours in adult volunteers given amoxicillin-clavulanic acid at a dose similar to those administered in the present study (20,21). The differences in the concentrations is most likely related to differences in the drug formulations, amount of dose given or to the analytical method of analysis. Based on recommendations of therapeutic effectiveness of beta-lactams in humans and experimental studies; the concentrations of antibiotic is required to be four to five times the MIC or higher when associated with improved outcome especially with resistant strains (23,24) thus, although levels of amoxicillin-clavulanic acid in plasma were higher than the MICs of the isolated species, this antibiotic did not attain effective concentrations in the site of infection (tonsils) to eradicate the resistant organisms (24). The *S. pyogenes* that was isolated from the patients was considered resistant according to the breakpoint therefore this antibacterial agent would not provide an effective treatment especially in recurrent cases with resistant organisms.

Furthermore, although plasma concentrations are generally a good indicator of drug effectiveness they are usually a poor indicator of intracellular concentrations, which is of major importance for intracellular pathogens including *S. aureus* as well as *S. pyogenes* that are also shown to be intracellular pathogen of tonsils (25, 26). One of the properties that characterize β-lactams including amoxicillin is that they are weak acids and quickly diffuse into cells and because the cell cytosol is more acidic than extracellular milieu they will be more in unionized form so they are prevented from accumulating in the cells and will readily be absorbed to systemic circulation (27, 28). Studies recommended administering amoxicillin-clavulanic acid at higher dose are based on their finding that one high dose of amoxicillin-clavulanic acid would inhibit the biofilm formed in the tonsillar tissue therefore exposing the bacteria to effective treatment since recurrent pharyno-tonsillitis and failure of treatment has been attributed to biofilm formation (29, 30).

Concerning azithromycin, the mean plasma concentrations of (0.27 ± 0.04 µg/ml) is close to those reported (0.24 µg/ml) in children receiving 30 mg azithromycin (31). However, lower concentrations in plasma (0.18 µ g/ml) were estimated by (32) and 0.13 µg/ml by (33). These differences are related to differences in dosing, different drug formulations and method of drug analysis. The mean azithromycin concentrations (13.96 ± 2.75 µg/g) in tonsillar tissues indicate this drug undergoes rapid uptake from systemic circulation into the infection site thus exposing the local compartment (tonsils) to azithromycin concentrations higher than plasma levels. High ratio of azithromycin concentration in tonsils to that in plasma was also reported and shown to be greater than plasma concentrations by many fold for all time intervals after administration (15, 33).

One of the remarkable features of azithromycin is its ability to accumulate in intracellular compartments, mainly in fibroblasts, phagocytic cells, and other white blood cells (34). This prominent character is explained by its dibasic molecular structure that allows azithromycin to be concentrated within the acidic lysosomes of white blood cells due to an ion-trapping mechanism from where it will be released very slowly from intracellular compartments (35). This characteristic is believed to account for the prolonged drug concentrations in tissues which are reported to persist long after the end of therapy and reflected by a long elimination half-life of up to 5 days (36).

In conclusion; although amoxicillin-clavulanic acid is clinically considered more effective against beta-lactamase producing organisms and is the most frequently prescribed antibiotic for such infection (9,37) azithromycin's good tissue penetration, once daily administration, besides its immunomodulatory effects provides further benefits along with its dual antibacterial mode of action (38, 39) and makes this antibiotic a good choice when the standard penicillin V therapy of tonsilo-pharyngitis fails. Furthermore, the consequences of the low amoxicillin-clavulanic acid levels in tonsils might lead to potentially negative effects on clinical response and emergence of resistances (40).

References

1- Huttner A, Harbarth S, Carlet J, Cosgrove S, Goossens H, Holmes A, et al. Antimicrobial resistance: a global view from the 2013 World Healthcare-Associated Infections Forum. Antimicrob Resist Infect Control. 2013; 2:31- 41.

2- Llor C, Bjerrum L. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther Adv Drug Saf. 2014; 5(6): 229- 41.

3- Thabit AK, Crandon JL, Nicolau DP. Antimicrobial resistance: impact on clinical and economic outcomes and the need for new antimicrobials. Expert Opin Pharmacother. 2015; 16(2):159-77.

4- Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015; 40(4):277-83.

5- Michael R. Wessels, M.D. Streptococcal Pharyngitis. N Engl J Med, 2011; 364:648-55.

6- Zautner AE, Krause M, Stropahl G, Holtfreter S, Frickmann H, Maletzki C, et al. Intracellular Persisting Staphylococcus aureus is the Major Pathogen in Recurrent Tonsillitis. PLos One. 2010; 5(3): e9452.

7- Alasil S, Omar R, Ismail S, Yusof MY, Ameen M. Bacterial identification and antibiotic susceptibility patterns of Staphyloccocus aureus isolates from patients undergoing tonsillectomy in Malaysian University Hospital. Afr J Microb Res. 2011; 5(27): 4748-52.

8- Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, et al. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin Infect Dis. 2012; 55(10): 1279-82.

9- Regoli M, Chiappini E, Bonsignori F, Galli L, de Martino M. Update on the management of acute pharyngitis in children. Ital J Pediatr. 2011; 37:10-17.

10- Aalbers J, O'Brien KK, Chan WS, Falk GA, Teljeur C, Dimitrov BD, Fahey T. Predicting streptococcal pharyngitis in adults in primary care: a systematic review of the diagnostic accuracy of symptoms and signs and validation of the Centor score. BMC Med. 2011; 9:67-78.

11- Babaiwa UF, Onyeagwara NC Akerele JO. Bacterial tonsillar microbiota and antibiogram in recurrent tonsillitis. Biomedical Research. 2013; 24 (3): 298-302.

12- Wallet F, Loiez C, Renaux E, Lemaitre N, Courcol RJ. Performances of VITEK 2 colorimetric cards for identification of gram-positive and gram-negative bacteria. J Clin Microbiol. 2005; 43(9):4402-06.

13- Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. Wayne, PA. USA. 2012; 32 (3): M100-S22.

14- Davies BE, Boon R, Horton R, Reubi,FC, C E Descoeudres CE. Pharmacokinetics of amoxycillin and clavulanic acid in haemodialysis patients following intravenous administration of Augmentin. Br. J. clin. Pharmac. 1988; 26: 385-90.

15- Blandizzi C, Malizia T, Batoni, G, Ghelardi E, Baschiera F, Paolo Bruschini P, et al. Distribution of Azithromycin in Plasma and Tonsil Tissue after Repeated Oral Administration of 10 or 20 Milligrams per Kilogram in Pediatric Patients. Antimicrob Agents Chemother. 2002; 46(5): 1594-96.

16- Jusko, WJ. Guidelines for collection and analysis of pharmacokinetic data. In: Shargel L, Wu-Pong S, Yu A, editors. Applied Biopharmacetics and Pharmacokinetics, New York: McGraw-Hill; 2005: 8-27.

17- Devi U, Borah PK, Mahanta J. The prevalence and antimicrobial susceptibility patterns of beta-hemolytic streptococci colonizing the throats of schoolchildren in Assam, India. J Infect Dev Ctries 2011; 5(11):804-08.

18- Hsieh TH, Chen PY, Huang FL, Wang JD, Wang LC, Lin HK, et al. Are empiric antibiotics for acute exudative tonsillitis needed in children? J Microbiol Immunol Infect. 2011; 44: 328-32.

19- Navarro SA. New formulations of amoxicillin/clavulanic acid: a pharmacokinetic and pharmacodynamic review. Clin Pharmacokinet. 2005; 44(11):1097-115.

20- Mostafavi SA, Dormiani K, Khazaie Y. Pharmacokinetics of Amoxicillin/clavulanic acid after oral administration

of new suspensions formulation in human volunteers. International J Pharmacology. 2007; 3(3): 265-69.
21- Kaur, RAO R, Nanda S. Amoxicillin: A broad spectrum antibiotic. Int J Pharm Pharm Sci. 2011; 3 (3): 30-37.
22- Averono G, Vidali M, Olina M, Basile M, Bagnati M, Bellomo G, Aluffi P. Evaluation of amoxicillin plasma and tissue levels in pediatric patients undergoing tonsillectomy. Int J Pediatr Otorhinolaryngol. 2010; 74(9):995-98.
23- Andes D, Craig WA. In vivo activities of amoxicillin and amoxicillin-clavulanate against Streptococcus pneumoniae: application to breakpoint determinations. Antimicrob Agents Chemother. 1998; 42: 2375-79.
24- Haeseker M, Havenith T, Stolk L, Neef C, Bruggeman C, Verbon A. Is the standard dose of amoxicillin-clavulanic acid sufficient? BMC Pharmacol Toxicol. 2014; 15: 38-46.
25- Fraunholz M, Sinha B. Intracellular Staphylococcus aureus: live-in and let die. Front Cell Infect Microbiol. 2012; 2:43- 50.
26- Fischetti VA, Dale JB. One More Disguise in the Stealth Behavior of Streptococcus pyogenes. mBio. 2016; 7(3): e00661-16.
27- Jensen A, Fago-Olsen, H, Sørensen CH, Kilian M. Molecular Mapping to Species Level of the Tonsillar Crypt Microbiota Associated with Health and Recurrent Tonsillitis. Plos One. 2013; 8(2): e56418.
28- Yamanaka, N. Moving towards a New Era in the Research of Tonsils and Mucosal Barriers. Adv Otorhinolaryngol. 2011; 72: 6-19.
29- Roberts AL, Connolly KL, Kirse DJ, Evans AK, Poehling KA, Peters TR, et al. Detection of group A Streptococcus in tonsils from pediatric patients reveals high rate of asymptomatic streptococcal carriage. BMC Pediatrics. 2012; 12: 3-11.
30- Alasil SM, Omar R, Ismail S, Yusof MY, Dhabaan GN, Abdulla MA. Evidence of Bacterial Biofilms among Infected and Hypertrophied Tonsils in Correlation with the Microbiology, Histopathology, and Clinical Symptoms of Tonsillar Diseases. Int J Otolaryngol. 2013; 2013:408238.
31- Liu P, Fang, AF, LaBadie RR, Crownover PH, Arguedas AG. Comparison of Azithromycin Pharmacokinetics following Single Oral Doses of Extended-Release and Immediate-Release Formulations in Children with Acute Otitis Media. Antimicrobial agents and chemotherapy. 2011; 55(11): 5022-26.
32- Danesi R, Lupetti A, Barbara C, Ghelardi E, Chella A, Malizia T, et al. Comparative distribution of azithromycin in lung tissue of patients given oral daily doses of 500 and 1000 mg. J Antimicrob Chemother. 2003; 51(4): 939-45.
33- Baschiera F, Fornai M, Lazzeri, G, Blandizzi C, Bruschinin P, Tacca MD. Improved tonsillar disposition of azithromycin following a 3-day oral treatment with 20 mg kg-1 in paediatric patients. Pharmacol Res. 2002; 46(1): 95-100.
34- Amsden GW. Advanced-generation macrolides: tissue-directed antibiotics. Int J Antimicrob Agents. 2001; 18 (S1): S11-15.
35- Hand WL, Hand DL. Characteristics and mechanisms of azithromycin accumulation and efflux in human polymorphonuclear leukocytes. Int J Antimicrob Agents. 2001; 18(5):419-25.
36- Bosnar M, Kelneric Z, Munic V, Erakovic V, Parnham MJ. Cellular uptake and efflux of azithromycin, erythromycin, clarithromycin, telithromycin, and cethromycin. Antimicrob Agents Chemother. 2005; 49(6): 2372-77.
37- Mollahaliloglu S, Alkan A, Donertas B, Ozgulcu S, Akici A. Assessment of antibiotic prescribing at different hospitals and primary health care facilities. Saudi Pharm J. 2013; 21 (3): 281-91.
38- Jelic D, Antolovic R. From Erythromycin to Azithromycin and New Potential Ribosome-Binding Antimicrobials. Antibiotics. 2016; 5(3): E29.
39- Kanoh S. & Rubin BK. Mechanisms of Action and Clinical Application of Macrolides as Immunomodulatory Medications. Clin. Microbiol. Rev. 2010; 23, 590-615.
40- Taccone FS, Laterre PF, Dugernier T, Spapen H, Delattre I. Insufficient beta-lactam concentrations in the early phase of severe sepsis and septic shock. Crit Care. 2010; 14:R126.