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ORIGINAL ARTICLE

Study of Tissue and the Plasma Concentrations of Cefotaxime to Assess Its Suitability for Prophylaxis in Cholecystectomy

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ABSTRACT

Background: Cholecystectomy is one of the frequent causes for abdominal surgery. It is generally thought that the antibiotic concentration used should be four to six times than the minimum inhibitory concentration (MIC) to be effective, when it is used as prophylaxis. Cefotaxime is a commonly prescribed agent for surgical prophylaxis.

Aim: The aim of this study was to measure the gall bladder tissue and the plasma concentrations of cefotaxime in cholecystectomy and to assess its suitability as a prophylactic agent.

Methodology: 24 patients who were undergoing Cholecystectomy were enrolled to collect plasma and gall bladder tissue samples. Cefotaxime levels in the Gall bladder tissue and plasma samples were estimated. These concentrations were compared against the Minimum Inhibitory Concentrations (MICs) of commonly isolated organisms from surgical wound samples.

Results and Discussion: Plasma concentrations to MIC ratios were calculated which were in the range of 10 to 21 times higher than MIC. Tissue drug concentrations to MICs ratios were also calculated and were found to be smaller. This indicated that the concentration of the drug in the tissues was less than the MIC during the sampling time. This situation of lesser tissue concentration of the drug might result in drug failure and subsequent infection if contamination occurs during surgery.

Conclusion: This study showed that even though cefotaxime remained at a microbicidal concentration in plasma, it was present in the tissues at ineffective concentrations with respect to MIC. These results showed that the practice of using cefotaxime as a prophylactic agent in cholecystectomy may be reconsidered.

Key Words: *Cefotaxime, Tissue concentration, Plasma concentration*

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and efficacy profile of the anti microbial agent, current literature evidence to support its use, cost and also institutional antimicrobial resistance patterns[2] . The occurrence of infection in postoperative wounds continues to be one of the serious complications from time immemorial. These infections occur in around 500,000 cases out of an estimated 27 million surgical procedures in the United States. Surgical site infections result in an extended period of hospitalization and increase health care costs significantly [3].

Surgical site infections are the second most frequent cause of nosocomial infections among hospitalized patients and the primary cause of nosocomial infection in surgical patients. The incidence of surgical site infections varies significantly between the western countries and the eastern countries 2%- 4% and 9-14% respectively [4], [5], [6]. Cephalosporins are one

of the most commonly prescribed agents for surgical prophylaxis because of their favourable pharmacokinetic profiles, low incidence of adverse effects and low costs [2]. Among various type of surgeries, cholecystectomy is one of the frequent causes for abdominal surgeries, where cefotaxime; a third generation cephalosporin is widely used as prophylaxis. Some studies from the western countries showed that surgical site infection (SSI) rates with cholecystectomy are 12%-15% without prophylaxis and 3%-6% with prophylaxis. In India, it is 12% without prophylaxis and 4.5% with prophylaxis [7], [8],[9].

It is generally thought that the antibiotic concentration should be four to six times as that of the minimum inhibitory concentration (MIC) to be effective, when it is used as prophylaxis¹⁰. Additionally, the concentration of the antibiotic in the serum and the tissue is very important in case of surgery, it is the strongest predictor of postoperative surgical site infections and also, it is clear that the clinical effectiveness of an antibiotic depends on its penetration into the tissues [1]. Cefotaxime follows time dependent killing, which means that its efficacy is closely related to the time during which its concentration remains above the minimum inhibitory concentration [10]. This study was planned to measure the plasma and gall bladder tissue concentrations of cefotaxime in case of cholecystectomy surgery to study its suitability as a surgical prophylactic agent.

Methodology

Patients and Samples

This study was conducted at the Department of General Surgery of Kasturba hospital, Manipal which is a tertiary care teaching hospital in South India. This was a prospective study carried out over eight months from September 2006 to April 2007. Ethical approval was obtained from the institutional ethics committee. Patients who were posted for cholecystectomy and who were receiving cefotaxime injection as IV prophylaxis were included in the study. Other inclusion criteria were ages above 18 years old and willingness to participate in the study. Patients who were posted for Cholecystectomy but are receiving any other antibiotics along with cefotaxime were excluded from the study. Patients with renal and hepatic complications were also excluded from the study.

Sample collection

During surgery, an investigator was present along with the surgical team. The investigator documented the time of prophylactic administration of cefotaxime and the surgical removal of the gall bladder tissue. The investigator also collected blood samples at the same time, along with the tissue samples, with the help of surgeons. The blood samples were collected in containers which were coated with EDTA, so as to separate plasma. The time for all sample collections was recorded. The collected tissue samples were immediately washed thoroughly with saline to remove blood and water was removed by wiping with dry tissue paper. These were packed in a container with ice packs and were then transported immediately to the lab for storage. The collected blood samples were processed immediately to separate plasma and were stored along with the tissue samples in a deep freezer (- 70 °C) till analysis. The tissue samples were homogenized and centrifuged to extract tissue fluids before analysis.

Analysis of samples

An analytical method for the determination of cefotaxime in human plasma and gall bladder tissues was developed and validated using the High Performance Liquid chromatography (HPLC) technique. The protein precipitation method using acetonitrile was used to extract the drug from the plasma and the tissue matrix. Cefuroxime was used as an internal standard. A mixture of 10 mM Ammonium acetate buffer, pH 5.0 and Acetonitrile (88:12, v/v) was used as a mobile phase and was filtered before use through a 0.45 µm membrane filter. The flow rate of the mobile phase was maintained at 1.0 ml/min. The detection wavelength was 236 nm, with a runtime of 15 minutes and the injection volume was 100 µL. The developed and the validated method was linear over the range of 1- 87µg/mL. The lowest concentration which could be quantified by this method was 1µg/mL. The developed method was specific, selective, accurate, precise and stable as per standard validation criteria [11].

Microbial Assays

The data on commonly prevailing organisms in surgical infections were collected. This data was collected from the culture reports of samples (wound swabs) which were sent from the department of surgery to the department of

microbiology for a period of three months. The organisms which were cultured from the swab samples were archived in the microbiology department. At the end of the three months, all the cultured organisms were collected and revived and the Minimum Inhibitory Concentrations (MIC) for cefotaxime was determined using the agar plate method. The MIC was measured as the lowest concentration of the cefotaxime that completely inhibited visible growth as judged by the naked eye, disregarding a single colony or a thin haze within the area of the inoculated spot. Finally, the MICs of the various organisms were estimated. Ratios like plasma and tissue concentrations to MIC were calculated to find out the anti-microbial efficacy of cefotaxime against the prevalent organisms of surgical infections.

Results

Patient data

During the study period, 253 patients underwent Cholecystectomy, out of which 24 patients were enrolled in the study. Gall bladder tissue and blood samples were collected from the recruited patients. All 24 patients underwent the laparoscopic Cholecystectomy procedure. 14 study subjects were males and 10 of them were females. The demographic data and clinical conditions of the patients are presented in [Table/Fig 1].

(Table/Fig 1) Demography of patients

| S.No | Demography of patients | No of patients (24) |
|------|---------------------------------|---------------------|
| 1 | <i>Gender</i> | |
| | Male | 14 |
| | Female | 10 |
| 2 | <i>Age group</i> | |
| | <40 | 10 |
| | >40 | 14 |
| 3 | <i>Diagnosis</i> | |
| | Cholelithiasis | 10 |
| | Acute Cholelithiasis | 8 |
| | Chronic calculus-Cholelithiasis | 6 |

Time of Tissue and Blood Collection

The usual length of the Cholecystectomy surgery ranged from 1-3 hours. Since the antimicrobial action is time dependent for cefotaxime, time

was considered as an important factor which affected the concentration of the drug in the blood as well as in the gall bladder tissues. Depending upon the length of surgery, the time of blood and tissue sampling varied from less than half an hour to more than three hours. The mean time of blood collection was 105.67 ± 54 minutes and the mean time of tissue removal was 97.6 ± 53.8 minutes. Various intervals of sample collection are represented in [Table/Fig 2].

(Table/Fig 2) Number of patients and various intervals of sample collection

| S.No | Time intervals | No of patients (24) |
|------|---------------------------------------|---------------------|
| 1 | <i>Blood sample collection</i> | |
| | <30 minutes | 1 |
| | 30 min-1 hour | 5 |
| | 1-2 hours | 11 |
| | 2-3 hours | 3 |
| | >3 hours | 4 |
| 2 | <i>Gall bladder sample collection</i> | |
| | <30 minutes | 1 |
| | 30 min-1 hour | 6 |
| | 1-2 hours | 11 |
| | 2-3 hours | 2 |
| | >3 hours | 4 |

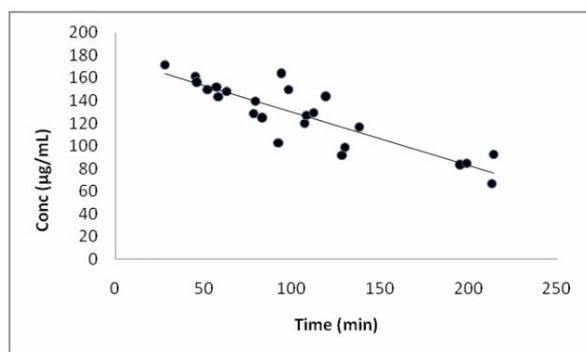
Plasma and Tissue Concentrations of Cefotaxime

The mean plasma cefotaxime concentration which was observed was $126.9 \pm 29.2 \mu\text{g/mL}$, with a range of 92.5 to $171.5 \mu\text{g/mL}$. The mean tissue concentration observed was $3.5 \pm 1.4 \mu\text{g/mL}$, with a range of 1.32 to $7.23 \mu\text{g/mL}$.

Correlation of Time with Plasma and Tissue Drug Concentration

The correlation between time and plasma concentrations was studied and it was found that there was a negative linear correlation with a Pearson correlation coefficient of $r^2 = -0.8322$ (95% CI=-0.9269 to -0.6380)], which is shown in [Table/Fig 3]. This showed that the rate of decline in plasma concentrations corresponded well with the passage of time. No such correlation was found between the time of tissue removal and the tissue concentration of the drug.

(Table/Fig 3) Correlation between time and plasma drug concentrations



Microbial Assays

The wound samples collected from the surgery department were cultured and the organisms were isolated. Totally, 9 common organisms were isolated from the swab samples and their MICs were determined. The organisms were considered to be susceptible to cefotaxime if the MIC was 16µg/mL or less, as moderately susceptible if the MIC was greater than 16µg/mL but less than 64µg/mL. The organisms are considered to be resistant if the MIC was 64µg/mL or more [12]. The sensitivity and MIC of the isolated organisms are shown in [Table/Fig 4].

(Table/Fig 4) Sensitivity and MIC of cefotaxime to isolated organisms

| S.No | Organisms Isolated From Surgery Department Samples | Sensitivity Pattern | MIC (µg/mL) |
|------|--|---------------------|-------------|
| 1 | <i>Pseudomonas aeruginosa</i> | R | 64 |
| 2 | <i>Escherichia. Coli</i> | S | 8 |
| 3 | <i>Aceneto bacter species</i> | R | 64 |
| 4 | <i>Pseudomonas aeruginosa</i> | MS | 32 |
| 5 | <i>staphylococcus aureus</i> | S | 8 |
| 6 | <i>Coagulase negative staphylococcus</i> | S | 8 |
| 7 | <i>Klebsiella pneumonia</i> | S | 8 |
| 8 | <i>Enterobacter species</i> | S | 8 |
| 9 | <i>Enterococci</i> | S | 8 |

R – Resistant; MS – Moderately susceptible;
S- Sensitive

Plasma and Tissue Concentrations to MIC Ratios

The plasma and tissue concentration to MIC ratio was calculated since this ratio is considered as one of the sensitive indices to find out the

effectiveness of an antibiotic. The mean plasma concentration to MIC ratio was 15.85. The tissue concentration to MIC ratio was very small when compared to the plasma concentration to MIC ratio. The results are shown in [Table/Fig 5].

(Table/Fig 5) Mean drug concentrations to MIC ratios in plasma and tissue

| S.No. | Medium | Mean Drug concentration (µg/mL) | Ratio (Mean drug concentration / MIC of sensitive organisms |
|-------|--------|---------------------------------|--|
| 1 | Plasma | 126.91 | 15.85 |
| 2 | Tissue | 3.52 | 0.43 |

Discussion

This study was conducted with an aim to assess the suitability of cefotaxime as a prophylactic agent in Cholecystectomy surgery, considering its pharmacokinetic property, tissue penetration and plasma concentration. For collecting gallbladder tissues and blood, the time of administration of the prophylactic dose was considered as the zero point to calculate other timings. The mean time of tissue removal was found to be 97.6 minutes, whereas the mean time of blood collection was found to be 105.6 minutes. During the study, it was observed that the duration of surgery ranged from one to three hours. It is generally advisable to use an antibiotic which has a longer half life than the length of the time of surgery [9]. In the present study, cefotaxime which has a half life of 80-100 minutes was administered as a prophylactic agent. Cholecystectomy took variable time periods, in many instances more than the half life of cefotaxime. The mean time of blood and tissue collection in this study was more than the half life of the drug itself and as a result, at the time of sample collection itself, half of the drug might have been eliminated from the system.

The time of tissue and blood collection was kept as close as possible so as to understand the drug concentrations at both the sites at a particular time. The negative correlation between time and plasma concentrations showed that the drug concentrations in plasma reduced linearly with the passage of time. Hence, time had to be taken as an important parameter in order to decide the regimen of the prophylaxis. There was no

correlation between tissue concentrations and time, since many of the tissue values were too low and highly variable. Since the tissue concentrations were at the lower end limit of the detection of our assay method, small variations in concentrations might have been missed and thereby, finding the correlation at this range was difficult.

The plasma concentration gives an idea about the quantum of the drug available in the circulation and also, it is the most dynamic parameter which changes with various other parameters like the distribution of the drug, plasma protein binding, clearance rate, etc. Cefotaxime has a protein binding of around 40%. This makes a reasonable quantity of the drug to be retained by plasma and the plasma concentration will be maintained as compared to other sites. For antibiotics with higher protein binding, it was proposed to give higher doses so as to reach tissues at higher concentrations [4]. Tissue concentrations were found to be lower in case of gall bladder tissues in the present study and this aspect had to be considered while choosing cefotaxime as a prophylactic antibiotic in gall bladder surgery, since the penetration of the drug was found to be inadequate.

The ratio of tissue to plasma drug concentration was too small in many patients. The traditional way of using the volume of distribution assumed homogenous distribution between the organs and blood.[1] But many studies including the present study showed uneven distribution of the drug between blood and the studied tissue. In the present study, when the correlation between plasma and the tissues was assessed, it was found that there was no significant correlation [Spearman $r = 0.3997$ (95% CI= -0.03979 to 0.7096)].

A total of nine organisms were isolated by the microbiology study. The organisms which were covered by cefotaxime only, were taken into consideration. Out of nine organisms, six were found to be sensitive to cefotaxime, with minimum inhibitory concentrations of $8\mu\text{g/ml}$. One organism was moderately susceptible and two organisms were resistant.

Regarding pharmacokinetic parameters, it has been demonstrated that the breakpoint for the increased probability of successful antimicrobial efficacy is a peak/MIC ratio which is >4 in case

of beta lactams and vancomycin and >10 in case of fluroquinolones [13], [14]. It was difficult to estimate the peak concentration in patients and so, it was decided to take the available plasma concentrations at the time of incision, to compare with MICs. The plasma concentrations to MIC ratios were calculated, which were in the range of 10 to 21 times higher than MIC. Tissue drug concentrations to MICs ratios were also calculated and were found to be smaller. This indicates that the concentration of the drug in the tissues was less than the MIC during sampling time. This situation of lesser tissue concentration of the drug might result in drug failure and subsequent infection if contamination occurs during surgery.

Previously reported studies have suggested that when cefotaxime was administered up to one hour before surgery, there was a reduction in the rate of infections by threefold. So, a prophylactic dose of cefotaxime was administered one hour before a surgical procedure [15] ,[16]. If the length of surgery exceeded two hours, it was also advised to give a second dose of cefotaxime, since it has shorter half life. Such a practice was not observed in the current study. Irrespective of the length of surgery, a second dose was given after the patient was shifted to the post surgical wards. Moreover, cephalosporins are classified as agents which have time dependent killing and therefore, the goal of therapy should be to maintain the levels of drug concentrations above MIC, as far as possible during the peri-operative period and in case of subsequent dose during surgery [17]. In this context, the prophylactic use of cefotaxime for cholecystectomy may be reconsidered, owing to less tissue penetration and shorter half life.

Conclusion

Cholecystectomy surgery, in many instances, was longer than the half life of cefotaxime and this fact needs to be considered while choosing a prophylactic agent for this surgery. This study showed that cefotaxime, even though it remained at a microbicidal concentration in plasma, was present in tissues at ineffective concentrations with respect to MIC. These results showed that the practice of using cefotaxime as a prophylactic agent in cholecystectomy may be reconsidered.

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