Journal of Chromatography B, 877 (2009) 3960–3964

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Penetration of cefuroxime in subcutaneous tissue during coronary artery bypass grafting surgery

Jorge Willian Leandro Nascimento[a,b,*], Maria José Carvalho Carmona[c], Tânia Mara Varejão Strabelli[c], José Otávio Costa Auler Jr[c], Sílvia Regina Cavani Jorge Santos[a]

[a] Pharmacy Department, School of Pharmaceutical Sciences, University of Sao Paulo, Av. Prof. Lineu Prestes, 580, 05508-000 Sao Paulo, SP, Brazil
[b] Post Graduation Program in Rehabilitation Sciences, Uninove, Av. Francisco Matarazzo, 612, 05001-100 São Paulo, SP, Brazil
[c] Heart Institute – HCFMUSP, Av. Dr. Enéas de Carvalho Aguiar, 44, 05403-900 São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 22 May 2009
Accepted 5 October 2009
Available online 9 October 2009

Keywords:
Cefuroxime
Subcutaneous tissue
HPLC
Tissue plasma ratio
CABG
CPB

ABSTRACT

A sensitive and rapid HPLC assay for determining cefuroxime penetration in the subcutaneous tissue near to surgical incision of patients submitted to coronary artery bypass grafting (CABG) with or without cardiopulmonary bypass (CPB) was performed. Blood and subcutaneous tissue samples were collected from 14 patients, in four periods during surgery. The analytical method presented linearity from 0.5 to 100 μg/g, LOQ = 0.50 μg/g, LOD = 0.25 μg/g, intra- and interday precision (%CV) ranged from 4.9 to 8.9% and 6.4 to 9.9%, respectively, and intra- and interday accuracy expressed as % of the nominal concentration ranged from 87.1 to 104.6% and 94.8 to 103.8%, respectively (mean of three concentrations). Relative recovery was 98.4%. Tissue/plasma ratios obtained for CPB and non-CPB were, respectively: 14.6% *vs* 19.0% (0.6 h); 15.7% *vs* 15.7% (2.1 h); 22.5% *vs* 19.9% (3.6 h); 15.7% *vs* 18.8% (4.5 h). Data obtained indicate that tissue/plasma ratio remains unchanged in CPB and non-CPB patients during all period of surgery and the CPB does not affect the penetration of cefuroxime in tissues close to the surgical wound.

1. Introduction

Postoperative infection has been demonstrated to be one of the factors responsible for the morbidity and mortality of patients undergoing cardiac surgery [1–3]. It is estimated that 5–10% of patients submitted to cardiac surgery develop nosocomial infection, especially in the region of the surgical wound [4–6].

Complex and invasive procedures are performed during cardiac surgery that exposes the patient to opportunistic and potentially infectious bacteria. In addition, cardiopulmonary bypass (CPB) is commonly installed during surgery, a fact that represents an additional risk factor for the patient by promoting marked physiological alterations that reflect on the kinetic disposition of drugs, including water-soluble antibiotics such as cefuroxime recommended for the prophylaxis of postoperative infections following cardiac surgery [7–9].

Cefuroxime, like other beta-lactam antibiotics, exhibits concentration-independent killing and the minimal inhibitory concentration (MIC) for cefuroxime ranges from 1 to 4 μg/mL, but the maximal killing effect is exhibited at concentrations approximately four times the MIC (4–16 μg/mL) [4].

To prevent infections after cardiac surgery, it is important to ensure the presence of adequate plasma concentrations of the antibiotic, thus permitting access of the drug to the site of highest bacterial exposure [10].

In this respect, the development of a HPLC method for the quantification of cefuroxime in plasma and tissues in the region surrounding the surgical wound may be a fundamental tool to optimize antibiotic prophylaxis, since so far no correlation has been established between the cefuroxime dose administered and the amount of drug fixed in proximal subcutaneous tissue. In addition, we evaluated the influence of CPB on the distribution of this antibiotic in subcutaneous tissue of patients submitted to this procedure.

2. Experimental

2.1. Patients

Fourteen adult (38–69 years) patients of both sexes with coronary disease, hepatic, renal and endocrine function within normal limits, who had a surgical indication for coronary artery bypass grafting, were studied. Eight of these patients were submitted to cardiac surgery with CPB (CPB group) and six without CPB (non-

* Corresponding author at: Pharmacology & Therapeutics Laboratory, Pharmacy Department, School of Pharmaceutical Sciences, Universidade de Sao Paulo-Brasil, Av. Prof. Lineu Prestes, 580, 05508-000 Sao Paulo, Brazil.
Tel.: +55 11 3091 2189; fax: +55 11 3091 2189.
E-mail addresses: jorgewillian@uninove.br, willian@usp.br, pharther@usp.br (J.W.L. Nascimento).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2009.10.005

CPB group). The patients signed a free informed consent form before the clinical trial. The protocol was approved by the Research Ethics Committees of the Institutions involved in the study.

Cefuroxime was evaluated in subcutaneous tissue using an open, prospective and rigorously controlled study protocol. The Hospital Infection Control Committee (HICC, Heart Institute/HC-FMUSP, Sao Paulo) recommends the intravascular administration of three doses of cefuroxime (1.5 g IV, bolus) each 12 h, in the transoperative period for antibiotic prophylaxis in patients submitted to myocardium revascularization without CPB (non-CPB group). The first dose was given at the anesthetic induction, while the second and the third doses were administered in the postoperative period; consequently 4.5 g were administered in a period of 24 h.

For patients undergoing CPB (CPB group), HICC recommends the administration of four doses of cefuroxime (1.5 g IV, bolus). The additional dose was given during surgery, immediately after the end of CPB, intending to prevent looses that could be resulted from the hemodilution and to ensure the plasma concentration of the antibiotic. In these patients, the third and fourth doses were administered 12 and 24 h after the additional dose.

2.2. Collection of blood and subcutaneous tissue samples

Subcutaneous tissue concentrations of cefuroxime were determined after collection of fragments (about 0.5 g/each) from the site of surgical incision in the chest area during surgery. These fragments were collected at determined periods according to the step of surgical procedure performed for both groups of patients and detailed as follows:

CPB group:

1st fragment (0.6 h): beginning of surgery, incision of the chest;
2nd fragment (2.1 h): beginning of CPB;
3rd fragment (3.6 h): 1 h after the beginning of CPB;
4th fragment (4.5 h): immediately before the suture.

In the non-CPB group, fragments were collected at times corresponding to those described above for the CPB group.

Non-CPB group:

1st fragment (0.6 h): beginning of surgery, incision of the chest;
2nd and 3rd fragments (2.1 and 3.6 h): during surgery;
4th fragment (4.5 h): immediately before the suture.

The collected tissue samples were immediately placed on sterile gauze for the removal of residual blood and then stored in a freezer (−80 °C) until the time of analysis.

Blood samples were collected from the venous central catheter in four periods during the cardiac surgery; simultaneously with the collection of subcutaneous tissue to determine plasma cefuroxime concentrations and to establish a correlation with the tissue concentrations obtained. The blood samples were centrifuged at 3000 rpm for 20 min for the separation of plasma, which was frozen and stored as detailed above.

2.3. Laboratory procedures

Validation of bioanalytical method for drug measurements in plasma was carried out and the analyte (cefuroxime) and its internal standard (vancomycin) were determined by a simple HPLC-UV technique according to FDA/ANVISA recommendations, and on the basis on methods reported previously by the same authors [8]. To determine cefuroxime in the subcutaneous tissue fragments, the bioanalytical method was adapted and re-validated as detailed below.

Cefuroxime calibration standards were prepared in the laboratory as described previously by Casal et al. [20], using subcutaneous tissue fragments obtained from dogs provided by the Animal House of Medical School (FMUSP) and not treated with the drug. The animals were previously anesthetized at the Laboratory of Experimental Anesthesia, Department of Anesthesiology, LIM – FMUSP, and subcutaneous tissue samples were collected from the chest region. Animals were used due to operational difficulty and ethical restrictions for obtaining blanks of human biological materials. Fragments weighing approximately 0.5 g were collected and stored in a freezer, following the same procedures as those used for the collection of tissue samples from patients during surgery.

2.4. Purification of biological materials

The calibration curve was obtained by adding cefuroxime standards to the subcutaneous tissue.

A fragment of subcutaneous tissue (0.5 g/each) obtained during the surgery by collection of the tissue piece was weighed (0.1 g) in an aluminum foil using a Shimadzu AX 120 analytical balance, then it was transferred to a 1.5-mL Eppendorf tube and the sample was finely sliced by a surgical knife, smashed for 2 min by adding 100 μL purified water plus 100 μL internal standard (vancomycin: 10 μg/assay). Precipitation of protein was performed by adding 200 μL of acetonitrile to the Eppendorf tube and the mixture was homogenized in a vortex mixer for 2 min. Tube was centrifuged for 30 min at 4 °C at 6000 rpm and 100 μL of the liquid phase was transferred to an Eppendorf tube for the concentration of purified extract to dryness in the water bath 37 °C under a nitrogen stream. The residue was dissolved with 200 μL volume of a mixture of acetonitrile and water purified and injected into liquid chromatography.

2.5. Chromatographic conditions

A Shimadzu liquid chromatograph was used which consisted of an LC-10 AVP pump, an SIL 10-AD automatic sample injector and an SCL 10 A-VP controller equipped with CLASS VP software. The peaks were monitored with an SPD-10A UV detector operating at 280 nm and the chromatograms obtained were printed with an HP 600 printer.

For quantitative analysis of cefuroxime, a 4-μm C18 Nova-Pak® column (150 mm × 3.9 mm; Waters Assoc., Milford, USA) and a binary mobile phase (0.375 M acetate buffer, pH 5.0: acetonitrile, 95:5, v/v) at a flow rate of 0.8 mL/min were used as described by Nascimento et al. [8] for the quantification of this drug in plasma.

2.6. Calibration curve and calculations procedures

Cefuroxime standard adding solution (0.1 mg/mL) was prepared from the convenient dilution of stock solution and the calibration curve was prepared to obtain the following concentrations 0.5, 2, 10, 20, 40, 80, 100 μg cefuroxime/g subcutaneous tissue fragments obtained previously from anesthetized dogs. Additionally, for the acceptation of the calibration daily curve, three internal standards were prepared to obtain 1.5, 50 and 90 μg cefuroxime/g tissue for low control, medium control and high control, respectively.

A linear regression line was obtained by plotting peak area ratio against drug concentration and the linear equation and linear correlation coefficient >0.99 for the acceptation of the curve (equation: $y = b + ax$, where x is the peak-height ratio, a the slope and b is the intercept) to quantify cefuroxime in the unknown samples and also for the acceptation of the daily curve based on three different internal quality controls (high, medium and low) were used. The acceptance limit was set at up to 15% of variation of the nominal value, with an analytical run above this limit being rejected.

2.7. Assay validation

Analytical method was validated on the basis of the confidence limits recommended by the regulatory agency. Linearity was obtained by using six concentrations (0.5–100 μg/g of tissue) in three replicates; precision and accuracy were determined by running three different concentrations (low, medium, high) in triplicates analyzed at the same day and for three consecutives days to obtain the intra- and interday variation. Accuracy and precision was accepted up to 20% of CV for the low, and 15% for the medium and high concentration.

Absolute recovery of cefuroxime extracted from the tissue fragment was estimated on the basis on the obtained peak area integrated from analysis of tissue purified extract *vs* the peak area obtained after direct injection of the cefuroxime standard solution, that was freshly prepared at the same concentration; relative recovery was measured by the peak area ratio of cefuroxime and its internal standard (vancomycin) and data were expressed as percentage for recovery studies.

Stability study was performed using different concentrations (0.5, 10 and 100 μg/g tissue) analyzed in triplicate, including short stability of samples on the bench (4 h), on the rack of the auto sampler at room temperature (time and conditions of analysis), samples after three thawing cycles and after storage in a deep freezer up to three months.

2.8. Calculations and statistical analysis

Based on the cefuroxime concentration obtained in subcutaneous tissue (T) and in plasma (P) at the same time points, the [T/P] ratio was calculated, which permitted to evaluate the extent of penetration of the antimicrobial agent in subcutaneous tissue.

Statistical analysis was performed with the GraphPad InStat 2.01® software using the nonparametric Wilcoxon and Mann–Whitney tests for paired and unpaired data, respectively. The results are reported as median, mean, standard error of the mean, and 95% confidence interval.

3. Results and discussion

A review of the literature demonstrated that there are some reports that describe the measurement of cefuroxime and other cephalosporins in several biological matrices such as bone tissue, abdominal fat, and heart tissue [10–17].

In a recent pilot study, Barbour et al. [18] evaluate six morbidly obese patients undergoing abdominal surgery that received a single intravenous dose of 1.5 g cefuroxime within 1 h of incision. Blood and microdialysis samples from the interstitial space fluid (ISF) of skeletal muscle and subcutaneous adipose tissue were collected and determined by HPLC-UV, revealing that cefuroxime distributes into the ISF of muscle and adipose tissue of this patients enough to prevent infections with Gram-positive organisms but may be insufficient to prevent infections with Gram-negative organisms.

Due to different methods found in the literature regarding the quantitative analysis of cefuroxime in subcutaneous tissue, the analytical method previously developed in our laboratories for the quantification of this antimicrobial agent in plasma was adapted to tissue samples [19]. Since no biological material from patients could be used for the development and optimization of the analytical method, in the present investigation subcutaneous tissue from dogs was employed based on the study of Casal et al. [20], who used biological materials obtained from birds and pigs for the development and validation of a method for the quantification of vitamins in human subcutaneous tissue.

Table 1
Validation of analytical method for quantification of cefuroxime in subcutaneous tissue.

Parameter	Obtained value
Linearity	0.5–100 μg/g
Linear correlation coefficient	$r = 0.9963$
Limit of detection	0.25 μg/g
Limit of quantification	0.5 μg/g
Stability	
Thawing cycles[a]	94.9–98.4%
After three months[a]	96.2–100%
Dry extract[a]	84.5–88.2%
On the rack[a]	94.4–96.2%
Precision (CV%)	
Within-day	
100 μg/g	4.9%
10 μg/g	5.5%
0.5 μg/g	8.9%
Between-day	
100 μg/g	8.2%
10 μg/g	6.4%
0.5 μg/g	9.9%
Accuracy (%)	
Within-day	
100 μg/g	104.6%
10 μg/g	95.8%
0.5 μg/g	87.1%
Between-day	
100 μg/g	103.8%
10 μg/g	101.6%
0.5 μg/g	94.8%
Relative recovery	98.4%

[a] Expressed as mean values relative to three concentration evaluated for each test (100, 10 and 0.5 μg/g).

3.1. Method validation

A good linearity was obtained after the analysis of cefuroxime in the tissue. The result was expressed by the intercept and also the slope of the linear function as mean, standard error of the mean (SEM) and the linear correlation coefficient (r) (Table 1).

The validation of the analytical method in this study was adequate for the determination of cefuroxime in these patients. The precision, accuracy and recovery rates obtained in the present study agree with those reported in investigations involving patients undergoing surgery of the neck and knee [11,12].

The analytical method shows specificity and selectivity, since any endogenous peak from the subcutaneous tissue was not co-eluted with cefuroxime or vancomycin after extraction. The retention times obtained for cefuroxime (12.8 min) and the internal standard (4.5 min) guaranteed an analytical run of only 15 min, thus permitting a large number of analyses per day (Fig. 1).

Although the internal standard vancomycin has a different structure from cefuroxime, this drug had a good recuperation without interference in the method validation or in the results of this study.

The reliability of the analytical method for the quantification of cefuroxime in this matrix was demonstrated by the following parameters: linearity (0.5–100 μg/g), limits of quantification (0.50 μg/g) and detection (0.25 μg/g), intra- and interday precision (low, medium and high concentrations) ranged from 4.9 to 8.9% and 6.4 to 9.9% (CV%), respectively, and intra- and interday accuracy ranged from 87.1 to 104.6% and 94.8 to 103.8%, respectively. Relative recovery of the analyte from the biological material was 98.4%.

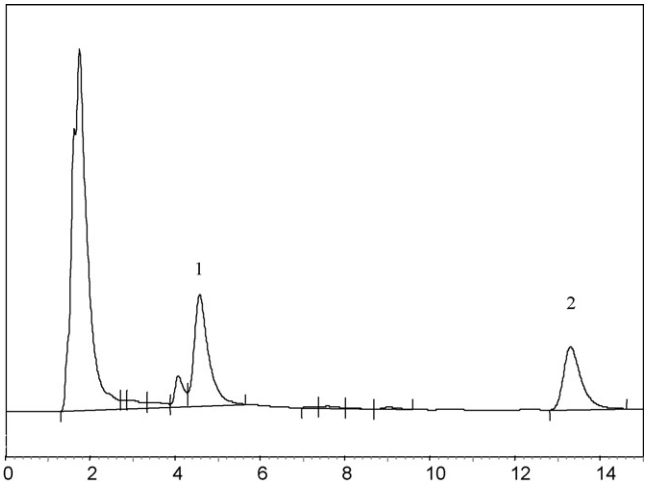

Fig. 1. Representative HPLC-UV chromatogram at 280 nm of patient's subcutaneous tissue sample using an eluent of 95:5 water/acetonitrile. 1: internal standard (vancomycin) and 2: cefuroxime.

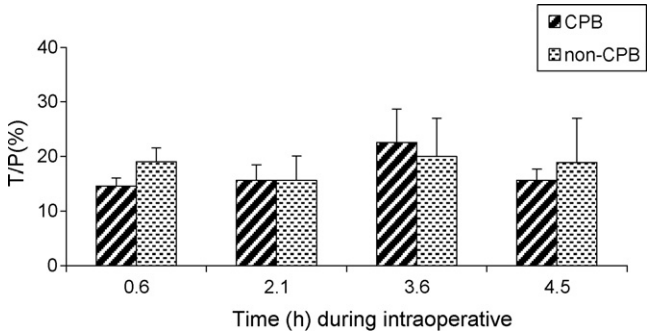

Fig. 2. Subcutaneous tissue/plasma concentration ratio (%) of cefuroxime in CPB and non-CPB groups in four moments during intra-operative. Values expressed as mean and SEM.

Short-term stability performed at room temperature of cefuroxime in the subcutaneous tissue and on the rack of autosampler was guaranteed up to 12 h. Three consecutive freeze–thaw cycles and after three months freezing showed good stability for cefuroxime in this matrix stored in −80 °C (Table 1).

3.2. Penetration of cefuroxime in the subcutaneous tissue

The penetration of the antimicrobial drug in the subcutaneous tissue during the intraoperative period and the respective concentration in plasma at the same time was investigated in both groups of patients. Maximal quantification of cefuroxime was observed in 0.6 h (1st sampling) after IV bolus administration of the drug. Comparison of the cefuroxime concentrations obtained upon 1st sampling showed no significant difference between groups, CPB and non-CPB (14.8 μg/g *vs* 13.2 μg/g, $p = 0.71$) (Table 2). Comparing both groups of patients, the results obtained for the 2nd and 3rd samplings, suggest that CPB procedure has no important influence on the distribution of the antimicrobial drug in subcutaneous tissue since no significant difference was observed between the CPB and non-CPB groups. However, a significant difference of cefuroxime concentration between both groups was observed for the 4th sampling (14.7 μg/g *vs* 3.9 μg/g), once only the CPB group received an additional dose of the drug immediately after the end of CPB (Table 2).

Cefuroxime quantified in tissue (T) and plasma (P) at the same moments permitted us to calculate the penetration ratio (T/P%). The data obtained shows that there are no difference in the percentage of penetration, independent of the group of patient or of the moment of the collection or the plasmatic bioavailability of the cefuroxime (Fig. 2).

Similar results have been reported by Lovering et al. [15] for 20 patients submitted to hip orthopedic surgery. These investigators reported a subcutaneous tissue concentration of cefuroxime of 15 μg/g, 10–30 min after administration of 1.5 g of the drug. The wide variation reported by these authors (14–79 μg/g) agrees with the present results showing cefuroxime concentrations for the 1st sampling ranging from 0.5 to 21 μg/g for CPB group and from 2.8 to 23.7 μg/g for the non-CPB group. For the other samplings, a similar variation was observed, respectively, for the CPB and non-CPB groups: 3.8–14.5 μg/g *vs* 9.0–14.0 μg/g for the 2nd sampling, 2.7–11.5 μg/g *vs* 2.4 to 7 μg/g for the 3rd sampling, and 11.5–18.0 μg/g *vs* 1.7 to 6.1 μg/g for the 4th sampling. Another investigation in which an identical dose of cefuroxime was administered to 15 patients submitted to neck surgery, the authors reported a subcutaneous tissue concentra-

Table 2
Cefuroxime in the plasma and subcutaneous tissue of patients during intra-operative coronary artery bypass grafting surgery.

Sample	*T* (h)	CPB	Non-CPB	*p*
		Mean (μg/g)[a] (CI 95%)	Mean (μg/g)[a] (CI 95%)	
Subcutaneous tissue				
1st	0.6	14.8 (9.5–21)	13.2 (2.8–23.7)	0.71
2nd	2.1	9.1 (3.8–14.5)	6.3 (9–14)	0.37
3rd	3.6	7.1 (2.7–11.5)	4.7 (2.4–7)	0.32
4th	4.5	14.7 (11.5–18)	3.9 (1.7–6.1)	<0.0001
Plasma				
1st	0.6	98.7 (102–121)	94.2 (80–150)	0.79
2nd	2.1	51.5 (33.6–69)	54.0 (14–94)	0.88
3rd	3.6	39.8 (17.4–62)	37.7 (10–65)	0.88
4th	4.5	90.7 (72.5–109)	25.8 (13.8–38)	<0.0001
Tissue/plasma ratio (%)[b]				
1st	0.6	14.6 (11–8)	19 (9.2–29)	0.18
2nd	2.1	15.7 (9.3–22)	15.7 (2.5–29)	0.99
3rd	3.6	22.5 (8–37)	19.9 (2.0–38)	0.78
4th	4.5	15.7 (10.7–20.7)	18.8 (2.1–34)	0.70
Mean (T/P)		17.1%	18.4%	

[a] μg of cefuroxime per gram of tissue or mL of plasma.
[b] Data expressed as means (μg/g of tissue) and CI 95%.

tion of the drug of 19.7 ± 7.1 μg/g 1.5 h after drug administration [12].

3.3. Clinical implications

Considering that the minimal inhibitory concentration (MIC) of cefuroxime ranges from 1 to 4 μg/mL for the main bacteria involved in surgical wound infections, the presence of the drug at this concentration permits adequate patient prophylaxis during surgery [4,15]. However, since patients of the non-CPB group receive the second cefuroxime dose only 12 h after the first one, the concentration of the drug at the site of surgical incision may have been lower than MIC at the postoperative period, with a consequent risk of postoperative infection, as reported by Nascimento et al. [21]. This fact compromises antibiotic prophylaxis since the efficacy of cefuroxime, like that of other beta-lactam antibiotics, depends on the period of time the drug remains in the organism. In addition, maintenance of a concentration about four times higher than the MIC is recommended to protect the patient against infections caused by more resistant bacterial strains [4,22]. Therefore, alteration in the dose scheme of cefuroxime is recommended as an alternative to maintain adequate subcutaneous tissue concentrations, since a similar and rapid loss of the drug is observed from both tissue and plasma, with a half-life around 1.5 h being observed in both groups of patients investigated.

In the present study, it was possible to determine the percentage of cefuroxime in tissue related to plasma using the calculated T/P ratio based on the concentration of the drug in subcutaneous tissue and plasma determined at the same time. The mean T/P ratios were similar in the CPB and non-CPB groups (17.1% *vs* 18.4%) throughout the intraoperative period. This ratio remained constant at all periods investigated (0.6, 2.1, 3.6 and 4.5 h, $p > 0.05$), suggesting that a fast distribution of drug from plasma to subcutaneous tissue occurs and the equilibrium is reached rapidly (Fig. 1). These data agree with the literature results showing a T/P ratio of 16% in a group of patients undergoing orthopedic surgery and treated with the same cefuroxime dose [15].

Additionally, as the cefuroxime has presented good penetration and its concentrations in blood and subcutaneous tissue during the surgery are over the recommendations, the additional dose administered after the cardiopulmonary bypass could be unnecessary.

4. Conclusion

The data obtained suggest that the distribution of the drug from plasma to subcutaneous tissue is fast and a fast equilibrium is reached after drug administration. In addition, a cefuroxime T/P ratio remained unchanged in the intraoperative period in patients undergoing coronary artery bypass grafting surgery, irrespective of the presence or absence of CPB. Finally, CPB does not seem to affect significantly the penetration of cefuroxime in tissues close to the surgical wound making unnecessary the additional dose administration.

Acknowledgments

To the Brazilians foundations FAPESP and CNPq for the support to the research project.

References

[1] D. Coskun, J. Aytac, A. Aydinli, J. Hosp. Infect. 60 (2005) 176.
[2] W.E. Trick, W.E. Scheckler, J.I. Tokars, J. Thorac. Cardiovasc. Surg. 119 (2000) 108.
[3] R. El Oakley, E. Paul, P.S. Wong, J. Cardiovasc. Surg. 38 (1998) 595.
[4] S.E. Pass, C.I. Miyagawa, D.P. Healy, T.D. Ivey, Ann. Pharmacother. 35 (2001) 409.
[5] H.G. Jakob, M. Borneff-Lipp, A. Bach, S. von Puckler, J. Windeler, H.-G. Sonntag, S. Hagl, Eur. J. Cardiothorac. Surg. 17 (2000) 154.
[6] I. Kriaras, A. Michalopoulos, M. Turina, S. Geroulanos, Eur. J. Cardiothorac. Surg. 18 (2000) 440.
[7] M.J.C. Carmona, L.M.S. Malbouisson, V.A. Pereira, M.A. Bertoline, C.E.K. Omosako, K.B. Le Bihan, J.O.C. Auler Jr., S.R.C.J. Santos, Braz. J. Med. Biol. Res. 38 (2005) 713.
[8] J.W. Nascimento, M.J. Carmona, T.M. Strabelli, J.O. Auler, S.R. Santos, J. Hosp. Infect. 59 (2005) 299.
[9] J.A.T. Sandoe, B. Kumar, B. Stoddart, R. Milton, J. Dave, U.R. Nair, M.H. Wilcox, J. Antimicrob. Chemother. 52 (2003) 877.
[10] C. Martin, X. Viviand, A. Cottin, V. Savelli, C. Brousse, E. Ragni, C. Richaud, M.N. Mallet, Antimicrob. Agents Chemother. 40 (1996) 1311.
[11] C. Hoddinott, A.M. Lovering, H.C. Fernando, J.H. Dixon, D.S. Reeves, J. Antimicrob. Chemother. 26 (1990) 823.
[12] W. Maier, J. Strutz, Chemotherapy 41 (1995) 421.
[13] H.J. Mann, H. Buchwald, Drug Intell. Clin. Pharm. 20 (1986) 869.
[14] J.P. Kaukonen, P. Tuomainen, J. Makijarvi, R. Mokka, P.T. Mannisto, Acta Orthop. Scand. 66 (1995) 14.
[15] A.M. Lovering, J. Perez, K.E. Bowker, D.S. Reeves, A.P. MacGowan, G. Bannister, J. Antimicrob. Chemother. 40 (1997) 99.
[16] W.K. Huizinga, A. Hirshberg, S.R. Thomson, K.I. Elson, R.T. Salisbury, J.G. Brock-Utne, J. Hosp. Infect. 13 (1989) 395.
[17] J.J. Andreasen, S. Møller, O. Hauch, L.N. Jørgensen, A. Lester, I. Mortensen, V.F. Thomsen, Antimicrob. Chemother. 22 (1988) 90.
[18] A. Barbour, S. Schmidt, W.R. Rout, K. Ben-David, O. Burkhardt, H. Derendorf, Int. J. Antimicrob. Agents (2009) (Epub ahead of print).
[19] J.W. Nascimento, M.J. Carmona, T.M. Strabelli, J.O. Auler, S.R. Santos, Braz. J. Pharm. Sci. 39 (2003) 265.
[20] S. Casal, B. Macedo, M.B. Oliveira, J. Chromatogr. B 763 (2001) 1.
[21] J.W. Nascimento, M.J. Carmona, T.M. Strabelli, J.O. Auler, S.R. Santos, Clinics 62 (2007) 257.
[22] M.R. Jacobs, Clin. Microbiol. Infect. 7 (2001) 589.