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Effectiveness of Prophylactic Intraosseous Antibiotic versus Intravenous Antibiotic in Knee Surgeries in Pigs: Experimental Study*

Eficácia do antibiótico profilático intraósseo versus endovenoso em cirurgias do joelho em porcos: Estudo experimental

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Abstract

Objective To demonstrate that the intraosseous (IO) access is more effective compared with the intravenous (IV) access for prophylactic antibiotic administration in knee joint surgeries, using 36 pigs as live models.

Materials and Methods Skin, subcutaneous tissue, cartilage, and bone samples were collected, analyzed and compared after the administration of IV or IO antibiotic in different groups.

Results When comparing the IO and IV groups, the IO group showed a higher concentration of prophylactic antibiotic in the skin ($p = 0.049$), cartilage ($p = 0.018$), and bone ($p = 0.002$), in the analysis of the first 24 hours after 30 minutes of infusion.

Conclusion Since complications regarding this practice are rare, the use of this pathway may be an alternative to reduce the risk of surgical site infection in orthopedic surgeries, leading to a decrease in morbidity and mortality and hospital expenses with readmission or prolonged hospitalization time. However, further research and further experimental studies in humans are required, as the effectiveness of the method in pigs has been proved.

Keywords
- antibacterial agents
- prophylaxis
- arthroplasty
- infection
- animals
- swine

Resumo

Objetivo Demonstrar, em 36 porcos usados como modelos vivos, que o acesso intraósseo (IO) é mais eficaz em comparação com o acesso endovenoso (EV) na administração de antibiótico profilático em cirurgias na articulação do joelho.

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Materiais e Métodos Foram coletadas, analisadas e comparadas amostras de pele, tecido subcutâneo, cartilagem e osso, após administração de antibiótico profilático EV e IO em grupos diferentes.

Resultado A comparação entre os grupos IO e EV indicou que no grupo IO houve maior concentração de antibiótico profilático na pele ($p = 0{,}049$), na cartilagem ($p = 0{,}018$) e no osso ($p = 0{,}002$), na análise das primeiras 24 horas após 30 minutos de infusão.

Conclusão Visto que as complicações dessa prática são raras, o uso dessa via pode ser uma opção para a diminuição do risco de infecção do sítio cirúrgico nas cirurgias ortopédicas, pois leva à diminuição da morbimortalidade e dos gastos hospitalares com reabordagens ou com tempo prolongado de internação. Contudo, são necessários mais pesquisas e novos estudos experimentais em seres humanos, dado que está comprovada a eficácia do método em porcos.

Palavras-chave
- agentes antibacterianos
- profilaxia
- artroplastia
- infecção
- animais
- suínos

Introduction

Infection at the surgical site is a significant cause of morbidity and mortality in knee joint surgeries, which has an indirect impact on the country's economy.[1] Patients with infection are more likely to die, require intensive care, and need retreatment. Orthopedic wards are classified as high-risk sites for this complication, especially in patients undergoing arthroplasties.[2] However, the administration of prophylactic antibiotic has shown to decrease the contamination and infection rates, and is the focus of many current researches.[1,3]

The most common bacteria that causes contamination and subsequent infection in total knee arthroplasties are *Staphylococcus aureus* and coagulase-negative staphylococci (CNSs).[3–5] Systemic administration of the first generation of cephalosporins and of vancomycin has been the most widely accepted recommendation regarding prophylaxis. Cephalosporins have a spectrum of activity against coagulase-negative staphylococci, methicillin-sensitive *S. aureus* (MSSA) and certain gram-negative bacteria, while vancomycin is active for methicillin-resistant *S. aureus* (MRSA).[3]

In order for the prophylactic antibiotic to be effective, its concentration in the tissue must exceed the minimum inhibitory concentration (MIC) of the organism commonly causing the infection, within the period between incision and wound closure.[1,4,6] Recent studies have questioned whether the antibiotic concentration achieved in the tissue, with IV administration as prophylaxis, is adequate for bactericidal activity.[3]

Young et al[7] have demonstrated that a higher concentration of prophylactic antibiotic in the tissue can be achieved with intraosseous regional administration (IORA) in patients submitted to knee surgeries, after placement of the tourniquet and before the incision in the skin.[1,3,7] A randomized study[3] with patients with total knee prosthesis compared IORA with IV and demonstrated that the IORA achieved tissue concentrations ten times higher than the IV (►**Table 1**).[3,7,8]

Considering the greater risk of complications in infected patients, it is fundamental to develop more effective measures to help prevent infections. Thus, the present study aimed to characterize, from a practical and quantitative point of view, the use of IORA so that greater tissue concentration could be obtained during these types of surgeries.

The objective of the study is to demonstrate that the IO access results in a greater local concentration of prophylactic antibiotic in swine knee surgeries compared with the IV administration.

Materials and Methods

The present study was performed in the "Basis of Surgical Techniques" discipline, at the animal house facility, and it was approved by the Ethics Committee for the use of animals, according to the normative resolution number 007/12 (approval protocol number 030/2016). The animal model was developed with 36 pigs (*Sus scrofa domesticus*), male or female, at 3 months of age and weighing $\sim$ 16 kg.

The animals destined to the study were fed with Presuntina feed and drinking water (provided by Sociedade de Abastecimento de Água e Saneamento S/A, SANASA) on demand, and they remained in individualized environments (stalls). The

Table 1 Antibiotic concentration per tissue (Wellman[3])

Type of tissue	250 mg IORA of vancomycin	500 mg IORA of vancomycin	1 g EV of vancomycin	1 g IORA of cefazolin	1 g EV of cefazolin
Subcutaneous fat (ug/g)	14	44	3.2	186	11
Bone (ug/g)	16	38	4	130	11

Abbreviations: EV, endovenous; IORA, intraosseous regional administration.

recommendations and norms prescribed by the Ethics Committee for the use of animals in scientific experiments and the protection of these animals were rigorously adopted and followed. All animals were sacrificed immediately after the procedures, also in accordance with the recommendations of the ethics committee.

For the experimental procedures, 3 groups were formed with 12 pigs each, and all of them were submitted to peripheral venous access, general anesthesia and orotracheal intubation, followed by antisepsis of the limb(s) treated with 2% aqueous chlorhexidine. In all pigs, the samples of material of the knee were taken in two moments. In each collection, two samples of skin were removed from the knee, two from the subcutaneous tissue of the knee, two from the cartilage of the tibial plateau, and two from the proximal end of the tibia. These samples followed the same pattern for removal (same equipment, similar size, same collection locations, and the same team). In addition, the instruments were cleaned with chlorhexidine, and the team wore clean surgical clothing and sterile gloves. In the first group, the control group (CG), only 0.9% saline solution was applied by the peripheral venous access (in the animal's ear). The first sample was collected 30 minutes after the end of the infusion, and the second, 1 hour after the end of the infusion. In the second group, the intraosseous group (IOG), a tourniquet was placed on the right lower limb (in the thigh) of the animal, and, afterwards, a 2 g cefazolin ampule diluted in 20 mL of 0.9% saline was dispensed intraosseously using the NIO Pediatric (PerSys Medical, Houston, TX, US) device in the medial region of the tibial plateau. The first collection was performed 30 minutes after the infusion of the antibiotic, and the second sample was collected 1 hour after the end of the infusion. In order to make a better analysis, a third group, the contralateral intraosseous group (CLIOG) was created, with samples collected at the same time as the IOG, but from the contralateral limb in which the IO antibiotic therapy had not been administered. In the fourth group (intravenous group, IVG) an ampule of 2 g cefazolin diluted in 250 mL of 0.9% saline solution was administered intravenously. The time elapsed between the beginning and the end of the infusion was, on average, 20 minutes. After 30 minutes of the end of the infusion of the antibiotic, the first collection of material was made, and the second sample was taken 1 hour after the end of the infusion (►**Fig. 1**). Mannitol salt agar plates were used for the analyses. These were incubated with *S. aureus* at a 4:1 dilution (4 parts of serum and 1 part of bacteria), and diluted with a sterile swab. Soon after the inoculation of the plates, tissue samples were inserted into them, and they were incubated at 37°C. After 24 hours of incubation, the halos were first analyzed. After the first analysis, the samples were incubated again for another 24 hours for the second analysis.[9–11] The formation of a red halo on the plates meant that the bacteria did not grow, so the medication in the tissue killed the bacteria that were in that location (►**Fig. 2**).

Results

The results were obtained by multiplying the largest diameter with the smallest diameter in centimeters of the red halo formed around each tissue (►**Fig. 3**).

The statistical test applied was the Mann-Whitney test, a nonparametric test with independent samples. Out of the 240 samples analyzed, 5 were discarded due to contamination of the plates, which were distributed as follows: 59 in the CG, 60 in the IOG, 56 in the IOCLG, and 60 in the IVG. In each group, one sample of skin, subcutaneous tissue, cartilage and bone were taken after 30 minutes in the cefazolin, and another sample of the same tissues was taken after 1 hour.

All samples were measured and weighed to see if they had no difference in size or weight that could influence the size of the halo (larger samples would have larger halos). The sample sizes, which were measured after 24 and 48 hours ($p = 0.715$ and 0.977 respectively), and the weights, which were measured after 24 and 48 hours ($p = 0.171$ and 0.623 respectively), when compared between the groups, were not statistically different; therefore it was proved that they were homogeneous (►**Fig. 4**).

The analysis of the size of the halo, with the sum of all the tissues together, showed that the IOG had the highest mean (25.57 cm), and the CG had the lowest mean (1.81 cm). In the analysis of all tissues together, more bacteria were killed around the tissue in the IOG than in all of the other groups.

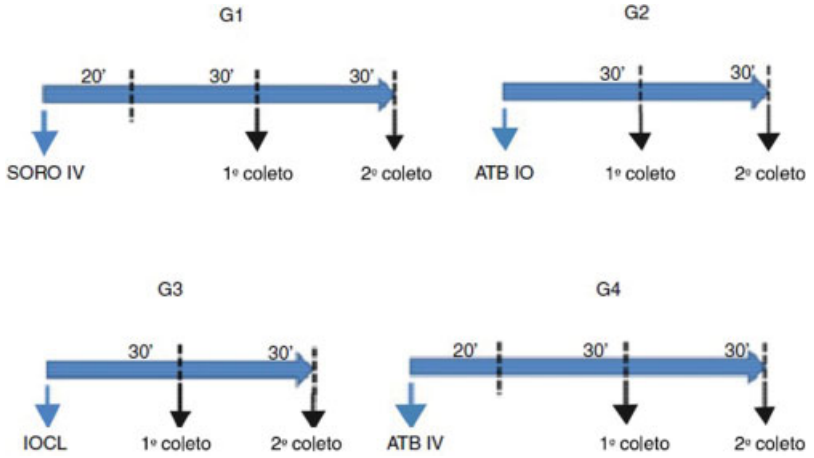

Fig. 1 Representative scheme of the collection flowchart. 1: SERUM IV. 2: 1st collection. 3: 2nd collection. 4: 1st collection. 5: 2nd collection. 6: 1st collection. 7: 2nd collection. 8: 1st collection. 9: 2nd collection.

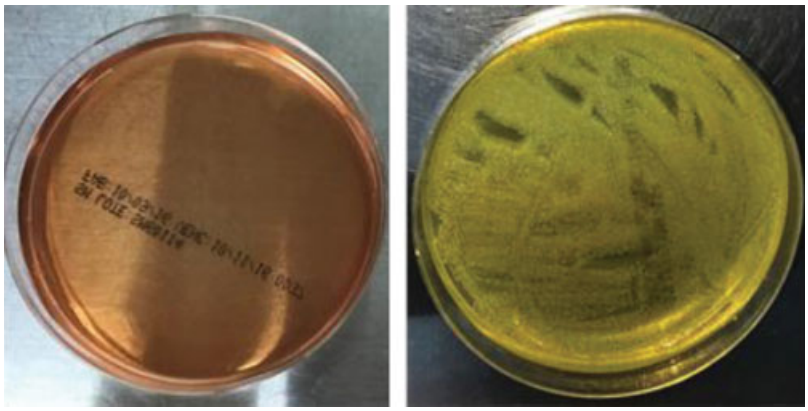

Fig. 2 Mannitol salt plate with and without *Staphylococcus aureus* respectively.

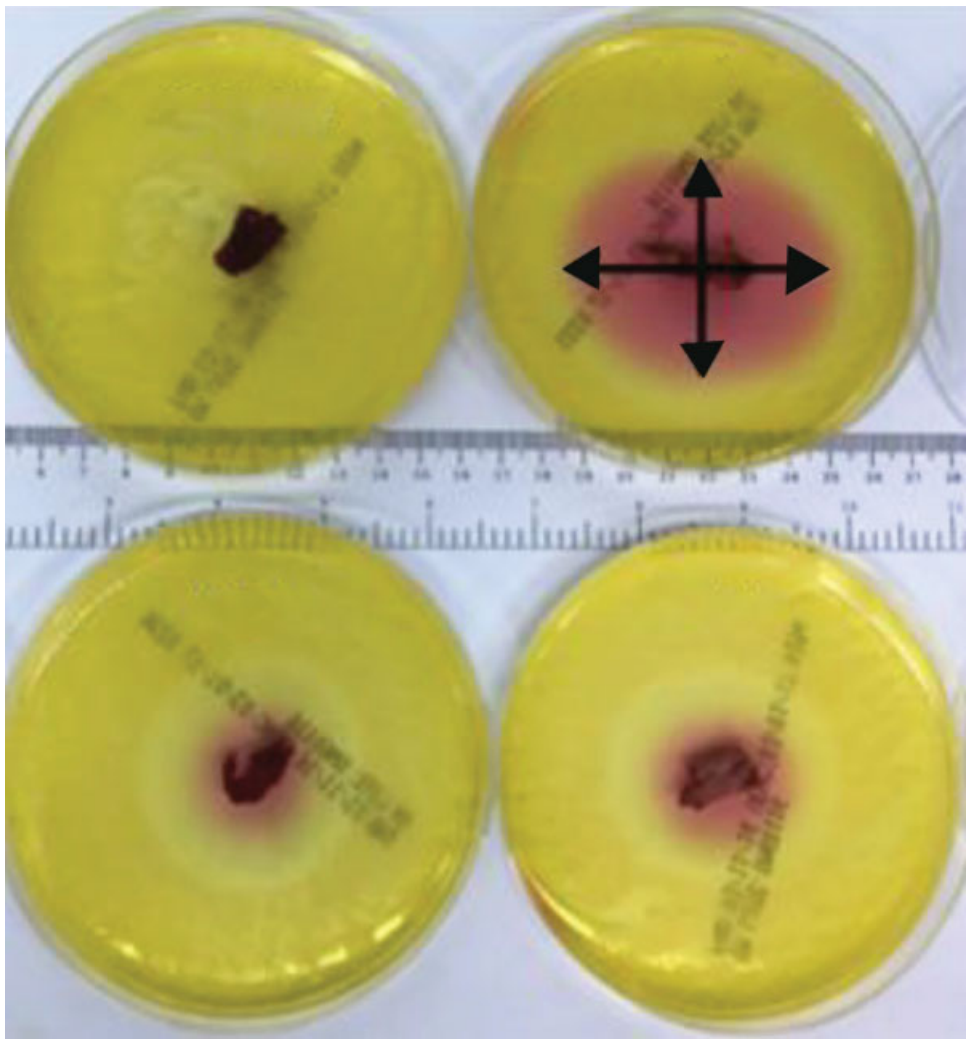

Fig. 3 Culture of bone tissue after 30 minutes of administration of cefazolin in 24-hour culture.

The statistical analyses of the means of the groups in relation to the tissue types are illustrated in ►**Table 2** and ►**Fig. 4**.

In order to know in which tissue the *p*-value was significant, we made an individual comparison by group and by tissue. There was no statistical significance when comparing the IOCLG and the IVG: all *p*-values, when compared with the individual tissues, were higher than 0.05. That is, the venous medication and the contralateral knee behaved in the same way. The CG only formed the halo in the skin tissues due to preoperative asepsis.

The IOG, when compared with the other groups, obtained a statistically significant result in all collections and at all time periods (►**Table 2**); the halo formed in the IOG was larger in all samples.

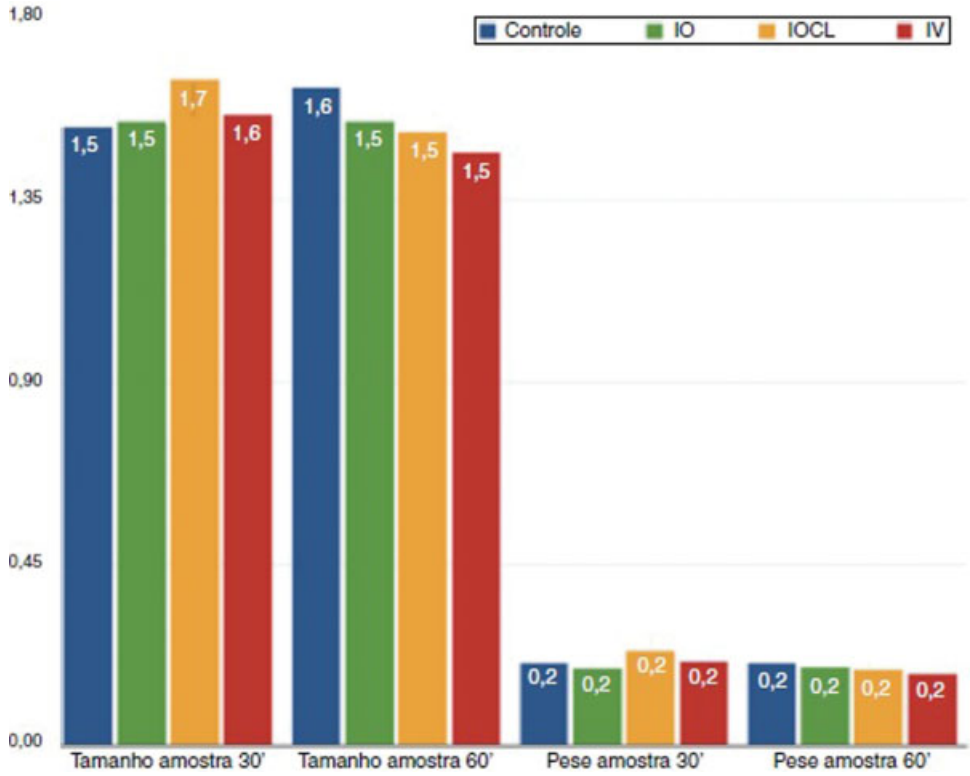

Fig. 4 Average per group concerning sample size and weight. 1: Control. 2: Sample size at 30 minutes. 3: Sample size at 60 minutes. 4: Sample weight at 30 minutes. 5: Sample weight at 60 minutes.

Table 2 Analyses of the IOG in relation to the IOCLG and IVG

Group	*p*-value
Time 24 h/30 min	
IOG x IOCLG	0.000000297
IOG x IVG	0.0000453
Time 24 h/1 h	
IOG x IOCLG	0.00000182
IOG x IVG	0.0003722
Time 48 h/30 min	
IOG x IOCLG	0.0042
IOG x IVG	0.005
Time 48 h/1 h	
IOG x IOCLG	0.04
IOG x IVG	0.047

Abbreviations: IOCLG, intraosseous contralateral group; IOG, intraosseous group; IVG, intravenous group.

In the comparison by tissue type of the IOG and IOCLG, the IOG was superior in the first 24 hours of the collection of 30 minutes in the skin ($p = 0.029$), and in the subcutaneous tissue, it was also superior in the first 24 hours, both in the collection of 30 minutes and 1 hour ($p = 0.016$ and 0.017 respectively). In the cartilage, in the first 24 hours, both in the collection of 30 minutes and 1 hour, it was also statistically significant ($p = 0.004$ and 0.002 respectively). When comparing the bone tissue with the other tissues at all time periods, the IOG was higher (►**Tables 3** and **4**). In the analysis of the values of the tables, we observed that in both collections (30 minutes and 1 hour), after IO medication, the tourniquet kept the medication more concentrated in the knee of interest, that is, the medication was more concentrated in these tissues, killed more

Table 3 Analysis of the IOG in relation to the tissues of the IOCLG

IOG x IOCLG	Tissue	*p*-value
Time 24 h/30 min	Skin Subcutaneous	0.029 0.016
	Cartilage	0.004
	Bone	0.002
Time 24 h/1 h	Skin Subcutaneous	0.052 0.017
	Cartilage	0.002
	Bone	0.002
Time 48 h/30 min	Skin Subcutaneous	0.096 0.476
	Cartilage	0.774
	Bone	0.008
Time 48 h/1 h	Skin Subcutaneous	0.275 0.655
	Cartilage	0.678
	Bone	0.034

Abbreviations: IOCLG, intraosseous contralateral group; IOG, intraosseous group.

Table 4 Comparison of means between the IOG and the IOCLG

Tissue	Sample 24 h/ 30 min	Sample 24 h/ 60 min	Sample 48 h/ 30 min	Sample 48 h/ 60 min
IOG skin	30.15	29.29	5.02	4.03
IOG subcutaneous	24.93	25.56	3.78	3.09
IOG cartilage	19.54	20.60	1.36	2.20
IOG bone	27.65	20.67	4.63	2.73
IOCLG skin	18.85	18.94	2.55	3.15
IOCLG subcutaneous	15.31	15.45	3.68	2.39
IOCLG cartilage	11.26	9.01	1.99	1.62
IOCLG bone	11.61	7.62	2.28	0.37

Abbreviations: IOCLG, intraosseous contralateral group; IOG, intraosseous group.

Table 5 Analysis of the IOG in relation to the tissues of the IVG

IOG x IVG	*p*-value
Time 24 h/30 min	
Skin	0.049
Subcutaneous	0.178
Cartilage	0.018
Bone	0.002
Time 24 h/1 h	
Skin	0.074
Subcutaneous	0.056
Cartilage	0.014
Bone	0.038
Time 48 h/30 min	
Skin	0.174
Subcutaneous	0.440
Cartilage	0.678
Bone	0.006
Time 48 h/1 h	
Skin	0.275
Subcutaneous	0.632
Cartilage	0.587
Bone	0.087

Abbreviations: IOG, intraosseous group; IVG, intravenous group.

bacteria, and increased the halo formed around the sample. After 48 hours, the concentration of the antibiotic decreases and the bacteria can grow, arriving close to the tissue, but the bone tissue was the only statistically significant in both collections ($p = 0.008$ and 0.034 respectively). In the comparison by tissue type between the IOG and the IVG, the IOG was superior in the first 24 hours of the collection of 30 minutes in the skin, with $p = 0.049$, but regarding the subcutaneous tissue, there was no statistical significance between the groups. In the cartilage, in the first 24 hours, both in the 30-minute and in the 1-hour collection, it was statistically significant ($p = 0.018$ and 0.014 respectively). When comparing the bone tissue, in the first 3 time periods, the IOG was superior, but in the second collection, with incubation of 48 hours, there was no statistical significance (►**Table 5**). We could observe that the halo of the bone tissue in the IOG was larger when compared with the other groups. This means that the concentration of antibiotics was higher in this tissue, it killed the bacteria around it, and increased the size of the halo, both in the collection after 30 minutes of the infusion and after 1 hour (►**Fig. 5**).

Discussion

Prophylactic antibiotics have been shown to reduce infection rates in arthroplasties,[1,7] and, to be effective, they must have adequate tissue concentrations at the operative site from incision until closure.[12,13] Although antibiotics, such as aminoglycosides and fluoroquinolones, are concentration-dependent, for b-lactam antibiotics such as cefazolin, the most important factor is the time beyond the MIC. As antibiotic resistance increases, the systemic administration of cephalosporins may no longer provide adequate tissue concentrations, whereas IORA reaches much higher tissue concentrations.[14] There is much evidence that prophylactic antibiotic therapy in osteomuscular surgeries, specifically in this case, in the knee, made by the IORA route, is more effective than when performed intravenously, as is conventionally done in Brazil.

This experimental study showed that the IORA provided greater bacterial inhibition, probably because it had a higher concentration of cefazolin present in the local tissues than the same dose of the antibiotic administered systemically, in the pig model, which was demonstrated by the growth of the staphylococci in Petri dishes; and that the IO plaques prevented, in greater quantity, the growth of *S. aureus*. Thus, it was deduced that the antibiotic concentration found in the tissues was higher in the IOG than in the IVG and IOCLG, which was also observed by Young et al.[1]

In all of the tissues studied, the concentration of the antibiotics in the group submitted to the IORA was higher when compared with the CG. In the skin, the concentrations were higher in the samples collected 30 minutes after administration of the medication, and this is the main moment when the concentration peak is necessary at this location: skin incision at the beginning of the procedure. In the subcutaneous tissue, cartilage and bone, the bacterial growth remained close to the samples collected at 30 minutes when compared with those collected after 1 hour of administration of the medication.

When the group submitted to the IORA and the IVG were compared, the IORA group showed greater inhibition of the bacterial growth in the first 24 hours after the collection at 30 minutes in the skin, cartilage and bone, as well as in the collection after 1 hour in the cartilage and bone. The

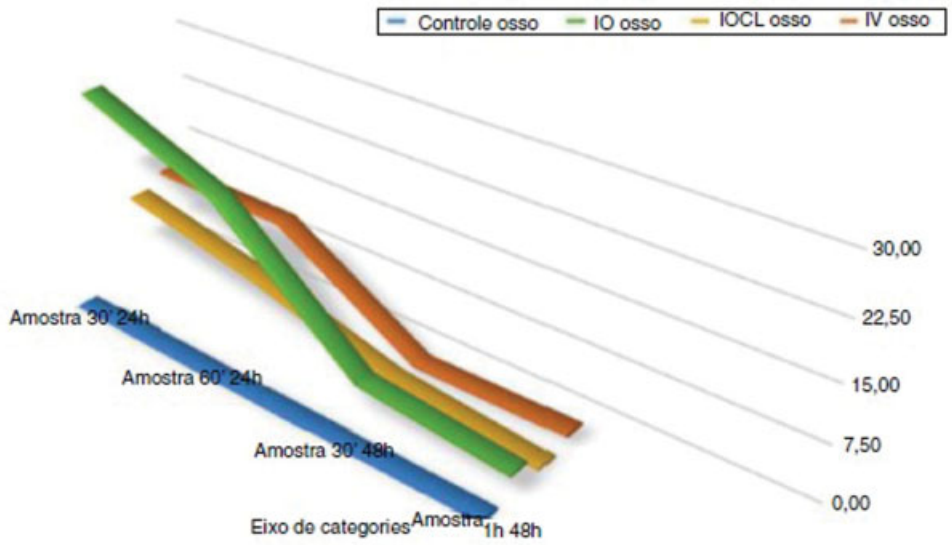

Fig. 5 Comparison of the bone tissue between groups. 1: Control bone. 2: IO bone. 3: IOCL bone. 4: IV bone. 5: Sample at 30 minutes/24 hours. 6: Sample at 60 minutes/24 hours. 7: Sample at 30 minutes/48 hours. 8: Sample at 60 minutes/48 hours. 9: Category axis. 10: 30.00. 11: 22.50. 12: 15.00. 13: 7.50. 14: 0.00.

medication in the sample of the IOG lost effect only after 48 hours of incubation, as well as in the samples collected after 1 hour of the infusion of the medication, that is, the medication remained active longer than in the other groups. This is one of the most important data obtained in the present study, since the main focus of it is the prophylaxis in musculoskeletal surgeries of the knee.

Another important data analyzed were the superiority of the IOG when compared with the IOCLG. The latter showed the same bacterial growth as the IVG, and showed the efficacy of the IORA and of the tourniquet, which maintained high concentrations of cefazolin in the local tissues and disseminated little medication to the opposite knee. The IOG was superior to the IOCLG regarding all tissues, in the readings after 24 hours of the collection at 30 minutes, and in the subcutaneous tissue, the cartilage and the bone, regarding the collection at 1 hour. On the reading after 48 hours of incubation, in both these collections, bacterial inhibition was significant in the bone tissue, demonstrating that the concentration of the antibiotics remained in the IOG, while it fell in the IOCLG and IVG.

Some biases were also observed in the present work. Although we have attempted to use doses of equivalent antibiotics and to simulate the clinical situation of a surgical procedure, it is not clear how close this approach is to the clinical situation in humans. In addition, although the IO route is reported to have pharmacokinetics for both fluids and for medicines like the IO administration has,[15] its use for the regional administration is not so well known, and there are few published works on it.

Conclusion

In the present study, the IORA of preoperative prophylactic antibiotics showed higher local concentration in the samples collected and resulted in a greater inhibition of bacterial growth in the tissues compared with the IV route. Since complications are rare with this practice, the use of this pathway may be an option to reduce the risk of infection in the surgical site in orthopedic surgeries. Thus, this type of approach becomes increasingly pertinent in orthopedics, and may later help change standard prophylaxis protocols in joint surgeries to reduce postoperative infection, considering the benefits to the patient and to the health system. However, since the effectiveness of the method in pigs was demonstrated, further experimental studies on humans are required, as well as the development of future works to confirm whether this translates into better prevention of infection. We believe that the results obtained with the present project will contribute to a better understanding of both ways studied and their effectiveness, and may open perspectives in the use of the access in question.

Conflicts of Interest

The authors have none to declare.

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