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EVALUATION OF NERIDRONATE ON THE OSSEOINTEGRATION PROCESS OF ENDEOUS TITANIUM IMPLANTS IN ANIMAL MODELS

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Bisphosphonates are compounds that inhibit bone reabsorption mediated by osteoclasts. The use of bisphosphonates in oral implantology is still in the experimental stage. The aim of this study is to evaluate the efficacy of an aminobisphosphonate to increase the ability of the drug to act on the implant and bone surfaces in the development of the osseointegration in sheep. Forty SLA titanium implants were used on sheep iliac crests. Neridronate added to connective gel (test 1) or to physiological solution (test 2) was used in order to increase the bone and implant adhesiveness. Physiological solution (control 1) or connective gel (control 2) alone was given to the control groups. A topical administration of Neridronate was made on the implant surface and in the implant site. Four Bergamasca sheep were used and were sacrificed by intravenous injection of 10 cc Tanax after 8 weeks from implantation. Histologic and histomorphometric analyses were carried out. The results did not show significant differences between the test group and control group. Our data are different from other similar studies obtaining statistically significant differences. These differences could depend on the procedure of application of the drug on the implant. This study demonstrates the poor efficacy of neridronate applied topically to the implant and implant site during surgery. Further studies using different fixation techniques of the drug may be necessary to confirm the present data.

Osseointegration is the direct contact between vital bone and the surface of the implant (1) and is influenced by many factors, such as anatomy of the edentular alveolar crest, the design of the implant, the volume, vascularization, and the mineral density of the bone (2). In fact, as indicated in a number of studies, the percentage of success of bone implants in the maxilla with low bone quality is lower than in the mandible (3-5). In particular, one study published in 1991 affirms that 35% of implants inserted in type IV bone failed, against a 3% failure rate of implants inserted in bone types I, II, and III (6). Clinically, many situations are far from "ideal", quantitatively as well as qualitatively, therefore, it is necessary to carefully evaluate the indications for bone implants in these patients (7). Bisphosphonates in this context can be of great help since they can increase bone density (8). In fact, in a double-blind, randomized controlled study with placebo as a control, Pols and others noticed that in all sites and in every given period of time the median percent modification of the BMD among the patients of the tested group were significantly higher than those found in the control group (8). Bisphosphonates

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are already considered the most suitable therapy for osteometabolic diseases such as: osteoporosis (9), Paget's disease (10), multiple myeloma (11), hypercalcemia (12) and metastatic bone tumors (13). Bisphosphonates have high affinity for mineralized bone and it is in this context that they show their biological functions (14).

Bisphosphonates have many biological effects which vary on the basis of the modality of administration, concentration and the type of drug molecule used (14). These drugs act on bone homeostasis, inhibiting the resorption of bone mediated by osteoclasts. They inhibit the activity of mature osteoclasts, increase the apoptosis processes of osteoclasts, and inhibit the formation and recruitment of new osteoclasts (15-16). Recent studies have shown that some of these compounds can stimulate the differentiation and the activity of osteoblasts (17) and that they have different and numerous extra-skeletal biological properties (18). The different molecular actions may suggest differences between the amino and non-amino bisphosphonates, drugs belonging to the same family but to different groups. These aspects are confirmed by the significant difference in the relative resorption pores that exist between the two groups. In fact, aminobisphosphonates have an anti-resorption property 10-100-10000 times higher than the non-aminobisphosphonates (19-20). Many published papers confirm (21-23) that aminobisphosphonates increase the bone-implant contact percentage compared to control groups. For these reasons it was planned to test the efficacy of neridronate, an aminobisphosphonate.

Various studies (24-30) have demonstrated how the implant surface is another important variable which could influence the release of the drug. In these studies, in which the aminobisphosphonate was fixed to implants with roughened surfaces or coated with HA, the release of the drug was gradual, constant and long lasting. On the contrary, in smooth implants the release was immediate and lasted only for the first hour (25). The response of the perimplant bone seems to be influenced, not only by the drug used, but also by the length of time it is released at the implant site. Pertaining to this, various methods have been discussed in literature which foresee almost all fixations of the molecule on the implant coating.

The aim of the present study is to evaluate the effects of local administration of an amino-bisphosphonate (neridronate) on improving the osseointegration of SLA titanium implants in an animal model characterized by low bone density.

MATERIALS AND METHODS

The pharmacological molecule we used was neridronate, an aminobisphosphonate which has an anti-resorption power higher than that of clodronate. The concentration of the drug was decided in collaboration with Abiogen Pharma (Abiogen Pharma s.p.a., Pisa, Italia). We chose to test the drug either combined with a connective gel or as a simple physiological solution.

The connective gel was used as a carrier to increase adhesion of the drug to the implant and to the implant site.

We used titanium implants with sanded and mordanted surfaces, not smooth surfaces, 3.8 mm in diameter and 9 mm in length. The implants were inserted into the iliac crests of four Bergamasca sheep. The average weight of the animals was 65 kg. We chose to study the iliac crest of sheep because the bone in this site is of low density and has a morphostructural characteristic which allows to evaluate the eventual influence of the drug on the osseointegration process. The animals were sacrificed after 8 weeks. Histomorphometric and biomechanical measurements were carried out on the samples obtained. For histomorphometric measurement the percentage of contact between the bone and the implant (B.I.C.) and the volume of perimplant bone were calculated (B.V\T.V). For the biomechanical measurement, we calculated the unscrewing torque of the implants using the removal torque, a device with a jack, which was supplied with a charged cell and a strain gauge. The jack was connected to a data logger connected to a PC. An appropriate software was installed on the PC (me.co.da.rec.- DSPM Industria-Milano, Italy) which allowed to acquire and elaborate the data to obtain the curve relative to the twisting moments of unscrewing

Group division and performed analyses

The four animals were penned. Each sheep was given 10 implants, 4 in the left iliac crest and 5 in the right iliac crest. We analyzed four different groups divided into test group 1 (T1), test group 2 (T2), control group 1 (C1) and control group 2 (C2). Group T1 was administered 50 µl Nerdronate in 600 µg connective gel. Group T2 was administered 50 µl Neridronato in 600 µg physiological solution. Group C1 was administered physiological

T1 50 µl neridronate in connective gel (600 µg)
T2 50 µl neridronate in physiological solution (600 µg)
C1 physiological solution
C2 connective gel
TOTAL NUMBER OF IMPLANTS: 4
NUMBER OF IMPLANTS PER SHEEP: 10 (5 for each iliac crest)
TOTAL NUMBER IF IMPLANTS PER GROUP: 10
TOTAL NUMBER OF IMPLANTS: 40

Fig. 1. *Experimental groups.*

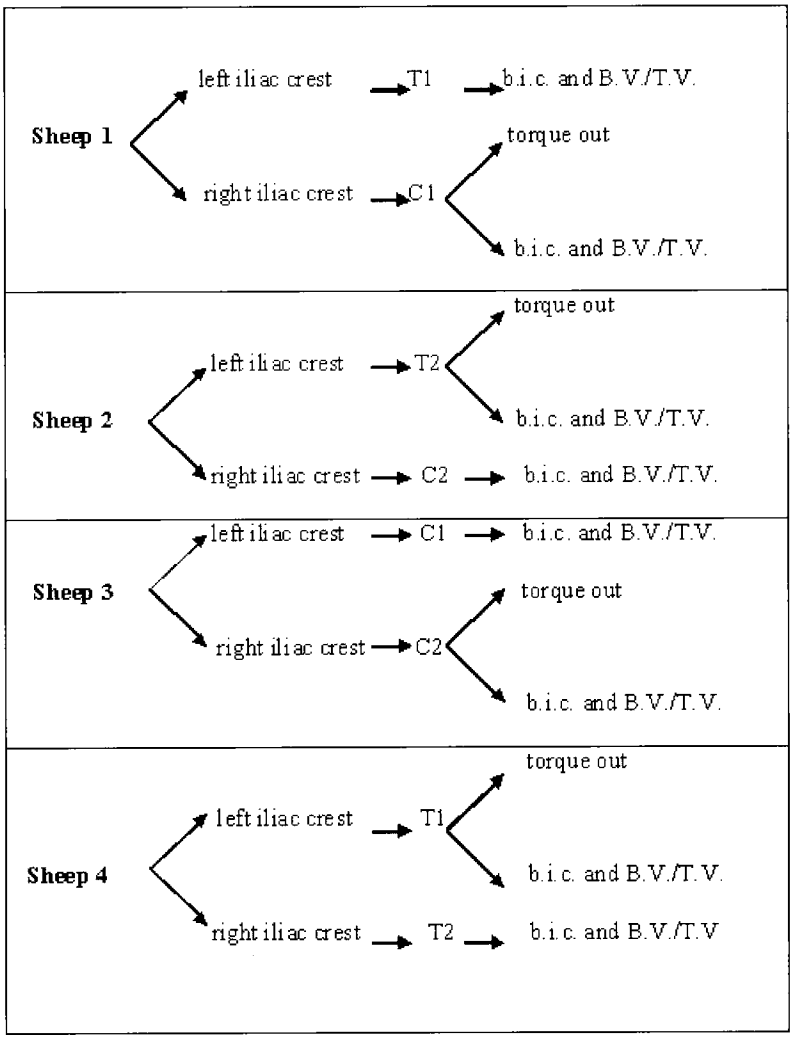


Fig. 2. *Group division and analyses performed.*

solution, and group C2 was administered connective gel. Each administration was carried out using an appropriate precision micropipette. For each group 10 implants were inserted, for a total of 40 units (Fig. 1).

The order in which the implants were inserted in each group is shown in Fig. 2. Each animal received implants belonging to two the different groups. With regard to the analyses carried out, on 5 implants of each group only histomorphometric evaluations were performed, and on the other 5 the torque out was carried out first, and then the histomorphometric analyses (Fig. 2).

Experimental procedure

The animals were anesthetized with Xilazine Chloridrate (Rompun S.S., BAYER, Germany) 0.1 mg/kg weight prior to operating. The operation site was then disinfected with Betadine (Asta Medica GmbH, Francoforte-Germania). An incision was made in the iliac crest of each animal. After exposing the bone floor, implant sites were prepared (3.8 mm x 9 mm) with cutters mounted on handles at a low rotation (800 rpm) and continually irrigated with a sterile physiological solution. At the implant site and on the implant surface we applied the different solutions as mentioned above. The implants were inserted on the iliac crest using jig surgery according to standard surgery protocol. After insertion, all the implants presented primary stability.

Finally, sutures of the muscular floor were made with absorbable thread in Dexon II polyglycolic acid (Kendall Company, US-Massachusetts), and of the soft tissue with a non-absorbable silk thread (Codisan S.p.a.,Belpasso(CT)-Italia).

Topical antibiotic therapy was then carried out with Kana spray (Gellini-Intervet, Milano, Italia), after which systemic antibiotic therapy consisting of 8 ml long-acting Clamoxil (Pfizer Italia S.r.l.,Roma-Italia) was administered to each animal for five days. After 8 weeks the animals were sacrificed with an intravenous injection of 10 cc Tanax (Intervet Italia Srl, Milano-Italia).

The analyzed samples were infiltrated with Remacryl resin, starting from a solution of 50% and, after 24 h, a solution of 100% resin. Photopolymerization was obtained by exposing the samples for 48 h to blue light and orienting them so that both sides were exposed contemporarily. After photopolymerization, the blocks were finished off by eliminating the excess resin and exposing the implants. They were then adhered to plastic plaques with a methacrylate-based glue. The resulting blocks were cut with the rotating blade at high speed of the Micromet microtome to obtain 250 μm sections (Remet, Bologna, Italia). The sections were reduced to about 40 μm using the LS-2 machine (Remet, Bologna, Italia) with sandpaper and cooled with water. After the finishing-off processes, each section was polished with specific paper and 3 μm granulometry polishing paste.

To analyse the various bone remodeling patterns, toluidine blue was used, while basic fuchsine was used to highlight the fibrous tissue to obtain a good contrast. The histomorphometric analyses were carried out by converting the analogical-digital images of a microscope, using a color Video Camera, and blocking them (JVC TK-C1380, Color Video Camera, IVC Victor Company, Japan). The images were obtained with a 10 X lens including the entire implant surface. The digital images were then analyzed by software (IAS 2000, Delta Sistemi, Italy).

Statistical analysis

The following factors were calculated and expressed as percentages of the body Bone-to-Implant Contact (BIC), Bone Volume (BV) and torque levels expressed Newton/cm. The average percentage of the BIC and BV are shown in Table I and Fig. 4, A and B by mean ± SD while the extraction Torque levels are shown in Table I and Fig. 3 by mean + ES.

The values found of these factors, considering the entire length of the implant surface, in respect to the variables with and without neridronate and in respect to the pre-treatment with physiologic solution or with connective gel, underwent statistical analysis by the two-way analysis of the variants (ANOVA). While the comparison of the 4 subgroups examined was evaluated by one-way ANOVA.

The statistical analyses of the data were carried out using the software packet SPSS® Advanced Statistics TM 13 (2004, Chicago, IL, USA).

RESULTS

No animal died in the post-operative period or in the following two-month penning. We saw no sign of discomfort in the days or the weeks following the operation. No implant was rejected. From the biomechanical tests (Table I) no statistically significant differences emerged between groups T1 and T2 and group C1 and C2. In fact, as shown in Fig. 3 relative to the dispersion graphic and the statistical mean, the best results were reported in group T2 with an average Torque out value of 116 N\cm (SD 20.7). 109 N\cm was the value reported in group C1, 108.2 N\cm (SD 6.7) in group C2 and 100 N\cm (SD 5.8) that reported in group T1(SD 38.43).

The histomorphometric results are in line with the biomechanical ones. In fact, as seen in the

Table I. *Mean percentage ± SD of all the morphologic and biomechanical values of the 4 groups studied and divided into screwed and non-screwed implants.*

	BV/TV (n=40)		TORQUE (n=40)		BIC (n=40)	
	With Neridronate	Without Neridronate	With Neridronate	Without Neridronate	With Neridronate	Without Neridronate
Physiological solution	36.9 ± 6.7	34.9 ± 3.6	116.6 ± 20.7	109.2 ± 5.8	57.4 ± 10.1	65.3 ± 13.0
Screwed	38.9 ± 3.1	34.8 ± 4.5	--	--	57.6 ± 11.9	62.8 ± 15.4
Non-screwed	34.9 ± 8.8	34.9 ± 3.0	--	--	57.2 ± 9.5	67.7 ± 11.5
Connective gel	37.2 ± 8.0	35.6 ± 5.3	100.4 ± 38.4	108.2 ± 6.7	60.7 ± 10.3	68.5 ± 4.1
Screwed	38.9 ± 11.1	36.0 ± 3.1	--	--	57.8 ± 9.4	68.4 ± 3.8
Non-screwed	35.4 ± 8.8	35.1 ± 7.4	--	--	63.6 ± 11.3	68.7 ± 4.9

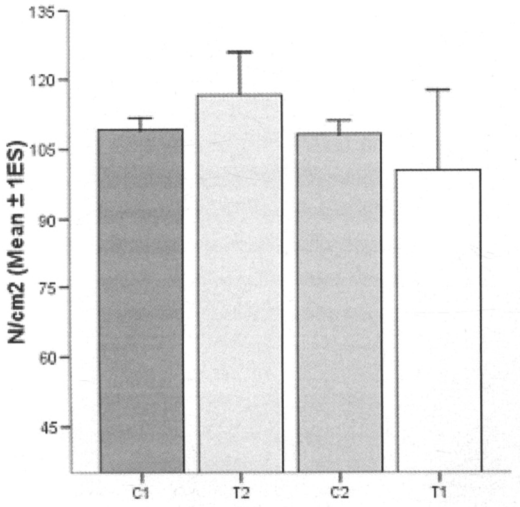


Fig. 3. *Mean ± 1ES of values (Torque N/cm) obtained from the biomechanic tests on the 4 groups studied (C1 – physiological, C2 –connective gel, T1 – physiological solution with neridronates,T2 – connective gel with neridronate).*

dispersion graph and the statistical averages (Fig. 4 A and B), the differences between the test groups and the control groups are minimum. The highest B.I.C. value, approx. 69% (SD 10.86), was registered in group control 2, i.e. that relative to connective gel alone, followed by control group 1, with approx. 63% (SD 15.38). The two test groups presented lower percentages with 58% (SD 11.86) for the group with neridronate in physiological solution and 61% (SD 9.44) for the group with neridronate in connective gel. In group T1, the mean percentage of B.V.\T.V. was 37.1% (SD 7.9), in group T2 it was 36.8% (SD 6.6), in C1 it was 34.8% (SD 4.5) and in C2 it was 35.1% (SD 7.3).

These data are also confirmed by the representative histological images (Fig. 5), where it can be seen that the bone implant contact as well as the peri-implant bone density are similar.

The pattern of the new bone was of the composite type, made of a core of interlaced bone surrounded by lamellar bone arranged in an osteonic pattern. Some secondary osteons were also visible in this context, showing a history of a remodeling process. Osteoid bands and osteoclasts were found on the bone surfaces surrounding the implant. These cells show that the remodeling processes were still present both at the interface and in the surrounding peri-implant bone. Some debris of drilled bone embedded in the newly formed bone matrix were still visible. The osteonic arrangement around the implant was different compared to the normal arrangement of the cortical bone at a distance from it, even though the walls of the drilled hole were no longer visible.

The cortical layer around the implant was restored.

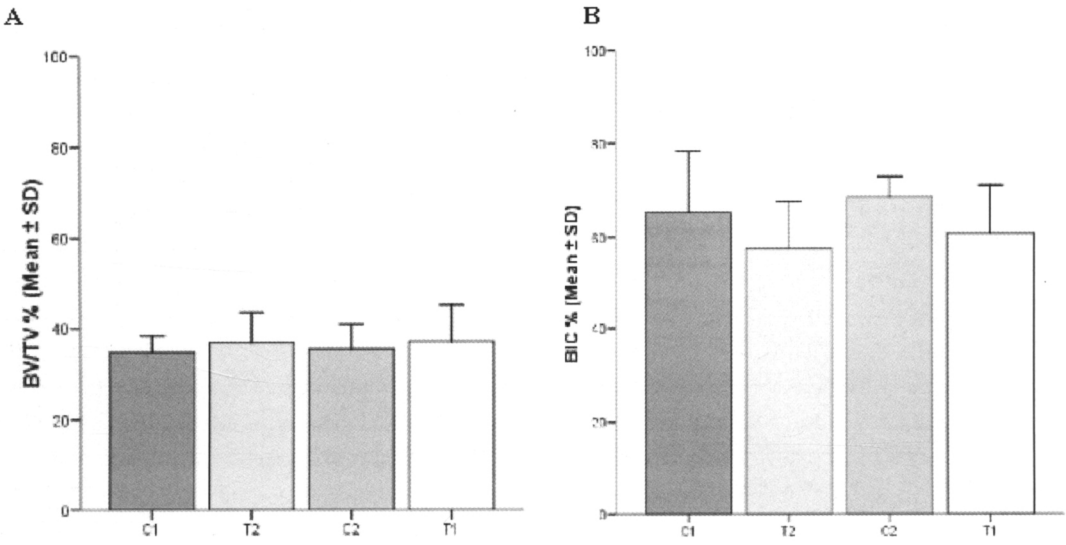


Fig. 4. ***A*** *and* ***B)*** *Mean ± SD of the B.I.C. values and BV/TV expressed in percentage for the 4 groups studied (C1 – physiological solution, C2 – connective gel, T1 – physiological solution with neridronate,T2 – connective gel with neridronate).*

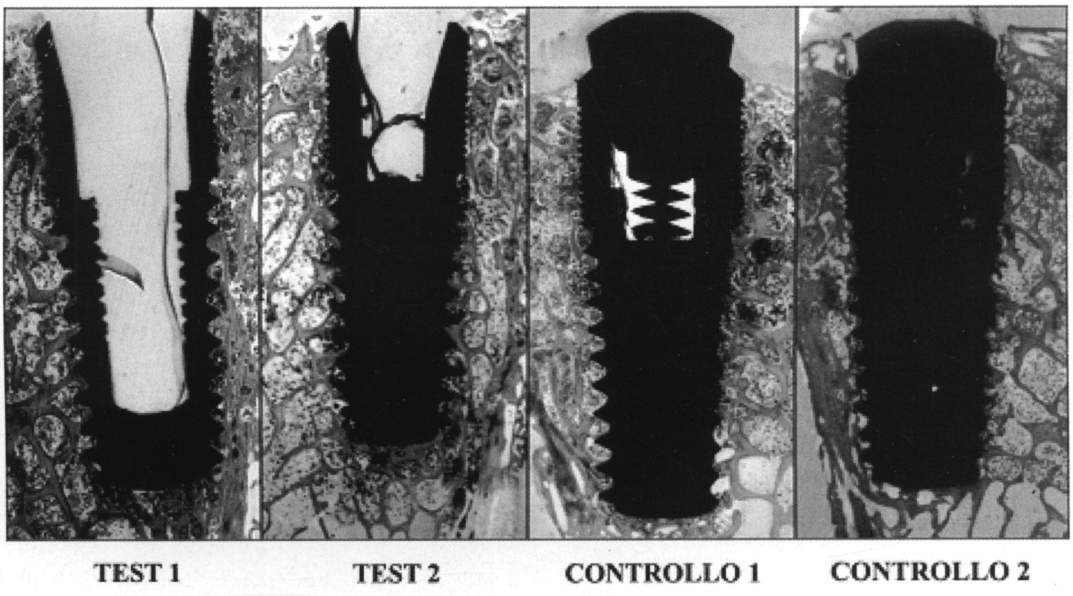


Fig. 5. *Histologic images.*

All the implants were in direct contact with the layer of peri-implant bone. The newly-formed bone, which penetrated deeply into the threads of the implant, also resulted to be in contact with the deepest part of the thread. The bone contact on the implant surface was not continuous, but interrupted by a small and thin medullary space containing fibroblasts, capillaries, osteoblasts and macrophages. There were no evident signs of osteoconductive activity on the implant surfaces. The pattern of the newly formed bone was of the composite type as it was composed of a centre of bone of interlaced fibres surrounded by

lamellar bone, organized in an osteonic model. Some secondary osteons were visible in this context, showing a re-modeling activity. Layers of osteoid matrix and osteoclasts were visible on the peri-implant bone. These cells show that the processes of remodeling were present on an interface level as well as in the surrounding peri-implant bone. Some fragments of "drilled" bone were also visible in the matrix of the newly-formed bone. The osteonic disposition around the implant was different to the characteristic model of the cortical bone away from the implant, even though the bone walls that were affected by the surgical operation were not distinguishable. Signs of an inflammatory reaction are visible around the implants.

DISCUSSION

The p-value obtained from the ANOVA results applied to the BV/TV data, with and without neridronte, physiological solution and connective gel were equal to 0.82 showing no significant difference from the four preceding groups. This demonstrates the homogeneity of the bones examined in the various parts of the experimentation. The p value (0.72) given for ANOVA applied to the data reported by the Torque in the four subgroups (with and without neridronte, physiological solution and connective gel) showed how the values of the biomechanical test was more or less similar in the four groups, while the p-value associated to the ANOVA applied to the results in terms of BIC relative to the four subgroups examined was equal to 0.080, denoting a trend towards a statistical significance.

The results show that the administration of modified Neridronate, used in a topical intra-operational way according to the technique used in this study, does not determine the increase in percentage of bone implant contact.

The data obtained by the experiments carried out lead to consider that the method of applying the drug is the one variable of the three that can most positively influence the process of osseointegration. From the above, it is therefore clear that the method of fixing the drug on the implant surface is the variable that can best guarantee the medium-long term stability of the drug in the implant site, a stability that is not obtained with the topical application used here, because the drug may be immediately washed away by biological fluids. This aspect is also analysed in a study by Tanzer (24) from which it emerges that a drug not fixed to an HA surface would be washed away from the surgical site after only an hour from its application. On the other hand, the release of a drug lasts for more than 15 weeks when the bisphosphonate is fixed to the hydroxyapatite lining. The poor results obtained in this study have led to planning a further experiment in which the same method of application will not be used, instead, the efficacy of clodronate, which is a drug not used as the aminobisphosphonate in this field of application because it is less effective, will be tested again, not using the same method but fixing it to the implant surface according to the techniques that are widely reported in the literature (22-30).

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