Bone densitometry in the evaluation of the results obtained with the use of bovine BMP in spine arthrodesis in rabbits

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SUMMARY

The object of this study is to evaluate the use of the bone densitometry as an evaluation method of the bone increment observed when we used bovine BMP in intertransverse arthrodesis of the rabbits' lumbar spine. Since the discovery of the BMP bone inductive properties, countless experimental models have been proposed. This caused the need of setting up evaluation methods to make possible a better understanding of the found results. Twenty female rabbits from New Zealand, divided in two groups, were submitted to the intertransverse arthrodesis of the lumbar column, segment L5/L6, posterior side. In the first group it was used autologous graft and in the second an association of autologous graft with biocompound (bovine BMP, 1.0 mg and hydroxyapatite, 9.0 mg). The animals were maintained in captivity, isolated and after 15 weeks submitted to the execution of bone densitometry by computerized tomography. 268 bone density measures of the normal bone, 134 measures of the newly formed bone by the association of the isolated autologous graft and 134 measures of the newly formed bone by the association autologous graft and BMP were obtained, what demonstrated a significant bone increment after statistical analysis ($p=0.034$) of the BMP/HAP group, when compared to the control group. We found this same variation of bone density analyzing the normal bone. The bone densitometry accomplished by the computerized tomography is an alternative method to assess the results when the BMP is used in experimental studies. Further studies should be accomplished for better understanding of the bone density variation found when the measures of the normal bone in the two groups are compared.

Keywords: Bone morphogenetic protein (BMP); Bone transplantation; Durapatite; Bone density; Tomography.

INTRODUCTION

Since the first reports of bone grafts, several materials have appeared as an alternative for bone grafts. Therefore, specific studies have been required to demonstrate the results obtained with the use of nonautologous grafts in the spine.

Several combinations of bone inducers and different carriers have been described in literature. In addition, the choice of an experimental model can vary according to the study objectives.

Some methods of evaluation, such as manual palpation, plain radiographs, computerized tomography, histological studies, and biomechanical assays, are often used with useful results and elucidate the alternatives for the use of BMPs in clinical practice.

MATERIAL AND METHODS

Twenty female white rabbits weighing 2.0-2.5 kg, aged approximately one year, were isolated and confined. They were fed *ad libitum* and received 100 g of pellets for rabbits and water. They were clinically and neurologically evaluated daily.

Two groups of ten animals were formed, the first of them was used as control. Animals from the control group were submitted to surgery under dissociative anesthesia, carried out with ketamine at the dose of 2.5 mg and xylazine at the dose of 1 mg, diluted into 0.1 ml of distilled water for each 100 g of animal's body weight and given by deep intramuscular injection. A variable latency time was seen, ranging from 30 to 40 minutes. After pain reflex disappeared, epilation of the lumbar region was carried out (approximately 15 cm^2) over the L5-L6 segment.

Animals were then carried to the operating room and skin was cleaned with the degerming agents povidone and chlorhexidine. A posterior incision was made in the midline and spinous processes and laminae were dissected so as to show L5 and L6 transverse processes. The latter were then decorticated and prepared for grafting.

Study carried out at the Federal University of São Paulo UNIFESP

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Homologous grafts were previously removed from the donator rabbit under general anesthesia. Animals were sacrificed, and grafts were aseptically removed consisting of long bones (femora and tibiae). Grafts were then fragmented and transferred to receptor sites (3 ml of graft for each side of the previously prepared transverse processes.

Nylon thread 3.0 was used to close the wound by planes, musculature and skin.

Animals from the second group were submitted to the same procedure described above. However, homologous grafts were combined with a BMP-hydroxyapatite mixture. The composition of the latter was the following: 0.25 cc of biocompound, consisting of 9.0 mg of pure hydroxyapatite and 1.0 mg of BMP.

All procedures were submitted to the Ethics Committee of the Federal University of São Paulo (UNIFESP - EPM) for opinion and approval.

Following procedures animals were isolated and confined. They were fed and received water *ad libitum* for 15 weeks.

During follow-up, one animal from each study group developed progressive neurological deficit of lower limbs and presented total paraplegia in the fourth week of follow-up after surgery. They also presented episodes of urinary tract infection. Both animals were excluded from the study. Other complications were not seen.

Animals were then submitted to multislice computerized tomography. The tomograph used was the model SYNERGY, manufactured by GE MEDICAL SYSTEMS, and had the following characteristics: 10cm DFOV, 100kv, 150mA, 3mm gap, and 1mm slice.

Each animal was anesthetized according to the technique previously described and submitted to tomography of lumbar spine.

After the lateral section was obtained (scalt), five tomographic sections were obtained with a 3-mm gap between each other so as to evaluate the L5-L6 segment.

For each of the tomographic sections, three measurements of bone mineral density of normal vertebra and newly formed bone were obtained according the radiological technique of Hounsfield[8] for both groups (Figures 1 and 2).

The following results were obtained: bone density values for normal bone of the species used as experimental animal in 268, bone density values of newly formed bone from homologous graft alone in 134, and bone density values of newly formed bone from combined homologous graft and BMP/HAP in 134.

RESULTS

Densitometric values were expressed in Hounsfield units. They are shown in Tables 1 and 2.

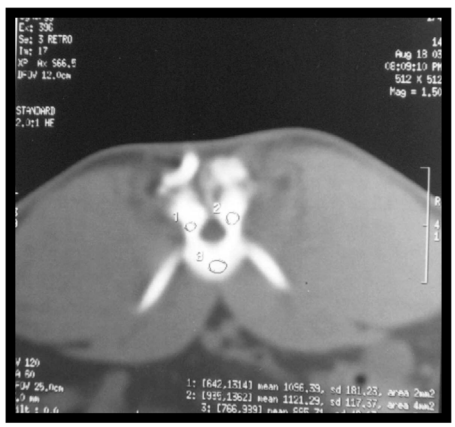

Figure 1 - *Sites where bone density measurements were taken in the normal bone.*

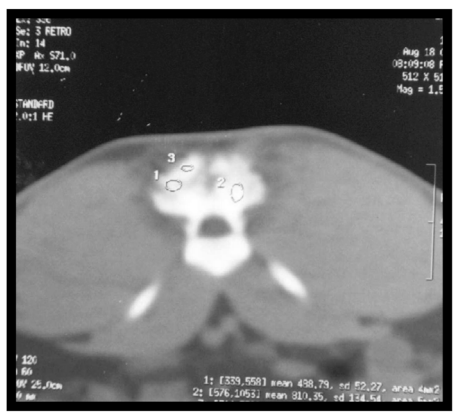

Figure 2 - *Sites where bone density measurements were taken in the graft area.*

Study results were statistically evaluated and are described below.

STATISTICAL ANALYSIS

A variance analysis model with repeated measurements with two factors (group and place) was used to compare results between the two groups. No difference was shown between the study groups when responses in the treated area and in normal bone were compared (p value for interaction factor = 0.859). In addition, a statistically higher mean was found in the group 2, as compared to the group 1 (p = 0.034), for normal bone and treated area measurements, the mean difference being 99 measurement units between the two groups.

DISCUSSION

The present study used bone densitometry to evaluate the use of BMP, at the dose of 1.0 mcg, combined with hydroxyapatite as carriers in spine arthrodesis in rabbits.

According to Boden et al.[1] and Wozney[13], there were three types of biocompounds with known bone-inducer activity: rhBMP-tipo2, rhBMP tipo7, or rhOP1, and a mixture of purified proteins extracted from bovine bone, chosen to be evaluated in the present study due to its easier availability and processing in our country.

Hydroxyapatite, used as a carrier is related to the category of ceramics, as described by Seeherman et al.[12], is able to act as a mechanical substrate for new bone growth. In addition, it remains in place for longer periods of time. According to reports by Helm et al.[6], it has pores able to retain BMPs, thus making its absoption difficult. These combined characteristics lead hydroxyapatite to have a great bone-conductor potential. For this reason, this was the product chosen to be used in the present study.

Several animal models have been described in literature. Sandhu and Khan[11] have suggested the use of rabbits as an experimental model for intertransverse arthrodesis. In addition, these models can be selected with uniformity and allow one to make evaluations that cannot be carried out in humans. However, Seeherman et al.[12] emphasize that these models are not predictive for humans. Boden[2] emphasizes that a judicious decortication of posterior elements is required for arthrodesis, thus avoiding pseudarthrosis.

Another point to be taken into account, according Boden et al.[1], is the choice of biocompound dosage to be used; according to Damien et al.[5],

		NORMAL BONE			GRAFT		
Rabbit 1	Sample 1	647.85	817.98	881.29	229.79	212.33	212.66
	Sample 2	736.03	907.67	904.96	838.82	280.00	752.15
	Sample 3	817.07	761.53	918.90	538.27	622.11	652.09
	Sample 4	624.94	729.94	367.72	390.47	516.77	434.45
	Sample 5	985.50	706.00	829.20	462.10	549.22	144.95
Rabbit 2	Sample 1	877.52	838.50	--------	538.43	493.14	227.04
	Sample 2	656.34	765.31	875.32	458.22	267.85	546.16
	Sample 3	871.49	836.95	793.95	413.63	581.31	324.06
	Sample 4	761.31	673.00	1126.03	416.35	420.32	436.94
	Sample 5	781.27	790.52	982.96	646.70	696.07	743.59
Rabbit 3	Sample 1	993.57	602.97	683.26	443.20	360.14	509.27
	Sample 2	917.33	989.89	988.59	445.20	803.69	313.80
	Sample 3	1223.50	1609.85	933.17	930.58	967.30	661.06
	Sample 4	1164.54	976.63	956.76	877.02	1341.36	1448.81
	Sample 5	806.47	1003.57	935.78	233.84	752.65	474.78
Rabbit 4	Sample 1	703.02	907.23	1140.57	488.79	810.35	559.86
	Sample 2	1096.39	1121.29	885.71	1025.17	453.03	593.04
	Sample 3	1168.78	1144.00	1013.58	1235.43	856.71	735.71
	Sample 4	1080.79	1092.10	1226.10	623.13	1142.37	1068.12
	Sample 5	979.07	991.63	693.14	923.41	801.19	1387.71
Rabbit 5	Sample 1	841.62	1010.95	1075.13	624.21	595.19	877.33
	Sample 2	1064.16	999.44	1347.50	1010.67	785.76	1631.33
	Sample 3	866.89	992.87	1333.15	384.07	446.31	180.43
	Sample 4	334.98	745.21	824.39	933.80	1060.91	554.42
	Sample 5	879.74	836.08	1204.25	236.57	158.17	208.93
Rabbit 6	Sample 1	830.09	887.69	693.32	692.73	313.29	335.59
	Sample 2	973.41	1211.37	995.82	498.62	293.08	513.00
	Sample 3	593.75	840.22	880.83	764.14	939.33	706.91
	Sample 4	1035.43	1119.18	755.55	673.18	638.89	360.79
	Sample 5	815.26	897.67	1270.43	600.00	441.48	480.80
Rabbit 7	Sample 1	712.04	710.89	733.11	505.00	929.53	398.90
	Sample 2	977.62	1075.58	1026.36	916.86	652.75	1182.55
	Sample 3	1064.06	1148.11	897.23	1005.82	1395.25	1382.78
	Sample 4	773.06	558.91	559.89	876.76	961.00	757.40
	Sample 5	714.56	858.68	723.29	362.67	569.92	356.50
Rabbit 8	Sample 1	737.00	712.12	645.29	440.61	388.86	368.05
	Sample 2	710.80	1010.81	731.66	491.22	704.71	585.14
	Sample 3	802.30	1218.31	909.50	405.31	562.38	471.00
	Sample 4	864.59	813.17	965.89	994.26	951.33	368.24
	Sample 5	925.25	894.64	831.55	96.50	179.67	410.23
Rabbit 9	Sample 1	887.16	800.14	744.62	885.57	349.30	251.07
	Sample 2	940.61	695.40	739.09	906.50	517.05	329.06
	Sample 3	779.56	1110.70	732.23	549.80	675.46	999.19
	Sample 4	795.97	1022.13	1074.93	896.83	764.51	438.58
	Sample 5	1016.01	520.59	520.01	562.90	359.11	270.14
Rabbit 10		Withdrawn from the study		Paraplegia and repeated UTI			

Table 1 *- Densitometric values in HU – Group I*

		NORMAL BONE			GRAFT		
Rabbit 1	Sample 1	765.50	1312.14	713.70	409.73	425.55	320.22
	Sample 2	843.45	759.56	932.35	807.56	835.14	573.85
	Sample 3	718.96	938.20	621.37	394.71	512.62	1104.24
	Sample 4	927.64	1461.67	1158.64	919.71	671.40	553.40
	Sample 5	775.51	462.80	543.62	391.86	331.03	692.73
Rabbit 2	Sample 1	1035.59	999.00	758.23	301.76	707.93	916.05
	Sample 2	1156.57	1144.00	996.88	646.86	1039.13	695.67
	Sample 3	988.67	1205.44	821.07	840.65	876.38	484.00
	Sample 4	706.09	1078.96	725.30	587.86	534.25	464.65
	Sample 5	979.95	1511.40	1098.56	669.57	469.92	605.38
Rabbit 3	Sample 1	932.33	933.77	857.34	805.52	364.10	348.04
	Sample 2	1045.67	1207.06	1127.27	697.00	285.00	469.56
	Sample 3	1009.31	1128.09	985.50	1057.75	462.97	509.76
	Sample 4	1118.73	1172.12	809.10	1076.62	397.78	660.35
	Sample 5	1068.56	1262.39	892.37	579.66	478.71	524.74
Rabbit 4	Sample 1	1068.15	1123.30	866.83	819.53	349.89	167.60
	Sample 2	1472.70	1145.44	870.38	754.93	1109.41	824.36
	Sample 3	1057.92	739.78	913.00	1081.00	827.29	802.65
	Sample 4	1024.57	645.00	580.69	369.06	777.97	555.37
	Sample 5	1252.74	1137.00	1011.77	351.40	177.32	621.90
Rabbit 5	Sample 1	1082.10	836.66	900.44	326.02	484.18	694.00
	Sample 2	1208.13	1317.65	1216.96	622.14	406.50	591.25
	Sample 3	670.58	1091.00	737.16	1548.07	630.91	1055.00
	Sample 4	1116.22	1175.06	738.38	984.57	870.18	1040.92
	Sample 5	346.80	199.00	748.39	214.65	457.40	212.33
Rabbit 6	Sample 1	922.25	903.65	859.37	1214.29	679.67	549.37
	Sample 2	1338.96	676.31	896.57	1120.13	776.17	756.83
	Sample 3	1363.12	1071.12	743.56	950.29	1415.82	683.77
	Sample 4	863.60	698.14	915.95	490.35	278.61	680.39
	Sample 5	1120.44	1084.32	955.32	334.08	349.39	907.82
Rabbit 7	Sample 1	1197.43	1129.48	918.43	1252.07	820.27	984.68
	Sample 2	1087.33	1045.41	451.35	1203.61	1234.76	1239.82
	Sample 3	1241.67	958.12	1204.44	557.63	670.38	1069.63
	Sample 4	1574.53	1122.45	1627.40	397.62	1100.50	970.30
	Sample 5	1043.39	1157.31	1241.70	208.90	735.85	938.62
Rabbit 8		Withdrawn from the study		Paraplegia and repeated UTI			
Rabbit 9	Sample 1	1707.89	849.33	598.18	1109.29	868.73	957.00
	Sample 2	702.93	764.43	605.87	936.10	880.89	974.35
	Sample 3	1033.54	849.86	1044.79	1078.12	1152.58	1168.38
	Sample 4	735.71	1000.12	356.12	778.82	1032.45	635.78
	Sample 5	632.64	473.83	675.95	1204.65	1031.54	819.02
Rabbit 10	Sample 1	348.94	479.55	792.77	889.11	807.93	1082.25
	Sample 2	1453.44	890.00	1306.96	636.35	1003.57	838.38
	Sample 3	1059.82	1436.50	1236.58	1394.38	1326.28	447.28
	Sample 4	114.75	1185.89	1001.28	889.71	752.76	491.45
	Sample 5	965.06	783.46	643.13	525.12	481.71	346.95

Table 2 - *Densitometric values in HU – Group II*

minimal doses of 0.15 mg of bovine BMP should be used for experimental models in rabbits.

In the present study, a dose of 1.0 mg of BMP was chosen because there is a threshold to be taken into account: proteins do not show any activity below the threshold while the increment in newly formed bone is directly proportional to the amount of implanted BMP when doses exceeding the threshold are used according to Boden et al.[1].

Computerized tomography was used, according to the technique described by Housfield[8], to assess bone densitometry so as to evaluate the results obtained in the present study because this method is not widely used and there are no precedents in literature establishing it as a technique of choice.

The most frequent ways to evaluate results suggested by Seeherman et al.[12] include the radiological study in two positions, histological preparations, computerized tomography, and biomechanical assays.

Some techniques are not routinely used, such as magnetic nuclear resonance, densitometry and even that carried out as described by Magim and Delling[10] who utilized bone scintigraphy with technetium so as to show the bone-inducer activity of type-7 BMP in pigs.

With the use of bone densitometry in the evaluation of bovine BMP, at the dose of 1.0 mg, combined with hydroxyapatite as a carrier in an experimental model in rabbits, a significant difference was shown in the bone increment in the treated group, as compared to the control group.

As for normal bone analysis, increased bone density was shown in the BMP-treated group, as compared to the control group.

Densitometry was also used by Cochran et al.[4] who reported findings consistent with those found in the present study, thus evidencing an increased bone mass in dogs used as an experimental model. They used metallic implants of titanium and BMP at the dose of 0.2 mg/ml. However, spine arthrodesis was not carried out in the mentioned study; implants were inserted into the mandible. Therefore, a direct comparison of results was not possible.

In a study carried out by Hong et al.[7], densitometry was used

in another experimental model to evaluate the use of type-2 BMP in the repair of bone defects previously induced in the skull in rabbits. Two different doses were used (0.5 and 1.0 mg of BMP). No significant differences, however, were found between the densitometric values obtained following the use of these doses.

Using a distinct experimental model in rabbits, Laffargue et al.[9] reported no significant differences in any of the study groups, as shown by *in-vivo* densitometry, when type-2 rh BMP at the doses of 10 mcg and 40 mcg combined with beta-tricalcium phosphate as a carrier was used to repair femoral bone defects. Both the characteristics of the mentioned study and its results differed from those of the present study.

The technique of densitometric evaluation described by Hounsfield[8] was used in a clinical study by Burkus et al.[3] who showed a significantly increased bone density in patients submitted to intersomatic arthrodesis where type-2 rhBMP, at doses ranging from 4.2 to 8.4 mg, combined with titanium cage and a collagen carrier was used, as compared to those submitted to autologous graft of iliac bone. They observed an increase of 213.9 Hounsfield units in the study group, as compared to an increase of 64.3 Hounsfield units in the control group, 24 months following surgery.

The use of densitometry to evaluate BMPs still causes some doubts.

Further studies will possibly elucidate some issues because densitometry cannot be able to quantify bone mass under certain circumstances and despite the fact that bone mass is increased. This is occurs because several variables should be taken into account, including the dose to be used, the animal species to be studied, type of protein used in the study, segment time, site of use of the biocompound, type of carrier, as well as standardization of densitometric measurements. These variables as a whole lead to the fact that several studies are carried out differently. Therefore, only few outcome parameters can be directly compared.

Studies similar to the present one, carried out using the same variables, are not available in literature, making the comparison of results difficult.

CONCLUSIONS

1. Bone densitometry through computerized axial tomography allowed us to show that bone mass was increased in the present study using an experimental model of rabbits submitted to lumbar spine (L5-L6) arthrodesis and implant of the biocompound BMP-HAP combined with homologous graft, as compared to autograft alone.

2. The increased bone mass shown in the study of normal bone can be related to other mechanisms of action of BMPs. Therefore, further studies are required to elucidate this issue.

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