ASSESSMENT OF NON-INVASIVE METHODS OF MEASURING BONE REPAIR IN NAVAL CASUALTY VICTIMS

MAJOR OBJECTIVES

The major objective of this report was to compare non-invasive quantitative methods of assessing bone and bone graft incorporation and strength with established invasive methods for assessment. Our ultimate goal was to develop a clinically applicable method for evaluating the quality of bone by valid and reliable, non-invasive methods which would correlate with the structural properties of those bones in vivo.

At the time of our original proposal, it was felt that the segmental cortical bone graft, both fresh and freeze-dried, would seem to be a suitable experimental model for the studies. The U.S. Navy Tissue Bank had a long history of clinically successful, freeze-dried bone allografts, but with the change in clinical needs, there was a greater demand for major segmental cortical bone grafts to replace large segmental losses resulting from trauma or massive resection for malignancy. Autologous cortical grafts are known to be slow to incorporate. It was, therefore, expected that freeze-dried allografts might be even slower. We felt that experimental research was needed in order to provide methods for evaluating these alloimplants and thereby give the clinician better parameters for patient mobilization subsequent to receipt of such grafts. As a second phase of this study, we also proposed to utilize electric current in an effort to accelerate the process of bone graft incorporation. Previous studies with such systems had suggested that bone was resorbed when the anode was attached, while bone was formed when the cathode was placed in the bone. Since creeping substitutions of a cortical grafts require porosity, it was felt that we could utilize both phases of the electrical cycle. To accelerate resorption we would use the anode pulse, and then, by switching polarity we could potentiate bone formation with the cathode current. This experimental group was proposed as a pilot project subsequent to our establishing non-invasive techniques for the quantitative evaluation of in vivo incorporation of the cortical bone. Since complications and infection rates with re-exposure of cortical allografts in the clinical setting approach 50%, and it seemed most appropriate that our research make an effort toward developing the methodology of non-invasive, quantitative assessment of healing or incorporating bone. Such a method would assist the clinician in post-operative rehabilitation phase of caring for patients who had received such major cortical bone grafts.

RESULTS OF STUDIES

In order to obtain a correlative analysis of segmental fibular autografts and freeze-dried allografts, four dogs underwent bilateral hind limb bone grafting. The purpose of this pilot project was to establish the methodologies of Tc bone scanning and skeletal trans-imaging using the pin-hole collimator on a high resolution gamma camera. In addition, the pilot project was necessary to train new technicians who had been hired after the loss of trained technicians in 1976. Finally, the project was undertaken to help define any problems with regards to radiation contamination, housing of animals after injection of radioisotopes, and other general animal logistics. All four dogs received bilateral fibular cortical transplants with a fresh autograft on the left and a fresh allograft on the right. Unfortunately one of these dogs became infected within a week post transplantation and had to be sacrificed. The
remaining three dogs were maintained for one year to give us a clue concerning the long term effects of such studies.

Biweekly x-rays were performed to define the course of repair for the segmental transplants (Type I, II, III). The three autografts were incorporated normally and were characterized as a Type I repair. In contrast, all three fresh allografts were indolently rejected, developing delayed unions at the graft-host junctions in some cases, and fully established nonunions in others.

At the time of sacrifice the segmental grafts were mechanically tested. The autograft strengths varied between 2.1 and 8.2 kg/cm which was not unusual when compared with previous animals undergoing the same protocol. In contrast, the mechanical strengths of the fresh allografts was definitely inferior to that of the autografts in two of the three samples. One allograft was entirely normal and equivalent to its autogenous control except that its histologic rating was a Type II repair rather than a Type I.

In the above grafts, microscopic analyses using microradiographic and tetracycline labeling techniques demonstrated that resorption of the allografts was far more severe than that found in the autografts. Although the amount of new bone formed in both autogenous and allogenic grafts was roughly equivalent, the new bone formed in the allograft was not enough to offset the resorptive process which left us with a significantly greater porosity in the segmental allografts. This increased porosity was related to the significantly decreased mechanical strength found in the segmental allografts. Animal BG-752-R was previously noted. These results as reported are consistent with data that have been previously published from this laboratory concerning the results of fresh allografts. The data from these animals are recorded in Table I. Comprehension of these data is enhanced by referral to the enclosed reprints which explain the methods of analysis and their interpretation.
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<th>DAY</th>
<th>L</th>
<th>R</th>
<th>TYPE</th>
<th>LOAD TO FAILURE</th>
<th>% POROSITY</th>
<th>% NEW BONE</th>
<th>GRAFT FIELD AND CALLUS PLUS GRAFT AREA (mm²)</th>
<th>% OF FIELD EQ. TO GRAFT</th>
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**TABLE I**
RESULTS OF 99-M TECHNETIUM BONE SCANS

With the use of the gamma camera, a number of Tc scans were acquired on animals who had received bilateral fibular bone grafts, and these scans were accomplished at different times subsequent to bone grafting. The scans enabled us to identify areas of active new bone formation, and likewise the lack of Tc concentration identified areas that showed little or no tendency for bone formation. The active sites were seen at the graft-host junctional zones early and in areas of stress fracture later, whereas the lack of concentration of the isotope was seen in areas of impending non-union or delayed incorporation. Because the computer was neither available or hooked up to the gamma camera, we were not able to perform quantitative analyses of the amount of new bone formation that was taking place within the bone grafts. We were able to get a qualitative assessment of the areas of activity, and by comparing sequential scans over time, we obtained an estimate of the rate of new bone formation occurring within the bone graft. Several dozen scans were accomplished in order to work out the technique, to identify the appropriate parameters for acquiring the best quality scan, and to train new personnel that would be carrying out these various studies.

RESULTS OF IODINE 125 TRANS-IMAGING FOR BONE MINERAL CONTENT

Preliminary studies were undertaken to demonstrate the capacity of performing trans-imaging analyses on fibular bone grafts in dogs. Unfortunately, without the hook-up between the gamma camera and the computer, we had no way doing the quantitative assessments that were proposed in the original contract. It then became apparent that there might be some other methods of determining bone mineral content and thereby quantitate the amount of bone resorption and bone formation that was occurring. It was essential that other methods be non-invasive also. It was about that time that we became aware of the photodensitometric system by Colbert and associates. After considerable correspondence and exchange of sample x-rays for analysis, a site visit was conducted of their laboratory at Wright State University in Yellow Springs, Ohio. Subsequently, we became convinced that their system was comparable to trans-imaging in sensitivity. In addition, it was far less cumbersome, more readily available clinically, and therefore commanded consideration as the method of choice for determining bone mineral content. With the placement of an aluminum wedge on the x-ray cassette at the time of standard x-ray exposure, a roentgenogram was acquired which could be placed on the drum scanner at the Radiological Research Laboratories in Ohio for analysis. The data print out from the computer was a transverse linear basis very similar to that we had acquired with trans-imaging.

A number of technical problems were encountered as we attempted to modify their software programs to analyse the canine fibula and simultaneously improve our radiological exposure system in order to insure the most accurate assessment of bone mineral content. These studies were conducted in collaboration with Dr. Colbert and Associates who we paid on a consulting basis instead of utilizing those funds for our own radioisotopic analyses.

Recent studies by Currey and Associates further documented that a direct correlation between bone mineral content and structural properties of bone exists. While in a holding pattern for conducting further canine studies with fibular bone grafts, we did conduct some preliminary studies with the Colbert system to correlate the bone mineral content of human cortical bone with the ultimate torque at failure in human metatarsals. With these samples of human bone, we were able to show a correlation coefficient of .96 and .97 between the methodologies of skeletal trans-imaging, Colbert’s photodensitometry, and CT scanning. When these same metatarsals were then
subjected to torsional loading to failure, the ultimate torsional stress correlated quite closely with the total bone mineral content. An expanded study along these lines, we believe, will enable us to achieve our ultimate goal of this contract. A change of methodology will be necessary in order to complete the goals of his contract.

RESEARCH FINDING FROM OTHER STUDIES IN OUR LABORATORY

With our own departmental funds we conducted experiments to evaluate segmental freeze-dried fibular bone grafts in dogs. The work was not supported by the Navy contract because we could not perform the non-invasive aspects of that contract. The results of these studies, however, have now been published and show that canine freeze-dried segmental fibular bone grafts did poorly compared to autogenous controls. These results have caused us to seriously question the validity of proceeding with the experimental groups as originally delineated in this contract.

Other studies with immunosuppression in conjunction with fresh fibular allografts suggest that this method of bone grafting is far superior to the results realized by the use of freeze-dried bone allografts. On the clinical level, of course, the evolution of vascularized fibular bone grafts has occurred which further outdates the proposals of this contract as far as the clinical relevance of freeze-dried bone is concerned. There is still the pressing question as to how to non-invasively evaluate the structural properties as well as the quality of a bone graft and its incorporation. Apropos to this specific need, one of the principal investigators (RWB) was recently invited to serve as a keynote speaker for the workshop on Biological Resurfacing of Human Joints which was sponsored by the Veterans Administration in Dallas, Texas. Our research into developing these methodologies was the topic presented to this meeting.

At the time of our initial proposal in 1975, we proposed the use of indwelling electrodes hooked up to a constant current battery pack to provide electric stimulation to the bone graft as described by Brighten and associates. By the time our revised proposal was submitted in 1977, we had conducted several preliminary studies in animals to test the capability of maintaining adult dogs with indwelling electrodes. This particular system turned out to have many technical problems in the canine model and therefore was replaced in our revised proposal with the electromagnetic field concept of Bassett and associates. Likewise, preliminary studies had been accomplished to several dogs using this non-invasive technology, and the system was found to be applicable in the canine model. Based on the encouragement from these preliminary studies, a grant proposal was prepared by other investigators in our laboratory to study the effects of the electromagnetic field enhancement of bone grafting incorporation. This proposal was funded by the Easter Seal Fund and 8 adult dogs received bilateral fibular grafts. One leg in each animal had electromagnetic coils attached over the graft site providing a constant current for 6 weeks while the opposite limb served as control. Data available from this study shows no statistical enhancement of bone healing at the junctional sites as assessed by torsional testing. In addition, several patients have had similar clinical problems and have undergone...
electromagnetic coil application over their bone grafts in an effort to promote healing. Unfortunately, none of these cases have come to a satisfactory union nor has there been any evidence of enhancement of bone graft incorporation. Thus, the proposed pilot project which was part of our Navy contract of 1977 does not seem to warrant further studies at this time by this technique or in this particular animal model.

A SUMMATION STATEMENT

In 1975 we proposed to evaluate freeze-dried fibular bone grafts in a canine model which had been well proven as an ideal system for assessing segmental cortical bone grafts. In addition, our original contract also called for the development of non-invasive technology to assess the quantitative incorporation of bone grafts and correlation of these findings with the structural properties of the tissue. Five years later, the proposed methodologies of this contract no longer seemed appropriate since other studies in our laboratory have shown that freeze-dried segmental fibular bone grafts in dogs do not incorporate well. In addition, other studies in our laboratory have shown that electromagnetic stimulation to a fibular bone graft does not enhance its incorporation.

Since substantial funds still remain in this contract, we are asking you to consider a one year, no cost extension. This will permit us to revise the methodologies and pursue our original goal of developing a simple non-invasive method for determining the qualitative and quantitative structural properties of bone in the in-vivo setting. The clinical implications of such technology would be far reaching and could include the clinical evaluation of the strength of healing fractures, as well as be capable of determining the strength of the patient's bone with metabolic bone disease (i.e.: osteoporosis) or advanced neoplastic disease.