INTRODUCTION

In current orthopedic practice, reconstruction of large skeletal defects is a challenging problem. All currently available methods have limitations. Transplantation of living xen- or allogeneic bone could potentially combine the healing and remodeling advantages of vascularized bone autografts. However, use of long-term immunosuppression may cause significant risk of opportunistic infections, malignancy and organ toxicity.

Angiogenesis is a biologic process of new capillary formation. Surgical transfer of vessels or well-vascularized tissue into avascular tissue induces neoangiogenesis and improved regional blood flow [1]. Implantation of vessels into allogeneic or xenogeneic bones induces neovascularization and subsequent new bone formation. We have recently demonstrated the repopulation of vascularized bone grafts with host-derived cells as a function of time [2]. In this study, a novel splenectomy protocol was employed to maintain viability of vascularized xenografts using a combination of surgical angiogenesis and short-term immunosuppression. Specifically, the effect of host-derived arteriovenous bundle (AV bundle) implantation on bone blood flow and bone viability in both immunosuppressed and non-immunosuppressed vascularized bone xenografts was evaluated.

MATERIALS AND METHODS

Outbred male Golden Syrian hamsters (HSD) and inbred male Lewis rats (RT11) were used. 141 heterotrophic vascularized femoral bone xenografts were performed with microvascular anastomosis. The contralateral saphenous AV bundle was placed through the medullary canal of the xenograft at the same time. Recipient rats were divided into four major groups; groups I and II, non-immunosuppressed and group III and IV, immunosuppressed. FK506 (im. 2mg/kg for 7 days) and then 1mg/day until sacrifice) and Cyclophosphamide (ip. 8mg/kg/day for 7 days) was used for immunosuppression. The implanted saphenous AV bundles were ligated in group II and group IV(control), and patent in groups I and III.

At 1 or 2 weeks after transplantation, we evaluated vessel patency, bone blood flow, capillary density, histologic grade of immune rejection in vascular pedicle and the bone viability. Bone blood flow was measured using microsphere technique and capillary density (area of vasculature/total area of bone segment) of each specimen was estimated on microangiographic images with NIH Image 1.62f. Histologic grading of vascular rejection and bone viability in H-E stained vascular and bone specimens were performed by three independent examiners using our criteria. The Institutional Animal Care and Use Committee approved the protocol and provided appropriate oversight.

Statistical Analysis: 112 rats were used in the analysis. For bone blood flow, capillary density, and histologic grade of bone necrosis, the rats were categorized into four groups based on intended patency of the pedicle and AV bundle. For histologic grade of vascular rejection, rats were categorized into three groups: (1) non-immunosuppressed; (2) immunosuppressed and patent pedicle; (3) immunosuppressed and occluded pedicle. Overall comparisons for each of the four measures of interest were made using a Kruskal Wallis test. Pairwise comparisons between categories of rats based on patency were made using a non-parametric Wilcoxon rank sum test with Bonferroni adjustment. The subset of 66 rats with intended patency of the pedicle and AV bundle was analyzed in a similar manner.

RESULTS

The overall patency rate of vascular pedicles was 75.4% (46 of 61) in immunosuppressed groups. No vascular pedicle remained patent in non-immunosuppressed animals. The patency rate of AV bundles, however, was lower than we anticipated. Only 48.6% (34 of 70) of these AV bundles remained patent at final evaluation. Microangiography revealed vascular patency and neoangiogenesis from implanted AV bundles (Fig. 1).

Bone blood flow: In Group I with patent AV bundle, median flow was 3.74 ml/min/100g. This value was statistically different from group II (p<0.001). A similar beneficial effect in immunosuppressed animals could not be demonstrated (P>0.63). The vascular pedicle remained open in most immunosuppressed animals (Group III and IV), resulting in blood flow significantly higher than those of group I and II (P=0.001).

Capillary density: Measured capillary density (neoangiogenesis) was highest in Group III (median: 0.32). It was significantly higher than that of group I (median: 0.11) (P<0.001), but not higher than that of group IV (median: 0.26) (P=0.32).

Histologic grading of vascular rejection: The histologic grade of vascular rejection in non-immunosuppressed groups was uniformly high (median: 3.00). In immunosuppressed animals with a thrombosed pedicle (median: 2.67), it was lower than that of non-immunosuppressed group (P<0.001), but higher than that of immunosuppressed, patent pedicle group (P=0.039).

Histologic grading of bone necrosis: Groups I and II showed a high grade of bone necrosis (median: 2.00, both). The extent of bone necrosis was less in immunosuppressed groups: (median: 1.00 in group III and 1.33 in group IV). The effect of immunosuppression on bone viability was statistically significant (P<0.001). However, there was no demonstrable benefit to bone viability derived from a patent AV bundle during the time periods studied (P=0.10).

DISCUSSION

Bone blood flow measurements demonstrate a significant benefit of a patent AV bundle in thrombosed nutrient pedicle groups but not in patent pedicle groups. This may reflect the limited revascularization in medullary space and the inner portion of endosteal bone during these short-term survival time periods. Microangiography and capillary density data demonstrate considerable capillary ingrowth from the patent AV bundles in both immunosuppressed and non-immunosuppressed groups. The low patency rate of the AV bundles might be associated with low blood flow, internal vessel compression and/or acute angulation of the AV bundle at its entry point. Inclusion of more perivascular tissue, more aggressive reaming of the medullary canal, and addition of antithrombotic treatment may result in higher AV bundle patency rates. A moderate grade of immune rejection in immunosuppressed groups suggests that our immunosuppressive treatment regimen suppressed but could not fully prevent rejection. The degree of bone necrosis seemed to reflect the microcirculatory status. The blood flow through the patent pedicle was important to maintain bone viability. In group I animals with a patent AV bundle, medullary osteocytes were alive, but most cortical bone were necrotic. A much longer time period may be necessary to revascularize the entire cortex.

SURGICAL ANGIOGENESIS IN VASCULARIZED BONE XENOGRAFT: AS A POSSIBLE WAY OF RESTORATION OF HOST-ORIGINED VASCULAR SYSTEM

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