THE EFFECT OF DEMINERALIZATION ON RETROVIRUS INFECTED CORTICAL BONE: IN VITRO AND IN VIVO STUDIES

INTRODUCTION: Viral and bacterial transmission are possible adverse outcomes of utilizing banked tissues for transplantation. Concerns that current freezing and storage practices may not be adequate to inactivate retroviruses were substantiated by recent animal model studies. These studies documented viral transmission from retrovirus-infected, transplanted allogeneic cortical and cancellous bone and connective tissue grafts to recipient animals. Demineralized bone matrix (DBM) is commonly used as an autograft extender or substitute. A specific process (D-Min®) previously was shown to inactivate viruses using a viral spiking technique. However, the effect of this process on viral inactivation of systemically infected bone was unknown. The objectives of the present study were to determine whether this demineralization process inactivated retrovirus in bones from feline leukemia virus (FeLV)-infected cats using both in vitro and in vivo test systems.

METHODS: The Michigan State University Animal Care and Use Committee approved all animal use protocols. Retrovirus-infected donor cats were generated by intravenous inoculation of infectious pooled plasma (0.2 ml) from cats infected with the Rickard strain of FeLV. Blood samples were collected prior to inoculation and weekly thereafter for 8 weeks to measure FeLV p27 antigen, antiviral antibody titers, and FeLV provirus using an ELISA test, a live-cell immunofluorescence assay, and real-time quantitative PCR, respectively. All donor cats became infected with FeLV as evidenced by positive antigen and antibody titers and FeLV provirus in blood samples. Long bones from each of 5 FeLV-infected donor cats were aseptically harvested after euthanasia, stored at –70°C, and cortical regions were harvested after euthanasia, stored at –70°C, and cortical regions were utilized as graft recipients. A 0.25” drill hole was made into the medial aspect of the proximal tibia exposing the medullary cavity under general anesthesia and using sterile surgical technique. In six animals, 0.5cc of morselized, untreated FeLV-infected MB was packed into the medullary space (positive controls). In fifteen animals, 0.5cc of DBM was placed into the medullary space. Three recipient cats received DBM from each of the 5 FeLV infected donors, while one additional cat received no graft material and was used as a sentinel animal (negative control). Blood samples were collected before surgery to confirm an FeLV-negative status. Serial weekly blood samples were collected from each cat to monitor the presence of FeLV p27 antigen (ELISA test) and antiviral antibodies against FeLV (live cell immunofluorescence assay).

RESULTS: In vitro: Neither FeLV p27 antigen nor provirus could be detected in negative control cultures or FEA cells cultured with DBM from FeLV-infected cats. In contrast, positive FeLV p27 antigen and provirus were detected in FEA cells cultured with untreated MB from FeLV-infected cats or supernatant fluid from an FeLV-infected cell line. Differences in FeLV p27 antigen (Figure 1A) and provirus (Figure 1B) between MB and DBM cultures were significantly different for all 4 passages.

**Figure 1 - Mean FeLV antigen S/P ratios (A) and mean FeLV copy numbers (B) for cultures at passage 4.**

DISCUSSION: Results of the in vitro studies demonstrated inactivation of the retrovirus following the demineralization process (D-Min®). Additional studies in our laboratory suggested that this may be due to denaturation of the DNA. The absence of viral transmission in DBM recipient animals confirmed the in vitro findings. These results suggest that the D-Min® process of bone demineralization is a highly effective technique for inactivation of retrovirus and prevention of transmission from infected bone.

REFERENCES:

**Laboratory for Comparative Orthopaedic Research, East Lansing, MI.**

**College of Veterinary Medicine, East Lansing, MI.**

**Osteotech, Inc., Eatontown, NJ.**

The amount of FeLV provirus was quantified by QPCR in DNA extracted from cells. Data were log transformed and significant differences between groups were determined using one-way ANOVA and Tukey’s post-hoc tests. Results were considered significantly different at p < 0.05.

**Figure 2 - Mean FeLV antigen S/P ratios (A) and mean antibody titers (B) from cats.**

**Table 1.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 0</th>
<th>Week 2</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td>0.2</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>DBM</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
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