Endothelial Progenitor Cell Therapy Combined with Local Antibiotics for the Treatment of Infected Nonunions in an Animal Model

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INTRODUCTION:

Infected nonunions remain a major clinical challenge, often requiring staged surgical treatment, with the initial stage consisting of surgical/medical treatment of infection followed by a second procedure to promote bone healing. A single stage treatment that could effectively eradicate infection and stimulate bone healing simultaneously would be highly desirable. Endothelial progenitor cells (EPCs) have been previously demonstrated to be highly effective for fracture healing and have been shown to possess immunogenic properties. In addition, the application of local antibiotics has been shown to be proficient at treating surgical site infection. This evidence suggests that EPCs, in combination with local antibiotics (ABX), could present an effective single stage treatment for infected nonunions. This study aimed to assess the efficacy of EPC therapy +/- local ABX in the treatment of infected critical size bone defects in an animal model. We hypothesized that EPC+ABX would be an effective single stage treatment for infected nonunions.

METHODS:

A 5-mm segmental bone defect was created and stabilized with a mini-plate in the femur of Fischer 344 rats. Subsequently, a control solution (Experiment 1: non-contaminated arm) or an inoculum of Staphylococcus epidermidis (Experiment 2: contaminated arm) was delivered at the defect site. Fourteen days later, we applied one of the following different treatments in both arms creating a total of seven groups; 1) Control (no treatment), 2) EPCs 3) EPCs + ABX (local vancomycin and rifampin) 4) ABX alone (this group utilized in the contaminated arm only). All animals were euthanized at 10 weeks after the second surgery and tissue samples were collected for microbiology. Radiographs were scored and assessed for union status, defect filling, and infection. Bone healing was also assessed with micro-CT and biomechanics. Our primary outcome in the non-contaminated arm was **radiographic union**, whereas in the contaminated arm our primary outcome was a composite of radiographic union and infection eradication (**non-infected union**).

RESULTS:

Non-contaminated: Both the EPC and EPC+ABX groups demonstrated significantly improved bone healing relative to controls with regards to union rates (EPC and EPC+ABX; 100%, Control; 0%). Similarly radiographic scores, micro-CT (bone volume (BV) and bone volume fraction (BV/TV)), and biomechanics (torque and stiffness) all demonstrated significantly improved outcomes in the EPC and EPC+ABX groups compared to the control group. There were no significant differences between the EPC and EPC+ABX groups (see Table 1 and Figure 1).

Contaminated: The results of the contaminated arm are shown in Table 2 and Figure 1. In terms of our primary outcome of non-infected union, the EPC+ABX group demonstrated the highest rate of success (46%), followed by the EPC group (33%), whereas the ABX and control groups had no non-infected unions (both 0%). A post-hoc analysis test showed that EPC+ABX treatment resulted in a significantly higher rate of non-infected union compared to ABX alone (0%) and the control group (0%) (p=0.003). There were no significant differences between any other groups in the rates of non-infected union. ABX treatment significantly improved infection outcomes and EPC treatment significantly improved bone healing outcomes. Of note, all but one of the animals in the EPC+ABX group had their infection eradicated, irrespective of whether or not the bone healed.

DISCUSSION AND CONCLUSION:

These results demonstrate two important findings. First, the ability of EPCs to heal bone defects is not negatively impacted by the addition local antibiotics. Second, the combination of EPC+ABX was most effective at achieving infection eradication and bone healing with a single stage treatment in the setting of infected nonunion, occurring nearly 50% of the time with a single treatment. In the instances where EPC+ABX did not achieve bone healing; infection was almost always eradicated. These findings strongly support continued investigation of EPCs combined with local antibiotics as a single-stage treatment for infected nonunions.

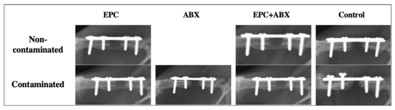

Figure 1: Representative 10-week radiographs of animals in both non-contaminated and contaminated groups treated with EPC, ABX, EPC+ABX and control.

Outcome	EPC (n=10)	EPC + ABX (n=10)	Control (n=10)	p-value
Union (%)	10 (100%)	10 (100%)	0 (0%)	<0.0001[a*]
Micro CT BV – mean ±SD	21.1 ± 2.6	22.1 ± 3.9	6.8 ± 2.4	<0.0001[b*]
Micro CT BV/TV – mean ±SD	40.7 ± 6.9	41.3 ± 8.3	12.6 ± 4.4	<0.0001[b*]
Radiographic Score – mean ±SD	7.5 ± 0.5	7.6 ± 0.6	2.5 ± 1.3	<0.0001[b*]
Maximum Torque – mean ±SD	110.0 ± 37.5	133.6 ± 61.3	0 ± 0	<0.0001[b*]
Stiffness – mean ±SD	26.5 ± 7.0	28.9 ± 5.6	0 ± 0	<0.0001[b*]

Table 1: Outcomes in non-contaminated arm.
[a]Fisher's exact test, Freeman-Halton extension
[b]one-way ANOVA test
*denotes significant difference

Outcome	EPC (n=12)	ABX (n=11)	EPC + ABX (n=13)	Control (n=12)	p-value
Uninfected Union (%)	4 (33%)	0 (0%)	6 (46%)	0 (0%)	0.003[a*]
Union (%)	6 (50%)	0 (0%)	6 (46%)	0 (0%)	<0.001[a*]
Radiographic Union Score – mean ±SD	5.0 ± 3.2	2.1 ± 1.3	4.4 ± 3.2	1.9 ± 0.8	0.005[b*]
Infection in Culture (%)	7 (58%)	0 (0%)	1 (8%)	11 (92%)	<0.001[a*]
Radiographic Infection Score – mean ±SD	1.5 ± 1.5	1.3 ± 1.0	0.8 ± 1.1	2.8 ± 1.0	0.001[b*]
Maximum Torque – mean ±SD	108.3 ± 129.3	0 ± 0	91.7 ± 121.3	0 ± 0	0.005[b*]
Stiffness – mean ±SD	16.0 ± 19.1	0 ± 0	16.4 ± 20.6	0 ± 0	0.004[b*]

Table 2: Outcomes in the contaminated arm.
[a]Fisher's exact test, Freeman-Halton extension
[b]one-way ANOVA test
*denotes significant difference