N-Acetylcysteine (NAC) Improves Free Autologous Fat Graft Survival: A Preclinical Study

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Abstract

Background: Autologous fat grafting is an increasingly popular technique for soft tissue reconstruction, but is limited by inconsistent graft take, under correction, and repeat procedures (1-3). The authors examined whether a widely available, clinically safe anti-oxidant, N-acetylcysteine (NAC), could be added to tumescent solution at time of harvest to improve fat graft survival in a mouse model.

Methods: Inguinal fat grafts were harvested from C57BL/6 mice using tumescent solution with or without NAC, and injected under recipient mouse scalps. Fat graft volumes were evaluated via microCT and Materialise's Interactive Medical Imaging Control System (MIMICS) volumetric analysis at 4 days (baseline), and 1 and 3 months post-injection. Explanted grafts were weighed and evaluated histologically for vascularity and quality. Flow cytometric analysis (FACS) and MTT assays were performed on adipose-derived stem cells (ADSCs) exposed to oxidative stress (hydrogen peroxide) with and without NAC. The effect of NAC on proliferation of a preadipocyte cell line was also analyzed using Oil Red-O staining and qualitative polymerase chain reaction (qRT-PCR).

Results: N-acetylcysteine resulted in improved fat graft retention, with 46% take in NAC animals, compared to 17% in controls ($p=0.027$) (Figure 1). Explanted grafts from the NAC group were significantly larger (46 vs. 8 milligrams, $p<0.01$) and had a 133% higher mean adipocyte density on histology ($p<0.0001$) than controls (Figure 2). No increase in vascularity was observed on histology. FACS analysis demonstrated that NAC protected ADSCs from oxidative stress in a dose-dependent manner. Combined exposure to both NAC and hydrogen peroxide led to a 200-fold increase in ADSC proliferation, significantly higher than with either agent alone. NAC significantly decreased the differentiation of pre-adipocyte cells into adipocytes compared to control ($p<0.05$).

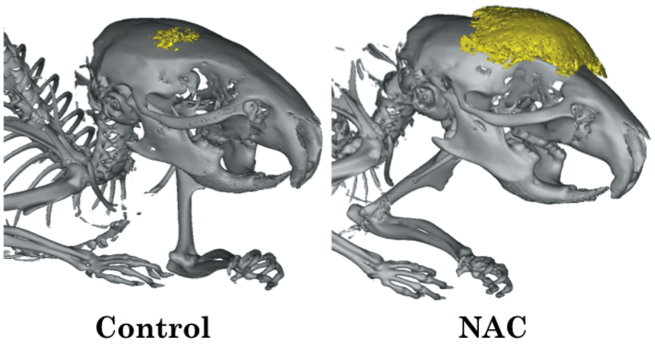

Figure 1: MIMICS three-dimensional reconstructions of control (left) and NAC (right) mice free fat grafts at 3 months post injection

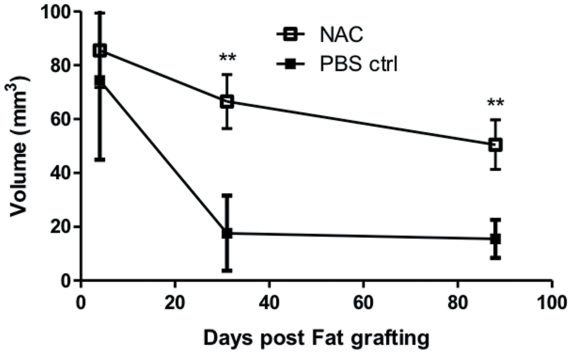

Figure 2: Free fat graft weights at 4 days, 1 month, and 3 months of control versus NAC based on three-dimensional reconstructed micro-CT scans of the grafts *in vivo*

Conclusions: Addition of NAC to tumescent fluid for harvest of fat improves graft survival and quality in a mouse model. Our in vitro findings suggest that this effect is the result of protection from oxidative stress and increased survival of ADSCs and adipocytes, while keeping the ADSCs in a pre-differentiation state to allow re-population of the graft over time. Thus, NAC is a clinically safe anti-oxidant that could be added to tumescent solution to improve fat graft survival over time, without any subsequent steps or additives *ex vivo*.

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