Sodium nitrate treatment escalates doxorubicin-induced cachexia in mice

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Introduction: Anthracycline chemotherapy instigates skeletal muscle pathology and wasting, which is independent of cancer-induced cachexia – this is best exemplified for the highly toxic agent, doxorubicin (DOX; reviewed in Sorensen *et al.*, 2006). Since anthracyclines are effective anti-cancer agents, investigating potential co-therapies to clinically address the skeletal muscle-specific side effects of chemotherapy treatment is important, particularly since lean mass is a predictor of patient morbidity and mortality. Sodium Nitrate (SN) treatment has been shown to afford protection against DOX-induced cardiomyopathy in mice (Zhu *et al.*,2011; Xi *et al.*, 2012). SN increases endogenous nitric oxide (NO) production, which is thought to increase anti-oxidative defence against the oxygen radicals produced during DOX metabolism by oxidases. As such, we aimed to investigate whether SN could also protect against DOX-induced skeletal myopathy.

Methods: Six-week old male Balb/C mice were treated with three intraperitoneal injections of either vehicle (0.9% NaCl, n=12), DOX (4mg/kg, n=12). To test the hypothesis that sodium nitrate treatment could protect the skeletal muscle against doxorubicin-induced myopathy, a group of mice (n=12) were treated with 1mmol.l^{-1} $NaNO_2$ in drinking water during DOX (4mg/kg) treatment. Mice were assessed for body composition using echoMRI before and after the treatment regimen. Thereafter, mice were anaesthetized with isoflurane (4% induction; 2% maintenance) and *m. extensor digitorum longus* (EDL) and *m. soleus* (SOL) were excised to investigate contractile properties. *M. flexor digitorum brevis* (FDB) was also excised, and fibres were isolated and cultured overnight to assess mitochondrial functional parameters.

Results: DOX treatment induced cachexia in mice as evidenced by a reduction in the % change in whole body composition (P<0.0001), lean mass (P<0.005) and fat mass (P<0.005) from pre- to post-treatment. SN treatment exacerbated the DOX-induced reduction in total body composition (P<0.05), yet not significantly so for the individual measures of lean and fat mass. There was no effect of DOX or DOX+SN treatment on specific force production in the EDL or SOL muscles, or on whole body grip strength. With respect to mitochondrial function, there was a strong trend for DOX treatment to increase the spare respiratory capacity (P=0.08) and the oxidative metabolic potential (P=0.06) in response to chemical uncoupling with FCCP. DOX+SN significantly increased the spare respiratory capacity (P<0.05).

Conclusions: We have demonstrated DOX-induced cachexia in mice which appears to be exacerbated by SN co-treatment. Thus, while SN appears to protect the cardiac muscle from DOX-induced myopathy, it affords no benefit to the skeletal musculature, but rather, exacerbates cachexia.

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