Glycine, a pharmaco-nutrient that protects muscle cells from cachectic stimuli *in vitro* and *in vivo*

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Cancer cachexia is a multi-factorial syndrome characterized by the loss of skeletal muscle mass that leads to progressive functional impairment. The loss of muscle significantly impairs quality of life and compromises the response of affected patients to chemotherapy and radiotherapy leading to increased morbidity and mortality (Murphy *et al.*, 2012). Cancer-induced inflammation reduces skeletal muscle protein synthesis and increases protein breakdown by increasing circulating inflammatory cytokines (*e.g.* TNFα and IL-1β) and increasing intracellular [Ca^{2+}] that trigger muscle degradative pathways and impair the normal anabolic response to food intake. Treatments that modulate inflammation and/or the anabolic response to food may have potential to counteract muscle wasting in cancer patients.

Amino acid availability plays a major role in the control of muscle protein synthesis and breakdown (Koopman, 2011). Although amino acids such as leucine, citrulline and arginine have purported anabolic properties, many of the non-essential amino acids are considered biologically neutral. Interestingly, preliminary data from our laboratory suggest that the non-essential amino acid glycine can reduce intracellular [Ca^{2+}] in muscle cells. We tested the hypothesis that glycine supplementation reduces the loss of muscle mass and maintains anabolic signalling during wasting conditions, such as cancer cachexia.

Confluent C2C12 myoblasts were cultured in differentiation media for 5 days to form mature myotubes. Atrophy was induced by incubating myotubes in: 1) serum free media for 48 h; 2) 1 $ug.ml^{-1}$ lipopolysaccharide for 24h to induce an inflammatory response; or 3) together with C-26 tumour cells (co-culture) for 24-96h. Media was supplemented with 2.5 mM glycine, alanine, or leucine. After the indicated time, cells were fixed in 3.7% formaldehyde and reacted with myosin antibodies to determine myotube diameter or prepared for western blot analyses. Protein synthesis was measured by incubating treated myotubes with puromycin for exactly 30 min immediately before the collection of cells. In addition, we determined the extent of loss of muscle and fat mass loss in a mouse model of cancer cachexia with mice treated with either with glycine or citrulline (1 g/day). All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Code of practice for the care and use of animals for scientific purposes, as stipulated by the NHMRC. CD2F1 mice were anaesthetized deeply with an intraperitoneal (*i.p.*) injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and received a subcutaneous (*s.c.*) injection of PBS (control, n=8) or C-26 tumour cells (n=24) in accordance with the methods of Murphy *et al.* (2012) for establishing the C-26 tumour bearing mouse model of cancer cachexia. After 21 days of daily treatment (*s.c.* injections) with glycine, citrulline or PBS, mice were deeply anaesthetized with an *i.p.* injection of sodium pentobarbital (60 $mg{\cdot}kg^{-1}$) and selected muscles, tumour and adipose tissue were excised and weighed. All mice were killed by cardiac excision, while under deep anaesthesia.

Serum withdrawal, lipopolysaccharide and c26-co-culture all resulted in a 15-25% reduction in myotube diameter. Interestingly, incubation with glycine prevented this wasting, whereas supplementation with equimolar amounts of alanine or leucine did not protect myotubes from wasting. After 48 h serum withdrawal, incubation with glycine completely prevented the 25% reduction in protein synthesis while leucine supplementation did not prevent this reduction. Glycine treatment in C26 tumour-bearing mice attenuated the loss of body mass by 40%, whereas citrulline administration increased tumour growth with 40%.

Glycine administration maintains normal protein metabolism and protects muscle cells from various cachectic stimuli *in vitro* and *in vivo.*

Murphy KT, Chee A, Trieu J, Naim T, Lynch GS (2012) *Disease Models & Mechanisms* **5**, 533-545.
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