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Baicalin supplementation reduces serum biomarkers of skeletal muscle wasting and may protect against lean body mass reduction in cancer patients: Results from a pilot open-label study

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Abstract

OBJECTIVES: Muscle wasting in patients with cancer has been linked to an increased activity of nuclear factor κB (NF-κB) and higher circulating levels of activin-A (ActA), a negative growth factor for muscle mass. Baicalin is a natural flavonoid that can reduce skeletal muscle atrophy in animal models of cancer cachexia by inhibiting NF-κB. This pilot open-label study assessed the effects of baicalin supplementation (50 mg daily for 3 months) in cancer patients who showed involuntary weight loss >5% over the past 6 months.
METHODS: A total of 20 patients were investigated. Participants were evaluated at baseline and at the end of the 3-month study period for the following endpoints: 1) changes from baseline in serum NF-κB and ActA levels; and 2) change from baseline in lean body mass (LBM).
RESULTS: We observed significant reduction in both NF-κB ($p<0.05$) and ActA ($p<0.05$) serum levels from baseline to 3 months. At 3 months, patients also showed a significant mean increase in LBM (+0.8 kg, $p<0.05$ compared with baseline).
CONCLUSION: Our pilot open-label data suggest that baicalin supplementation is potentially useful for contrasting lean body mass reduction in cancer patients with involuntary weight loss, an effect which is likely mediated by the inhibition of negative growth factors for muscle mass.

ORIGINAL ARTICLE

INTRODUCTION

Skeletal muscle wasting and weight loss associated with lean body mass (LBM) reduction remain major challenges in the care of cancer patients and portend increased morbidity, mortality, and poor quality of life (Johns *et al.* 2013; Dodson *et al.* 2011). The pathogenesis of skeletal muscle wasting in patients with cancer is multifactorial and has not yet completely elucidated (Burckart *et al.* 2010). Because energy supplementation and nutritional support alone fail to increase body weight

in patients with malignancies, it has been hypothesized that a combination of tumor- and host-related metabolic changes may hamper appropriate utilization of energy supplements, ultimately impairing their clinical benefits (Rüegg & Glass 2011).

Growing evidence indicates that skeletal muscle wasting occurring in tumor-induced cachexia is linked to both inflammatory (Onesti & Guttridge 2014) and endrocrine alterations (Utech *et al.* 2012). In this regard, animal models of cancer cachexia syndrome have shown a marked activation of the inflammatory pathways orchestrated by the activation of the transcription factor nuclear factor κB (NF-κB) (Zhou *et al.* 2003; Guttridge *et al.* 2000). Notably, Rhoads *et al.* (2010) reported that NF-κB signalling is involved in the initiation and progression of cancer cachexia in humans. In addition, He *et al.* (2013) demonstrated that NF-κB may drive cachexia by causing overexpression of the transcription factor Pax7 in the muscle, ultimately impairing the regenerative capacity of myogenic cells in the muscle microenvironment. Similar to NF-κB, circulating activin-A (ActA) – a member of the transforming growth factor β superfamily – may act as a negative growth factor for muscle mass (Chen *et al.* 2014). Recently, Loumaye *et al.* (2015) have reported that plasma ActA concentrations in patients with cancer cachexia are approximately 40% higher than in non-cachectic patients. Intriguingly, circulating ActA levels showed a positive correlation with the severity of weight loss (Loumaye *et al.* 2015).

Baicalin (5,6-dihydroxy-4-oxygen-2-phenyl-4H-1-benzopyran-7-beta-D-glucopyranose acid) is a bioactive flavonoid glycoside extracted from *Scutellaria baicalensis* with anti-inflammatory, anti-oxidative, and anti-proliferative activity (Srinivas 2010). Growing evidence suggests that the anti-inflammatory effects of baicalin are mediated by inhibition of NF-kB activation (Lim *et al.* 2012; Hao *et al.* 2012). Interestingly, Li *et al.* (2014) have recently reported that baicalin can effectively prevent skeletal muscle atrophy in an experimental cancer cachexia model, most likely by inhibiting activation of NF-κB. Based on baicalin's postulated mechanisms of action, we designed the current pilot, open-label study aimed at assessing whether baicalin supplementation for 3 months could reduce serum biomarkers of muscle wasting (NF-κB and activin A) and increase LBM in a sample of cancer patients who experienced a recent involuntary weight loss >5%.

METHODS

Study design and participants

Twenty patients (18 males and 2 females, mean age: 61±8 years) with pathology-proven head and neck cancer who experienced a recent involuntary weight loss >5% were included. All participants were required to be at least 18 years of age, with no previous history of any other cancer and an expected survival of more than 6 months. Exclusion criteria were as follows: other causes of malnutrition or artificial nutrition, gastrointestinal disorders, malabsorption, major depression, hyperthyroidism, end-stage renal disease, liver cirrhosis, severe heart failure, and use of systemic steroids. TNM stage II and III disease were diagnosed in 4 and 16 patients, respectively. The treatment approach was primary concurrent chemoradiotherapy (CCRT) in 14 patients and postoperative CCRT in 6 patients. Patients undergoing CCRT received a dose of 64–68 Gy in 32–34 fractions over an 8-week period. Patients received a chemotherapy regimen consisting of cisplatinum infusion (50 mg/m^2) plus oral tegafur-uracil (250 mg/m^2/day) on day 1 and oral calcium folinate (90 mg/day) on days 1–14 every 2 weeks, concurrently with radiotherapy. Patients received antibiotics, analgesics, mouth rinses, and topical steroids when clinically required at the physicians' discretion. The study protocol followed the tenets of the Declaration of Helsinki and was approved by the local institutional review board. Written informed consent was obtained from all participants.

Protocol

Patients received oral baicalin 50 mg once-daily about 1 h before breakfast for 3 months. Baicalin was provided by Biodue S.p.A. (Tavarnelle Val di Pesa, Italy) in the form of a sachet containing a powder ready to mix drink. The supplement was mixed with 8 oz of water and then consumed. Compliance with the study protocol was assessed by supplement count. Outcomes were assessed at baseline and at 3 months. Dose reductions or interruptions were not permitted per protocol. The study had three endpoints, as follows: 1) change in serum levels of NF-κB and ActA from baseline to the end of the study; 2) change in LBM from baseline to the end of the study; and 3) tolerance (assessed by asking patients about any signs or symptoms of systemic or local adverse reactions).

Serum biomarkers

Venous blood samples were collected from an antecubital vein between 8:00 and 9:00 a.m. after an overnight fast. Samples were centrifuged at 2 500 *g* for 10 min and serum aliquots were stored at −80 °C until immediately before analysis. Serum levels of NF-κB were measured with a commercially available enzyme linked immunoassay (Abbexa Ltd, Cambridge, UK) according to the manufacturer's instructions. Similarly, serum ActA levels were measured using a commercial kit (R&D Systems, Minneapolis, MN, USA). All measurements were performed in duplicate and the results were averaged. The intra- and interassay coefficients of variation for both assays were <6% and <8%, respectively. Since laboratory personnel were blinded to the participants' status, any possible measurement error was likely to be nondifferential.

Lean body mass measurement

Whole-body LBM was determined using dual X-ray absorptiometry with narrow fan beam technology (Hologic QDR-Series, Hologic Inc., Bedford, MA, USA). All scans were performed using the standard thickness mode, which was automatically chosen by the software. Subjects were fasted and rested (no exercise) for at least 3 h before the scans. They were also instructed not to drink any fluid during this period. All subjects wore light clothing, and all jewelry and metal objects were removed. All patients voided their bladder before scanning.

Statistical analysis

Results are given as means and standard deviations or counts and percentages. Pre- and post-treatment data were analyzed using paired Student's *t*-tests. Linear mixed models were used to detect potential interactions, which might influence the relation between treatment and change in the study variables. All analyses were conducted with the SPSS version 17.0 statistical software (SPSS Inc., Chicago, IL, USA). Two-tailed *p*-values <0.05 were considered statistically significant.

RESULTS

The general characteristics of the study patients are shown in Table 1. No participants dropped out from the study. We observed significant reduction in both serum levels of NF-κB (baseline: 2.11±0.99 ng/mL; 3 months: 1.57±0.85 ng/mL; $p<0.05$, Figure 1) and ActA (baseline: 464±112 ng/mL; 3 months: 392±101 ng/mL; $p<0.05$, Figure 2) from baseline to 3 months. Age, sex, tumor stage, current smoking, and tumor differentiation had no effect on change in serum biomarkers (data not shown). At 3 months, patients also showed a significant mean increase in lean body mass (from 42.9±7.4 kg to 43.7±7.7, difference: +0.8 kg, $p<0.05$ compared with baseline). Of note, the correlation between the increase in lean body mass and the reduction in serum NF-κB was significant (r=–0.42, $p<0.05$). A similar relationship was identified between lean body mass increase and ActA reduction (r=–0.53, $p<0.05$). Baicalin supplementation was well-tolerated in all participants. No patient discontinued treatment due to adverse local or systemic effects.

DISCUSSION

The results from this pilot open-label study suggest that supplementation with baicalin may potentially be useful for preserving and/or improving LBM in cancer patients who experience an involuntary weight loss >5%. Our findings also indicate that baicalin reduced serum levels of NF-κB and ActA, two molecules involved in the pathogenesis of muscle wasting. Intriguingly, we demonstrated that the observed changes in LBM correlated well with the reduction of NF-κB and ActA concentrations.

Tab. 1. Clinical characteristics of the 20 study patients.

Characteristic	No. of Patients	%
Age, years Mean ± standard deviation	58±8	
Body mass index, kg/m^2 Mean ± standard deviation	21.5±3.1	
Lean body mass, kg Mean ± standard deviation	44.1±7.4	
Sex		
Male	18	90
Female	2	10
Tumor stage		
II	4	20
III	16	80
Current smoking		
Yes	15	75
No	5	25
Tumor differentiation		
Good	11	55
Moderate	8	40
Not specified	1	5

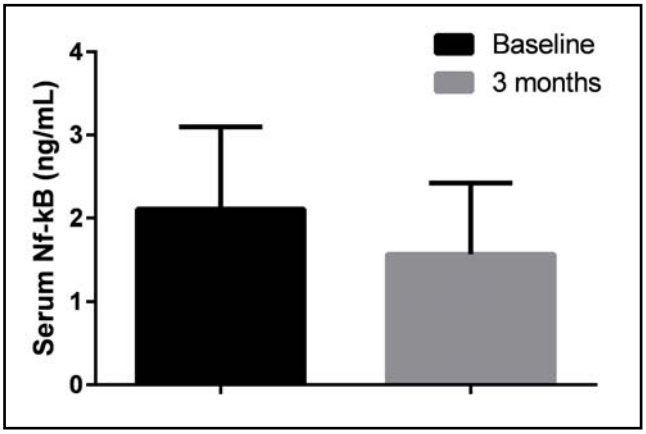

Fig. 1. Changes in serum NF-κB levels throughout the study.

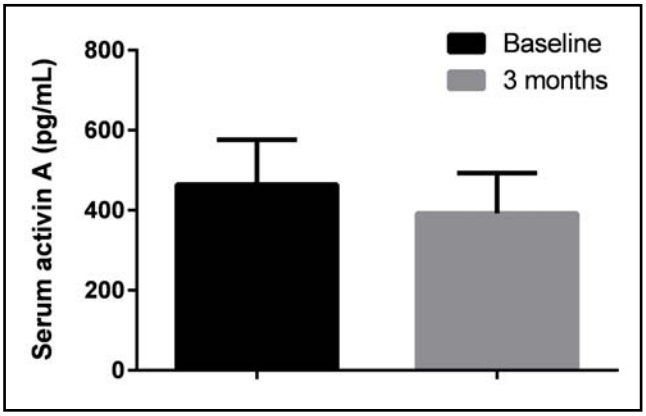

Fig. 2. Changes in serum ActA levels throughout the study.

Owing to the multifactorial nature of cachexia in patients with malignancies, a multifaceted approach to its clinical management is warranted (Dodson *et al.*

2011). In this scenario, novel suppressors of negative regulators of muscle mass are eagerly awaited. Based on previous data obtained in an animal model (Li *et al.* 2014), we reasoned that baicalin could be effective in inhibiting NF-κB-induced loss of lean body mass. In addition, ActA – a cachexia-promoting molecule – is known to be upregulated by NF-κB (Wamsley *et al.* 2015). We therefore hypothesized that baicalin could not only directly lower NF-κB expression but also promote an indirect reduction of ActA. Consequently, both serum NF-κB and ActA were selected as biomarkers in the current study. Consistent with our hypothesis, baicalin supplementation for 3 months resulted in a statistically significant lowering of both serum markers. Interestingly, LBM in the study participants increased significantly throughout the study period. The negative correlations between serum levels of negative regulators of muscle mass and LBM suggest that the increase in the latter parameter was driven at least in part by baicalin-induced changes in increases in NF-κB and ActA concentrations. Further studies are necessary to identify the exact molecular mechanisms responsible for the observed changes. Importantly, baicalin supplementation was safe and no adverse events and/or discontinuations were observed.

Our report has a number of major limitations. Owing to the open-label nature of the study and the small sample size, our preliminary results should be interpreted with caution. Nonetheless, the significant response seen in our subjects suggests that direct reduction of circulating negative growth factors for muscle mass may be useful for cancer patients with weight loss. Well-designed, placebo-controlled randomized studies are required to confirm and expand our preliminary findings. In addition, we solely focused on a small sample of patients with head and neck malignancies. The potential confounding effect of concomitant therapies needs to be addressed in larger studies. Finally, we did not specifically investigate the effects of baicalin of appetite. These limitations notwithstanding, we believe that our pilot data have at least two important implications. First, our findings expand previous observations implying a role of NF-κB and ActA in cancer-associated muscle wasting. Second, the current results suggest that baicalin may be worth of further scrutiny in the management of low lean body mass in patients with different solid malignancies.

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