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β-Hydroxy-β-methylbutyrate supplementation reduces tumor growth and tumor cell proliferation ex vivo and prevents cachexia in Walker 256 tumor-bearing rats by modifying nuclear factor-κB expression

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Abstract

Cancer cachexia syndrome contributes to wasting and weight loss leading to inefficacy of anticancer therapy. In this study, the anticatabolic agent β-hydroxy-β-methylbutyrate (HMB) was supplemented to adult Walker 256 tumor-bearing rats during 8 weeks aiming to determine if tumor burden could be reduced. Male Wistar rats were randomly assigned to nontumor and tumor-bearing groups and fed regular chow or regular chow plus HMB supplemented (76 mg/kg body weight). β-Hydroxy-β-methylbutyrate supplementation induced a lower tumor weight and tumor cell proliferation ex vivo, totally prevented glycemia reduction, as well as blunted the increase in the serum lactate concentrations and also preserved glycogen stores in tumor-bearing rats. Reduction in tumor cell proliferation ex vivo was accompanied by increased nuclear factor-κB inhibitor-α content by more than 100%. In contrast, nuclear factor-κB p65 subunit content was suppressed by 17% with HMB supplementation. In conclusion, HMB supplementation, at a similar dose used in humans to increase muscle mass, caused antitumor and anticachectic effects, with tumor-cell nuclear factor-κB pathway participation, which might be a potential nutritional strategy in cancer therapy.

Keywords: Cancer cachexia; β-Hydroxy-β-methylbutyrate (HMB) supplementation; Tumor growth; Cell proliferation; Walker 256 rat

Abbreviations: C, control group; H, HMB-supplemented group; HMB, β-hydroxy-β-methylbutyrate; HW, tumor-bearing HMB-supplemented group; IκB-α, nuclear factor-κB inhibitor-α; NF-κB, nuclear factor-κB; PIF, proteolysis inducing factor.

1. Introduction

The cachexia syndrome may be associated with tumor growth contributing to physical disability and mortality [1]. Cachexia is also seen in a variety of other conditions such as surgery, trauma, sepsis, and AIDS. It is characterized by anorexia, weight loss, early satiety, changes in taste perception, weakness, anemia, edema, skeletal muscle wasting, and depletion of carbohydrate, protein, and lipid stores [2,3]. The basic mechanism responsible for the establishment of cancer cachexia is poorly understood. Humoral factors such as tumor necrosis factor, interleukin-6, interferon-γ, ciliary neurotrophic factor [4,5], increased plasma levels of vasopressin, prostaglandin E_2 [6], glucagon, cortisol, catecholamines, and reduced insulin levels [7,8] have been reported to contribute to the development of cachexia. These humoral features result in catabolic pathway stimulation, promoting intense mobilization of metabolites, leading to cachexia.

Wasting and weight loss are associated with decreased efficacy of anticancer therapy [9]. Indeed, the importance of

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preventing weight loss in cancer patients has been recognized for more than 70 years [10]. In addition, the advanced muscle mass loss can compromise cardiac and respiratory functions [9,11]. The specific mechanisms inducing muscle protein loss in cancer cachexia are still unknown; however, evidence points to participation of proinflammatory cytokines and tumor factors in the activation of proteolytic pathways inside the skeletal muscle cell [9,12].

β-Hydroxy-β-methylbutyrate (HMB) has been used to prevent body weight loss, to reduce skeletal muscle damage, to increase skeletal muscle mass and strength, and to decrease skeletal muscle protein breakdown [13]. The mechanism of action involved in this process is unclear, but it is assumed that HMB acts like an anticatabolic agent [2]. β-Hydroxy-β-methylbutyrate is a leucine metabolite formed by transamination to α-ketoisocaproate in muscle, followed by oxidation of the α-ketoisocaproate in the cytosol of hepatic cells in liver, and perhaps in other tissues, resulting in HMB [14]. Both leucine and α-ketoisocaproate have been suggested to decrease nitrogen and protein loss, and such effects seem to demand production of HMB [15]. Cancer patients undergoing weight loss (stage IV) supplemented with a mixture of HMB, arginine, and glutamine show increased body weight [2]. A few years ago, a direct action of HMB was reported in the ubiquitin-proteasome-ATP–dependent system component expression and activation, which could explain the antiproteolytic action of HMB. These actions occur due to inhibition of the nuclear factor-κB (NF-κB) pathway [16]. Recently, a specific role of NF-κB activation in tumor development was demonstrated using several animal models [17].

Previous studies investigating the effect of HMB administration have shown that supplementation using doses of 320 mg/kg of body weight per day increases the lifespan in Walker 256 tumor-bearing rats [18]. However, that dose is approximately 4 times what is typically administered to humans.

In the Walker 256 rat, tumors initially arose spontaneously in the mammary gland of a pregnant albino rat. This tumor was transplanted and grew with the morphology of a carcinosarcoma exhibiting 2 to 3 cell types forming independent patterns [19]. In rats inoculated with Walker 256 tumor cells, the solid tumor mass grows without causing apparent physiological disturbances for a certain period (usually approximately 10 days), which is suddenly interrupted by the initiation of a period of rapid tumor growth and marked metabolic changes in the host; both protein and fat tissues decline, and eventually, the tumor-bearing animal dies [20]. These characteristics make the Walker 256 tumor an excellent model to study cachexia and tumor growth. β-Hydroxy-β-methylbutyrate supplementation is an efficient strategy to decrease protein degradation in MAC16 tumor-bearing mice because this compound modifies the NF-κB system in skeletal muscle [16]. Because activation of the NF-κB system induces tumor growth [17], we decided to investigate whether HMB could decrease the body weight reduction, induced by Walker 256 tumor, and whether NF-κB proteins, inside tumor cells, might be involved in this process. To our knowledge, there is no study that has investigated the effect of HMB supplementation on susceptibility to cachexia establishment as well as the tumor cell proliferative response ex vivo, based in doses applied in some human trials, that is, 76 mg kg^{-1} d^{-1} [13]. Also, few studies on HMB supplementation have reported the effects on metabolic responses typical of the tumor-bearing state. Therefore, in this study, we provided HMB supplementation to adult rats for 8 weeks and examined the effects on Walker 256 tumor growth, tumor cell proliferation ex vivo, tumor NF-κB protein expression, and metabolic indicators of cancer cachexia.

2. Methods and materials

2.1. Chemical and enzymes

Chemicals and enzymes used were obtained from Sigma Chemical (St Louis, MO), unless otherwise indicated.

2.2. Study design

All procedures involving animals were approved by the Animal Ethics Committee of the Biological Science Building, Federal University of Paraná. Wistar male rats (70 days old), from the Animal House of the Biological Science Building, Federal University of Paraná, were maintained under controlled temperature (23°C), humidity (55% ± 10%), and a 12-hour light/12-hour dark cycle. The rats had free access to water and to a standard commercial chow (Nutrilab-CR1; Nuvital Nutrients Ltda, Curitiba-PR, Brazil) containing the following: carbohydrate (660 g/kg), protein (230 g/kg), fat (40 g/kg), fiber (60 g/kg), and vitamins plus minerals (10 g/kg). Thirty-two rats were randomly assigned to 1 of the 4 experimental groups: control (C), tumor-bearing (W), HMB-supplemented (H), and tumor-bearing HMB-supplemented (HW). Supplemented rats received HMB at a level of 76 mg per kg body weight per day in a 10% sucrose solution, which was provided as a single daily bolus by gavage for 8 weeks, always at 0800 hours. Rats that did not receive HMB were fed 10% of sucrose solution as a "sham" gavage. The calcium HMB monohydrate, food grade–based supplement, was supplied by Metabolic Technologies Inc (Ames, IA).

At the sixth week of supplementation, tumor cells were inoculated in tumor groups subcutaneously in the right flank with a sterile suspension of 3×10^7 Walker 256 tumor cells, obtained from a rat where cells grew in the peritoneal cavity. Fifteen days after tumor inoculation, the animals were killed by decapitation using a guillotine. Body weight was measured during the experimental period, and tumor weight was determined after euthanasia. Blood was collected and serum prepared to measure the concentrations of glucose and lactate. Liver, soleus, and white portion of gastrocnemius muscles were dissected out and used immediately