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Des-acyl-ghrelin (DAG) normalizes hyperlactacidemia and improves survival in a lethal rat model of burn trauma

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ABSTRACT

Critical illness, including burn injury, results in elevated plasma lactate levels. Dysregulation of PI3K/Akt signaling has been shown to play a predominant role in the inactivation of skeletal muscle PDC and, hence, in hyperlactacidemia in rat models of sepsis and endotoxemia. This observation, and our previous finding that DAG can reverse burn-induced skeletal muscle proteolysis through the activation of PI3K/Akt pathway, led us to hypothesize that DAG may also attenuate hyperlactacidemia in burn injury. Our investigations revealed that burn injury significantly elevated both skeletal muscle lactate production and plasma lactate levels. Moreover, this was accompanied in skeletal muscle by a 5–7 fold increase in mRNA expression of pyruvate dehydrogenase kinases (PDK) 2 and 4, and a ~30% reduction in PDC activity. DAG treatment of burn rats completely normalized not only the mRNA expression of the PDKs and PDC activity, but also hyperlactacidemia within 24 h of burn injury. DAG also normalized epinephrine-induced lactate production by isolated skeletal muscles from normal rats. Moreover, DAG also improved survival in a lethal rat model of burn trauma. These findings with DAG may have clinical implications because chances of survival for critically ill patients are greatly improved if plasma lactate levels are normalized within 24 h of injury.

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Introduction

Highly elevated blood lactate concentration (hyperlactacidemia) is common in patients with critical illness including those with burn injury, sepsis and hemorrhagic shock. Moreover, the duration and degree of increased plasma lactic acid have been considered as useful markers to predict the outcome in critically ill patients. In one study, analysis of the blood lactate data for 166 severely burned patients revealed that there was >70% survival rate if the serum lactate concentration normalized within the first 24 h, and that it decreased to <30% if lactate levels remained supra-normal past 24 h [13]. Another study reported that there was 100% mortality rate in the surgical intensive care unit if lactate levels failed to normalize, and that mortality increased depending on the time taken for normalization [3.9% (<24 h), 13.3% (24–48 h) and 42.5% (48–96 h)] [21]. Nevertheless, it appears that the etiologies contributing to increased lactate production, rather than lactate per se, are responsible for increased morbidity and mortality of critically ill patients.

In general, hyperlactacidemia has been attributed to increased anaerobic glycolysis, mainly by skeletal muscles, due to poor tissue perfusion and oxygen supply [22]. On the other hand, it has also been apparent since the early 1990s that aerobic glycolyis as well as organelle dysfunctions may also contribute to enhanced lactate levels in critical illness [7,9,15,18]. Well-oxygenated muscles from rat models of sepsis, burn or hemorrhagic shock exhibit increased production of lactate due to increased intracellular concentration of Na^+ ($[Na^+]_i$) and increased Na^+, K^+-ATPase activity [11,12,17–20]. In addition, burn and sepsis have been associated with muscle mitochondrial dysfunctions, which could lead to increased lactate production through glycolysis [2,23,26]. Nevertheless, increasing tissue perfusion and oxygen supply to tissue is still used as the preferred method to attenuate hyperlactacidemia, and improve

Abbreviations: DAG, des-acyl-ghrelin; EDL, extensor digitorum longus; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PDP, pyruvate dehydrogenase phosphatase; TBSA, total body surface area.

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survival. This mode of treatment, however, may not be adequate as it may have little or no effect on lactate produced by aerobic glycolysis or organelle dysfunctions.

Pyruvate dehydrogenase complex (PDC) plays a pivotal role in oxidation, since it catalyses irreversible oxidative decarboxylation of pyruvate to acetyl-coenzyme A, thus linking glycolytic and citric acid cycles [1,6,27]. Therefore, any dysregulation in PDC activity can lead to hyperlactacidemia. This, indeed, has been found to be the case in several pathophysiological states including sepsis and endotoxemia. Moreover, it has been shown that downregulation of PI3K/Akt signaling under cachectic conditions results in increased FOXO-mediated transcription of muscle specific pyruvate dehydrogenase kinases (PDK) genes 2 and 4. The increased PDK2 and 4 activities results in enhanced phosphorylation and inactivation of PDC.

It is well known that burn injury also results in the downregulation of the skeletal muscle PI3K/Akt pathway, and hyperlactacidemia. However, to the best of our knowledge, little or no work has been done to investigate the role of PDC in burn-induced hyperlactacidemia. Moreover, we have shown that des-acyl-ghrelin (DAG), the precursor peptide of ghrelin, can attenuate muscle cachexia in burn injury through the downregulation of elevated inflammatory cytokines and the upregulation of repressed PI3K/Akt signaling [24]. Based on these observations, we hypothesized DAG may also normalize hyperlactacidemia in burn injury through reversing dysregulated skeletal muscle PDC activity. The present investigations show that DAG treatment can in fact normalize skeletal muscle lactate production and plasma lactate levels elevated by burn injury. Moreover, we also demonstrate that DAG can normalize epinenephrine-induced lactate production by EDL muscle. DAG also improved survival in a lethal rat model of burn trauma.

Materials and methods

Rat burn model

Twenty four Sprague-Dawley male rats weighing 50–60 g (Harlan, Indianapolis, IN) were housed individually in a temperature controlled room (25 °C) under 12-h light/dark cycle, and maintained on standard rodent chow (Harlan Teklad Rodent Diet) and water ad libitum for 48 h before experiments. We used young rats in order to estimate lactate production by skeletal muscles during in vitro incubations. Young rats possess lower extremity muscles which are thin enough to permit diffusion of oxygen from the medium, thus preventing development of hypoxic regions in the muscles [3,4,24]. All animal procedures used in this study were approved by the University of Cincinnati Animal Care and Use Committee. All rats were anesthetized with pentobarbital (50 mg/kg) and their back and abdomen were shaved. Eight rats were implanted subcutaneously with a primed Alzet osmotic minipump (Catalog # 2001D, Durect Corp., Cupertino, CA) containing DAG (0.5 mg/rat) in saline, while the other sixteen were implanted with pumps containing saline. DAG used in this study was synthesized and characterized in our laboratory as reported previously [4]. Contents of the minipumps (200 μl) are nominally delivered at 8 μl/h over 24 h. Shortly (<5 min) after implantation of minipumps, a total of 16 rats (8 DAG-burn and 8 saline-burn) were subjected to a 15 s third-degree open flame burn on the back, affecting 30% of total body surface area (TBSA). The surface area of burn was controlled using a layer of kerosene soaked gauze applied to the dorsum of the rat [8]. The remaining 8 rats with minipumps containing vehicle, which were not burned, constituted the control sham group. All the rats were then resuscitated with 0.3 M NaCl (10 ml/100 g body weight, *ip*) to compensate for hypovolemia due to post-burn edema, and the rats were provided with rat chow equivalent to ~10% of their body weight, based on previous observations that burned rats consumed food ≤10% of their body weight within the first 24 h after burn injury [3,4,24]. After ~24 h, rats were re-anesthetized with pentobarbital (50 mg/kg), and the gastrocnemius muscles were dissected and rapidly frozen in liquid nitrogen. The dissected extensor digitorum longus (EDL) muscles were used for lactate production studies, described below. One gastrocnemius muscle from each rat was used in RT-PCR studies, while the contralateral gastrocnemius muscle was used for determining total PDC activity. Blood was collected and centrifuged, and the plasma was used to determine lactate concentrations. Mortality rate of burned rats was ≤5%.

A similar study was conducted with 24 adult male rats (225–250 g), but in this instance, rats were subjected to a 25 s flame burn to produce 30% TBSA burn injury. Moreover, the 2001D minipumps were filled with either 200 μl saline or 200 μl saline containing DAG (2.50 mg/rat). The plasma collected was used to determine effects of DAG on plasma lactate levels in adult burn rats.

Another study was performed using 16 unburned young rats (50–60 g) to study the effects of DAG on epinephrine-induced EDL muscle lactate production. EDL muscles isolated under pentobarbital (50 mg/kg) were tied to metal clamps and incubated as described below.

Determination of skeletal muscle lactate production

To assess lactate production, EDL muscles of young rats were tied by the tendons at resting length to stainless steel supports and preincubated in a shaking water bath for 30 min at 37 °C in individual stoppered 25 ml flasks containing 3 ml oxygenated (95% O2–5%CO2) Krebs-Henseleit bicarbonate buffer (pH 7.4) with 10 mM glucose [3,4,11,12,19,20,24]. Muscles were then incubated in fresh buffer containing 0.5 mM cycloheximide for 2 h. At the end of the incubation, muscles were weighed, and the incubation medium collected for lactate analysis. Incubation studies were carried out using 6–8 muscles per group, each EDL muscle obtained from a separate rat.

In the case of epinephrine studies, both EDL muscles from eight rats were incubated for 2 h as above in the buffer alone (control) or buffer containing DAG (1.0 μM) [12]. EDL muscles from another eight rats were incubated for 2 h as above in buffer containing epinephrine (0.50 μM) or DAG (1.0 μM) + epinephrine (0.50 μM).

PCR investigations

The skeletal muscle mRNA expression of PDK2 and 4, and PDP1 was determined by quantitative real-time reverse transcription (RT) polymerase chain reaction (PCR) [3,4,24]. Total RNA from gastrocnemius muscles was isolated using TRI Reagent® (Molecular Research Center Inc., Cincinnati, OH) according to the protocol provided by the manufacturers. The yield and the purity of the RNA was determined by absorbance at 260 nm and the 260/280 ratio, respectively. Five micrograms of RNA was used to prepare the complementary DNA (cDNA) using Superscript Reverse Transcriptase (Invitrogen, Carlsbad, CA). Resulting cDNA was subjected to PCR amplification with the appropriate specific forward and reverse primers. Proprietary primers of PDK2 and 4, and pyruvate dehydrogenase phosphatase 1 (PDP1) were obtained from Super Array Bioscience Corp, Frederick, MD. The Real-time RT-PCR was carried out using a SYBR green based kit (Brilliant QPCR Master Mix, Stratagene, CA). The threshold cycle, the cycle at which the PCR reaction emits a fluorescence signal greater than background, was used for the quantification of mRNA. The relative quantities of mRNA were calculated using a built-in formula in the Stratagene real-time PCR instrument, and the expression of cyclophilin mRNA was used to