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Butyrate Ameliorates Lipopolysaccharide-induced Myopathy through Inhibition of JNK Pathway and Improvement of Mitochondrial Function in C2C12 Cells

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Inflammation induced by metabolic syndromes, cancers, injuries, and sepsis can alter cellular metabolism by reducing mitochondrial function via oxidative stress, thereby resulting in neuropathy and muscle atrophy. In this study, we investigated whether butyrate, a short chain fatty acid produced by gut microbiota, could prevent mitochondrial dysfunction and muscle atrophy induced by lipopolysaccharide (LPS) in the C2C12 cell line. LPS-activated MAPK signaling pathways increased the levels of the mitochondrial fission signal, p-DRP1 (Ser616), and the muscle atrophy marker, atrogin 1. Interestingly, butyrate significantly inhibited the phosphorylation of JNK and p38 and reduced the atrogin 1 level in LPS-treated C2C12 cells while increasing the phosphorylation of DRP1 (Ser637) and levels of mitofusin2, which are both mitochondrial fusion markers. Next, we investigated the effect of MAPK inhibitors, finding that butyrate had the same effect as JNK inhibition in C2C12 cells. Also, butyrate inhibited the LPS-induced expression of pyruvate dehydrogenase kinase 4 (PDK4), resulting in decreased PDHE1α phosphorylation and lactate production, suggesting that butyrate shifted glucose metabolism from aerobic glycolysis to oxidative phosphorylation. Finally, we found that these effects of butyrate on LPS-induced mitochondrial dysfunction were caused by its antioxidant effects. Thus, our findings demonstrate that butyrate prevents LPS-induced muscle atrophy by improving mitochondrial dynamics and metabolic stress via the inhibition of JNK phosphorylation. Consequently, butyrate could be used to improve LPS-induced mitochondrial dysfunction and myopathy in sepsis.

Key words : Butyrate, lipopolysaccharide, mitochondrial dynamics, mitogen activated-protein kinases, myopathy

Introduction

Skeletal muscle plays crucial roles in movement in mammals such as humans, as well as acting as a reservoir of amino acids and regulating the systemic metabolism of glucose and lipids. Muscle atrophy is a degenerative process of muscle loss associated with increased morbidity and mortality that can occur due to aging, denervation, muscle injury, an inactive lifestyle, malnutrition, and various systemic diseases such as cancer, diabetes, and sepsis [19]. This process is caused by impaired protein synthesis and/or accelerated protein degradation in skeletal muscle fibers.

Under both physiological and pathological conditions, two important proteolytic systems are involved in decreasing skeletal muscle mass; ubiquitin-proteasome machinery and autophagy-lysosome machinery [4]. The ubiquitin-proteasome system has been extensively studied for over two decades and includes the muscle-specific ubiquitin E3 ligases atrogin-1 (also known as MAFbx) and MuRF1, which are among the most commonly induced atrophy-related proteins, also known as atrogines. These E3 ligases increase the ubiquitin-mediated degradation of MyoD [40], eIF3f [9], and sarcomeric proteins, which play pivotal roles in protein synthesis during muscle differentiation and homeostasis [3]. Numerous studies in the last decade have examined the effect of the autophagy-lysosome system on skeletal muscle development and its physiological function; however, the role of autophagy in skeletal muscle has remained largely unclear. A recent study found that the muscle specific inhibition of autophagy by genetic modification attenuates muscle regeneration after acute injury and accelerates muscle loss during starvation [28]. Unfortunately, the fundamental underlying mechanism remains unclear since multiple mechanisms could be involved in muscle atrophy.

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It has recently emerged that mitochondrial dysfunction is an early indicator of life-threating diseases, such as myopathies and neuropathies [1, 22]. In addition to ATP generation, mitochondria play key roles in the regulation of fundamental cellular processes, including cell survival, the production of reactive oxygen species (ROS), apoptosis, and Ca^{2+} homeostasis [27]. During these processes, mitochondria are continuously exposed to ROS-mediated damage and damaged mitochondria are removed through tightly-regulated fission and fusion activities, known as mitochondrial dynamics [23]. Together, the regulation of mitochondrial fission and fusion maintains mitochondrial homeostasis between biogenesis and selective mitophagy by coordinately altering mitochondrial content in response to stresses and extra-/intra-cellular signals [22, 36]. Major mitochondrial dysfunction is initiated by changes in these processes; for instance, dynamin-related protein 1 (DRP1) is an important regulator of mitochondrial dynamics that induces and inhibits mitochondrial fission when phosphorylated at Ser616 and Ser637, respectively [20]. Conversely, Mitofusin1/2 (MFN1/2) are GTPases in the mitochondrial membrane that are responsible for mitochondrial fusion [13]. The dysregulation of proteins that regulate mitochondrial fission and fusion in skeletal muscle causes the deterioration of the normal mitochondrial network (mitochondrial content, shape, and localization) and induces skeletal muscle atrophy [12, 39].

Acute and chronic inflammation induced by metabolic disorders, cancers, and sepsis have been shown to disturb mitochondrial homeostasis, resulting in organ failure and muscle loss [34]. Indeed, uncontrolled mitochondrial fragmentation caused by inflammation leads to the loss of skeletal muscle by activating ubiquitin-proteasome machinery, indicating that inflammation is an important risk factor for muscle atrophy [16]. Accumulating evidence has suggested that inflammation significantly reduces oxidative phosphorylation (OxPhos) in skeletal muscles by depleting several protein components of the electron transport chain in mitochondria, thereby depleting cellular ATP levels [2]. Lipopolysaccharide (LPS) is a bacterial endotoxin that is expressed in the outer membrane of gram-negative bacteria and has been implicated in the pathogenesis of inflammation and septic shock, one of the most life-threatening diseases caused by super-bacteria in hospitalized patients. LPS is recognized by Toll-like receptors (TLRs, i.e. TLR4) on the surface of innate immune cells which activate downstream mitogen-activated protein kinases (MAPKs), leading to the induction of inflammation [33]. Although LPS signaling pathways have been extensively studied, numerous trials to prevent LPS-induced inflammation and sepsis have so far failed to develop a safe therapeutic drug [26].

Previously, we reported that LPS-induced inflammation increases the expression of pyruvate dehydrogenase kinase 4 (PDK4), a protein that regulates the pyruvate dehydrogenase complex (PDC) and thus glucose metabolism [29]. Pin *et. al.* [31] also found that increased PDK4 expression has deleterious effects on skeletal muscle; however, the exact relationship between altered glucose metabolism and muscle atrophy has not yet been elucidated. Butyrate is a short chain fatty acid bacterial metabolite that is produced by gut microbiota through the fermentation of dietary fibers [15]. Although butyrate has potent anti-inflammatory properties and was found to exert beneficial effects in chronic inflammatory diseases, such as colitis and inflammatory bowel syndrome [15], no studies have yet examined its anti-inflammatory effects against mitochondrial dynamics and the depletion of muscle cell volume. Here, we explored the effects of butyrate on LPS-induced mitochondrial dysfunction and skeletal muscle atrophy.

Materials and Methods

Materials

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Hyclone Laboratory (Logan, UT, USA). Butyric acid, 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bacterial lipopolysaccharide (LPS), and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). MAPK inhibitors such as the c-Jun N-terminal kinase (JNK) inhibitor (SP600 125), p38 inhibitor (SB202190), and ERK inhibitor (PD98059) were purchased from Abcam (Cambridge, MA, USA). Antibodies against DRP1, p-DRP1 (Ser616), p-DRP1 (Ser637), p-JNK (Thr183/Tyr185), JNK, p-ERK (Thr202/Tyr204), ERK, p-p38 (Thr180/Tyr182), p38, and Bcl-2 were purchased from Cell Signaling Technology (Danvers, MA, USA); human atrogin-1 from ECM Bioscience (Versailles, KY, USA), and β-actin from Sigma-Aldrich (St. Louis, MO, USA). Secondary antibodies specific for mouse- and rabbit-antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Butyrate solution (1 M) was prepared by neutralizing butyric acid with 30% KOH solution, followed by syringe filter (0.2 μm) sterilization.

Cell culture

The mouse skeletal muscle cell line, C2C12, was cultured in DMEM (high glucose, 4.5 g/l) supplemented with 10% FBS, 100 μg/ml streptomycin, and 100 U/ml penicillin (Gibco, Grand Island, NY, USA) in a humidified incubator with 5% CO_2 at 37℃. Cells were sub-cultured for maintenance before they reached 70% confluence.

Cell viability analysis

Cell viability was analyzed using MTT assays. Briefly, C2C12 cells were seeded in a 96 well culture dish (3×10^3 cells/well) and cultured for 24 hr. After treatment with LPS, butyric acid, or butyrate at the indicated concentrations for a further 24 hr, the cells were washed with serum free media and stained using MTT solution (100 μl serum free medium containing 0.5 mg/ml MTT reagent). The plate was then wrapped with aluminum foil and incubated at 37℃ for 2-3 hr until a purple precipitant was visible. To detect formazan formation, the staining solution was carefully removed and the cells were incubated with MTT dissolving solvent at 37℃ in a shaken incubator until the formazan had completely dissolved. Formazan concentration was determined by measuring the absorbance at 590 nm using a microplate reader (Versa Max, Molecular Device LLC, San Jose, CA, USA).

Western blotting

To isolate proteins for western blot analysis, C2C12 cells were seeded in 100 mm culture dishes (5×10^5 cells/dish), cultured until they reached 80% confluence, pretreated with 1 mM butyrate for 1 hr, and then treated with 40 μg/ml of LPS for the indicated length of time. After the cells had been washed twice with ice-cold phosphate buffered saline (PBS) and harvested in 1 ml of ice-cold PBS using a cell scraper, the PBS was completely removed by centrifugation at 500× *g* for 5 min at 4℃. The cells were then lysed using RIPA buffer (Thermo Scientific, Waltham, MA, USA) containing 10 mM β-glycerophosphate, 50 mM potassium fluoride, 1 mM sodium orthovanadate, 0.5 mM EDTA, 1× Xpert protease inhibitor, and 1× phosphatase inhibitor. Cell lysates were clarified by centrifugation at 12,000× *g* for 10 min at 4℃ and protein concentration was determined using a BCA protein test kit. Proteins (20 μg) were separated using 12.5% SDS-PAGE and transferred to PVDF membranes, which were blocked with 5% skimmed milk in tris-buffered saline solution containing 0.05% Tween-20 (TBST) for 1 hr, incubated with specific primary antibodies overnight at 4℃, and incubated with anti-rabbit or anti-mouse IgG as secondary antibodies for 1 hr at room temperature. The resulting blots were visualized using ECL reagent and band density was analyzed using ImageJ software (Ver. 1.53; NIH, Bethesda, MD, USA).

Measurement of lactate concentration

C2C12 cells were grown to 80% confluence in DMEM complete medium, washed with HBSS, and then pretreated with 1 mM butyrate or 20 μM JNK inhibitor for 1 hr in DMEM without pyruvate and serum, followed by treatment with LPS (40 μg/ml) for 24 hr. The culture medium was then collected and deproteinized using perchloric acid (6% final concentration). After centrifugation, the supernatant was neutralized using 30% KOH solution and lactate concentration was measured enzymatically using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Jp) [14].

Statistical analysis

Data were expressed as the mean ± SEM. Statistical analyses were performed using unpaired Student's *t*-tests. *P* values of $\leq$ 0.05 were considered statistically significant.

Results

LPS decreases cell viability and increases markers of mitochondrial fission

First, we examined the effect of LPS on cell viability using MTT assays, finding that LPS concentrations greater than 20 μg/ml significantly decreased C2C12 cell viability in a dose-dependent manner (Fig. 1A). In addition, we examined whether treatment with 5, 10, 20, or 40 μg/ml of LPS stimulated mitochondrial fission in C2C12 cells. High LPS concentrations significantly increased DRP1 (Ser616) phosphorylation and atrogin1 levels, markers of mitochondrial fission and muscle atrophy, respectively, compared to untreated controls (Fig. 1B). These results suggest that high LPS concentrations induce muscle atrophy, potentially via mitochondrial fission; therefore, we used 40 μg/ml of LPS for subsequent experiments.

LPS induces mitochondrial fission and muscle atrophy by activating JNK signaling

Since LPS is known to induce cellular inflammation via the TLR4-MAPK signaling pathway [11], we examined the phosphorylation status of MAPKs in LPS-treated C2C12

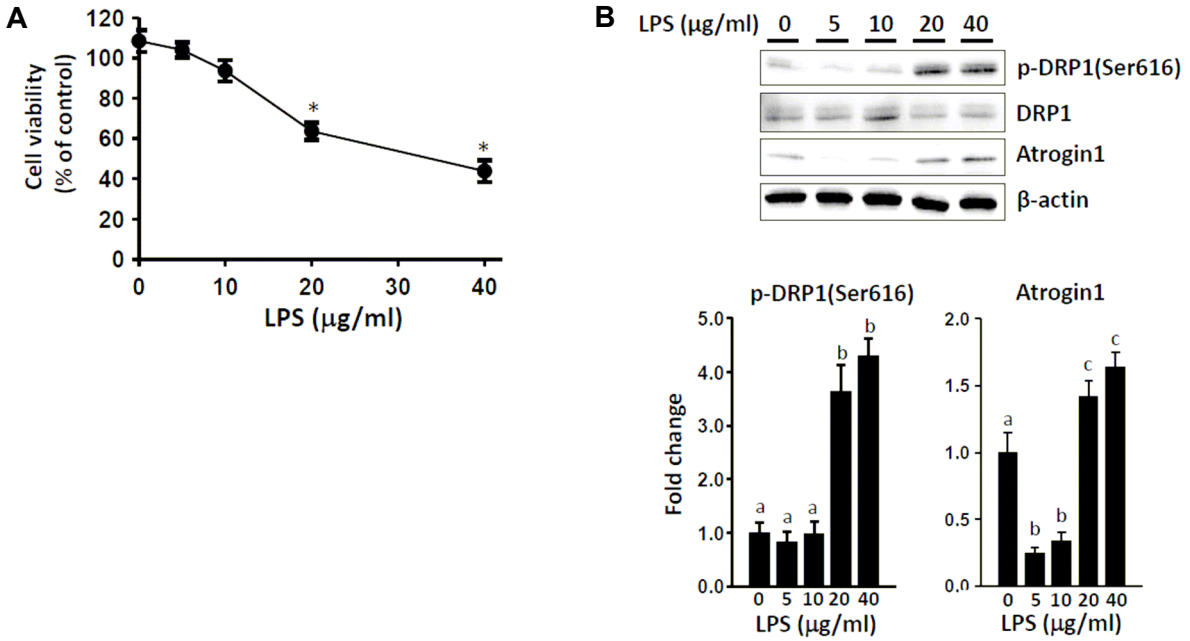

Fig. 1. Effect of LPS on cell viability, mitochondrial dynamics, and muscle atrophy in C2C12 cells. (A) C2C12 cells (3×10^3 cells/well) were seeded in a 96 well dish and cultured for 24 hr before treatment with the indicated concentration of LPS for another 24 hr. Cell viability was assessed using MTT assays. Results represent the mean ± SEM. *p<0.05 compared to the control. (B) C2C12 cells (~ 80% confluence) were treated with the indicated concentration of LPS for 24 hr. Proteins were collected using RIPA buffer containing protease and phosphatase inhibitors and separated using 12.5% SDS-PAGE. Western blot analysis was performed using the indicated antibodies and band density was determined using ImageJ. Results represent the mean ± SEM. Different letters represent significant differences (p<0.05) compared to the control.

cells. LPS treatment (40 μg/ml) for 30 min significantly increased JNK, p38, and ERK phosphorylation (Fig. 2A). Within MAPK signaling, the JNK pathway is the main cause of LPS-induced inflammation in C2C12 cells [29, 38]; therefore, we treated C2C12 cells with a JNK inhibitor (SP600125). As shown in Fig. 2B, JNK inhibition significantly decreased DRP1 (Ser616) phosphorylation and atrogin-1 levels induced by LPS, but significantly increased DRP1(Ser637) phosphorylation, which inhibits mitochondrial fission. Conversely, treatment with Erk (PD0325901) or p38 (SB203580) inhibitors did not significantly change LPS-induced markers of mitochondrial fission and muscle atrophy (data not shown). Thus, LPS appears to stimulate mitochondrial fission and muscle atrophy by activating JNK.

Butyrate inhibits LPS-induced JNK and p38 MAPK phosphorylation in C2C12 myoblasts

Butyrate has been reported to exert strong anti-inflammatory effects against macrophages [37]; therefore, we examined whether butyrate prevents LPS-induced muscle atrophy. Before examination this hypothesis, we first tested the cytotoxic effects of butyrate in C2C12 cells. Neither butyric acid nor butyrate showed any cytotoxic effects at concentrations up to 1 mM, but both displayed comparable levels of cytotoxicity at concentrations above 2 mM (Fig. 3A). Consequently, 1 mM of butyrate was used for subsequent experiments to examine whether butyrate could inhibit LPS-induced MAPK phosphorylation and cell apoptosis. Although butyrate significantly inhibited LPS-induced JNK and p38 phosphorylation, it did not inhibit LPS-induced ERK phosphorylation (Fig. 3B). LPS-induced JNK phosphorylation is known to increase oxidative stress in C2C12 cells and result in apoptosis, a risk factor for muscle atrophy [38]. As shown in Fig. 3B, butyrate significantly restored LPS-induced reductions in Bcl2, an anti-apoptotic marker, suggesting that butyrate effectively inhibits LPS-induced muscle cell death by inhibiting JNK phosphorylation.

Butyrate ameliorates mitochondrial function by inhibiting LPS-induced JNK phosphorylation

To identify the mechanism via which butyrate inhibits LPS-induced muscle atrophy, we examined changes in mitochondrial dynamic regulators, such as DRP-1 phosphorylation and Mitofusin2 (Mfn2), with LPS-induced JNK phos-

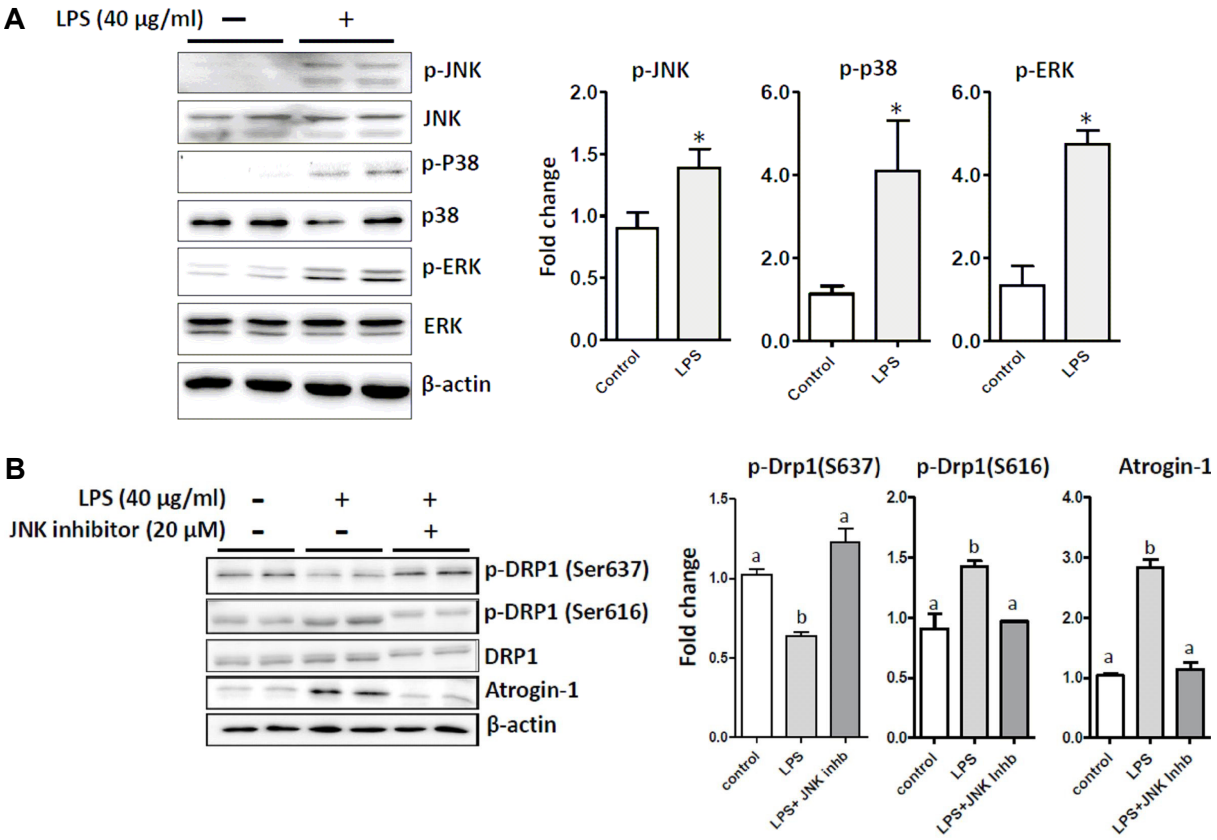

Fig. 2. Effect of LPS on MAPK pathway activation in C2C12 myoblasts. C2C12 cells (~80% confluence) were treated with LPS (40 μg/ml) for 30 min. Proteins were collected using RIPA buffer containing protease and phosphatase inhibitors and separated using 12.5% SDS-PAGE. Western blot analysis was performed using the indicated antibodies and band density was determined using ImageJ. (A) MAPK phosphorylation was analyzed using western blotting with specific antibodies. Results represent the mean ± SEM. *p<0.05 compared to the control. (B) Effect of a JNK inhibitor on LPS-induced mitochondrial dynamics and muscle atrophy. C2C12 cells were pretreated with the JNK inhibitor for 1 hr and then treated with LPS (40 μg/ml) for 24 hr. Results represent the mean ± SEM. Different letters represent significant differences (p<0.05) compared to the control.

phorylation. Interestingly, butyrate treatment significantly inhibited DRP-1(Ser616) phosphorylation induced by LPS in a comparable manner to JNK inhibition (Fig. 4). In addition, butyrate significantly increased LPS-induced reductions in mitochondrial fusion markers, such DRP-1 (Ser637) phosphorylation and Mfn2 levels, compared to the control or JNK inhibitor-treated cells (Fig. 4). Furthermore, LPS-induced atrogin-1 expression was significantly reduced by butyrate and JNK inhibition, suggesting that butyrate inhibits JNK phosphorylation to reduce LPS-induced mitochondrial fission and muscle atrophy and induce mitochondrial fusion, thereby ameliorating mitochondrial function.

Butyrate inhibits the LPS-induced Warburg effect by exerting anti-oxidative effects

Previously, we showed that LPS-induced JNK activation shifts glucose metabolism from oxidation to lactate formation, also known as the Warburg effect, by inducing PDK4 expression [29]. Since changes in glucose metabolism have been shown to induce inflammation via ROS production in metabolic diseases such as diabetes, sepsis, and cancer [18], we explored the effect of butyrate on the changes in glucose metabolism induced by LPS by measuring PDK expression, PDHE1α phosphorylation, and lactate production. LPS-induced PDK4 expression was significantly reduced by treatment with butyrate or JNK inhibitors, while butyrate also slightly altered the expression of other PDKs, such as PDK1 and PDK3. Furthermore, butyrate treatment inhibited PDHE1α (Ser300) phosphorylation induced by LPS (Fig. 5A) and significantly reduced LPS-induced lactate production in a similar manner to JNK inhibition (Fig. 5B). These results suggest that butyrate may restore OxPhos glucose metabolism by reducing PDK4 expression and inhibiting JNK activation induced by ROS production. Finally, we tested

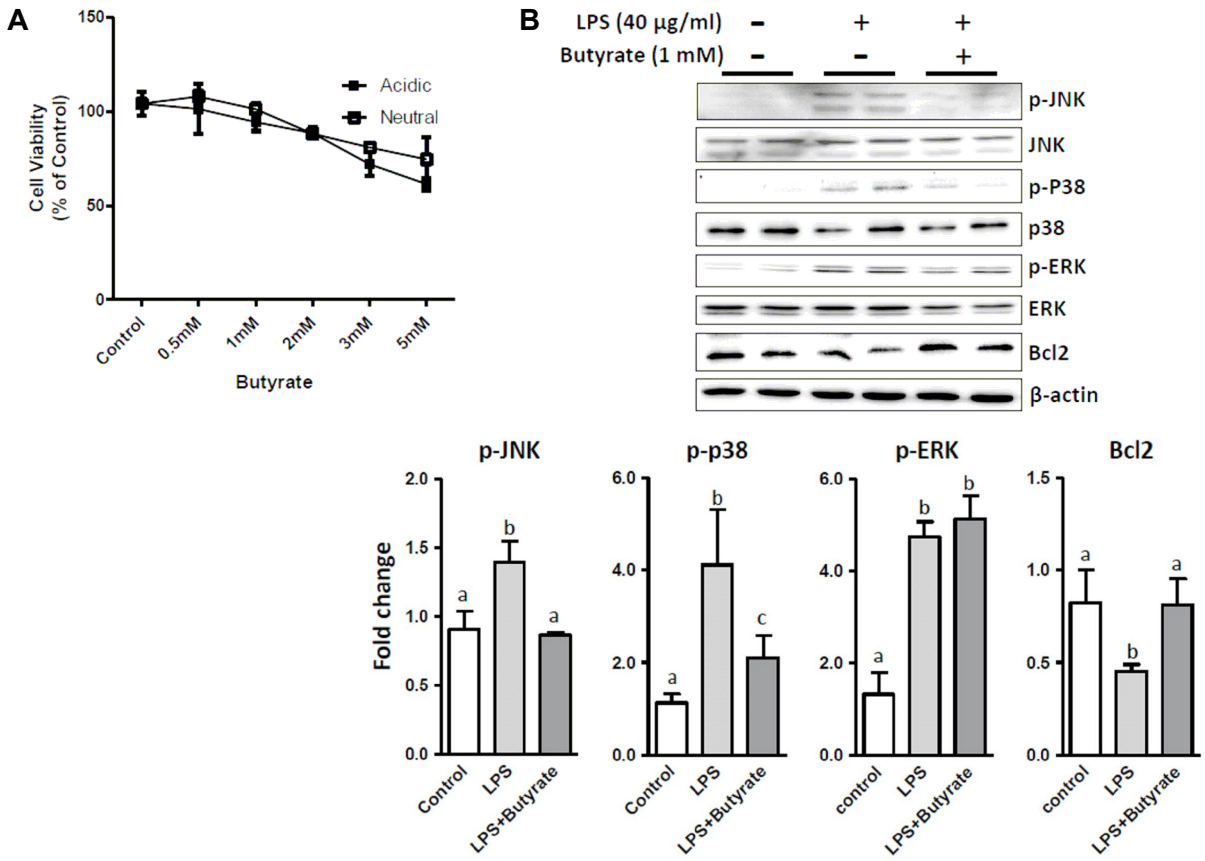

Fig. 3. Effect of butyrate on C2C12 cell viability and MAPK phosphorylation induced by LPS. (A) C2C12 cells (3×10^3 cells/well) were seeded in 96 well dishes and cultured for 24 hr before treatment with the indicated concentration of butyrate or butyric acid for another 24 hr. Cell viability was assessed using MTT assays. Results represent the mean ± SEM. (B) C2C12 cells (~80% confluence) were pretreated with 1 mM butyrate for 1 hr followed by LPS (40 μg/ml) treatment for 30 min. Western blot analysis was performed using the indicated antibodies and band density was determined using ImageJ. Results represent the mean ± SEM. Different letters represent significant differences ($p<0.05$) compared to the control.

whether butyrate could affect LPS-induced ROS generation in C2C12 cells, finding that butyrate treatment inhibited JNK and DRP1 (Ser616) phosphorylation induced by LPS in a similar manner to the antioxidant, NAC (Fig. 5C). Together, these findings indicate that butyrate inhibits LPS-induced ROS production and JNK phosphorylation to ameliorate mitochondrial function and skeletal muscle atrophy.

Discussion

Butyrate is a short chain fatty acid produced by the fermentation of dietary fibers in the intestine. Accumulating evidence has suggested that butyrate exerts strong anti-inflammatory effects in intestinal diseases [6], neuronal diseases [43], and muscle atrophy [42] by inhibiting JNK activation induced by pathological stimuli. However, the exact molecular mechanism underlying this process remains unclear. Since numerous recent studies have suggested that inflammation could involve in mitochondrial dysfunction, or vice versa [24, 41], we decided to investigate the protective effects of butyrate against LPS-induced mitochondrial dysfunction in skeletal muscle and subsequent protection against inflammation-induced muscle atrophy.

It was previously reported that LPS induces inflammation in C2C12 cells by activating MAPK signaling pathways [29, 38]. Consistently, we found that LPS administration activated MAPKs in C2C12 cells (Fig. 2A) and induced muscle cell death (Fig. 1A), yet this LPS-induced MAPK activation was dramatically inhibited by butyrate treatment (Fig. 3). In addition, butyrate treatment restored Bcl2 expression reduced by LPS treatment, indicating that butyrate inhibits LPS-induced muscle cell death. Among the MAPKs activated by LPS, JNK appeared to play a major role in LPS-induced inflammation in C2C12 cells [29]. Indeed, we ob-

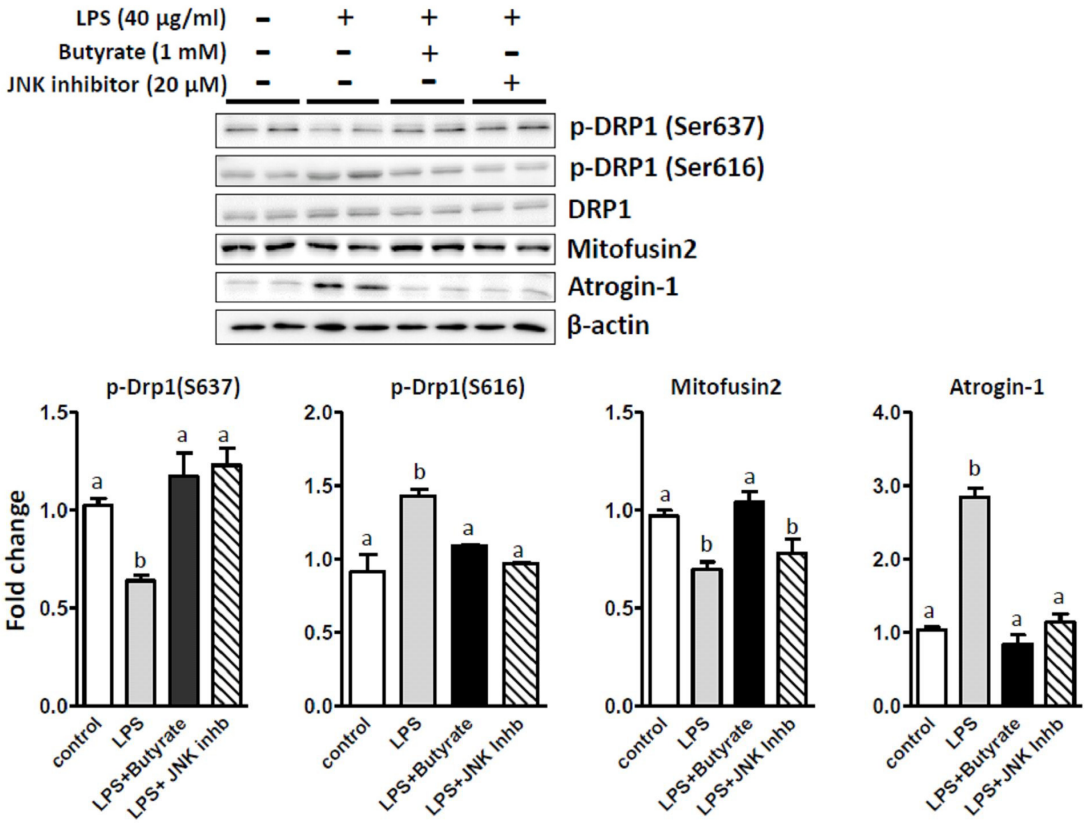

Fig. 4. Effect of butyrate and JNK inhibition on mitochondrial dynamics and muscle atrophy in LPS-treated C2C12 cells. C2C12 cells (~80% confluence) were pretreated with butyrate (1 mM) or a JNK inhibitor (20 μM) for 1 hr, followed by LPS (40 μg/ml) treatment for 24 hr. Western blot analysis was performed using the indicated antibodies and band density was determined using ImageJ. Results represent the mean ± SEM. Different letters represent significant differences (p<0.05) compared to the control.

served that LPS-induced inflammation increased mitochondrial fragmentation and muscle atrophy by activating JNK in C2C12 cells (Fig. 1B, Fig. 2). These findings were confirmed by treatment with a JNK inhibitor, which completely abolished LPS-induced DRP1 (Ser616) phosphorylation and atrogin-1 expression (Fig. 2B).

Mitochondrial dynamics refers to the cycle of mitochondrial membrane fission and fusion that controls mitochondrial number, shape, and functionality in response to intra- and extra-cellular stresses, such as nutrient supply, energy demand, and oxidative stress. The regulation of mitochondrial homeostasis plays a crucial role in the control of cell viability, growth, differentiation, and death [5, 7, 8]. Previous studies have reported that mitochondrial fragmentation is increased in cancers and oxidative stress, and that DRP1 is activated by Ser616 phosphorylation through ERK1 and ERK2 in cancer cells [21]. However, we found that DRP1 (Ser616) phosphorylation was increased by LPS treatment in C2C12 cells in a JNK-dependent manner, whereas DRP1 (Ser637) phosphorylation and Mfn2 expression, markers of mitochondrial fusion, were significantly decreased (Fig. 4A). These effects of LPS were restored by treatment with butyrate or a JNK inhibitor, indicating that butyrate may inhibit JNK activity. Moreover, butyrate treatment and JNK inhibition reduced LPS-induced atrogin-1 expression, suggesting that the inhibition of mitochondrial fission reduces muscle atrophy, consistent with previous studies [10, 35, 38].

Skeletal muscle is one of the most metabolically active organs in animals, in which the dysregulation of mitochondrial metabolic function can evoke metabolic syndromes such as diabetes and muscle atrophy. Skeletal muscle generally utilizes glucose and/or free fatty acids as a fuel source via OxPhos in response to their availability and physiological conditions. However, the impairment of this metabolic process reduces ATP generation, leading to oxidative stress and mitochondrial dysfunction. LPS has been shown to dysregulate the electron transport chain in mitochondria and increase the production of ROS in many cell lines [25].

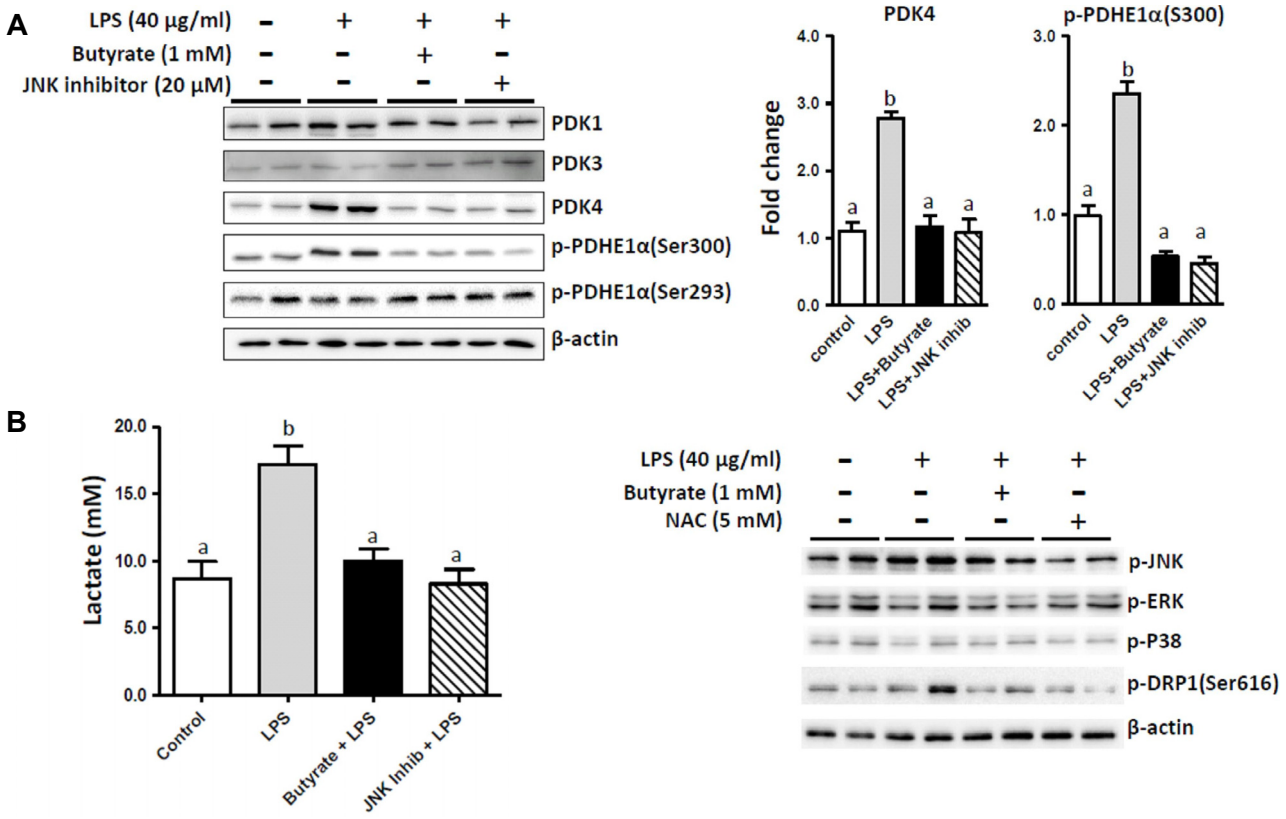

Fig. 5. Butyrate restores glucose metabolism altered by LPS treatment in C2C12 cells. (A) C2C12 cells (~80% confluence) were pretreated with butyrate (1 mM) or a JNK inhibitor (20 μM) for 1 hr, followed by LPS (40 μg/ml) treatment for 24 hr. Western blot analysis was performed using the indicated antibodies. Band density was determined using ImageJ. Results represent the mean ± SEM. Different letters represent significant differences (p<0.05) compare to the control. (B) C2C12 cells (~80% confluence) were washed with HBSS, added to DMEM without pyruvate and serum, and pretreated with butyrate (1mM) or a JNK inhibitor (20 μM) for 1 hr, followed by LPS (40 μg/ml) treatment for 24 hr. Culture media were collected and deproteinized using perchloric acid and neutralized. Lactate was measured enzymatically using a spectrophotometer. (C) C2C12 cells (~80% confluence) were pretreated with butyrate (1 mM) or NAC (5 mM) for 1 hr, followed by LPS (40 μg/ml) treatment for 24 hr. Western blot analysis was performed using the indicated antibodies.

Together with our previous studies [29], we found that LPS increases PDK4 expression to inhibit PDC activity, which shifts glucose metabolism from OxPhos to aerobic glycolysis. In skeletal muscle, PDK4 expression is induced by obesity, insulin resistance, and diabetes, while its inhibition has been shown to ameliorate metabolic diseases and cancers. Thus, PDK4 may be over-expressed in response to reduced OxPhos in mitochondria, thereby increasing ROS production [17, 30]. We also found that butyrate repressed LPS-induced PDK4 expression, supporting the theory that reduced PDK4 expression could restore OxPhos and inhibit ROS production in skeletal muscle during LPS treatment. Other studies have suggested that PDK4 overexpression induces muscle atrophy by elevating atrogin-1 and MuRF1 expression to increase protein catabolism [31]. We also observed that butyrate dramatically repressed LPS-induced atrogin-1 expression by reducing PDK4 expression; however, the exact mechanisms responsible for this phenomenon remain unclear. Since the effects of butyrate were similar to those of JNK inhibitors and the anti-oxidant NAC, we hypothesize that butyrate may exert anti-oxidant effects by inhibiting MAPK.

In conclusion, this study explored the effects of butyrate on LPS-induced mitochondrial dysfunction and skeletal muscle atrophy. We observed that butyrate could inhibit LPS-induced mitochondrial fragmentation and muscle atrophy by inhibiting MAPK signaling and PDK4 expression, indicating that butyrate improves mitochondrial structure and metabolism. Together, these findings suggest that butyrate could be used to improve mitochondrial dysfunction and myopathy induced by inflammatory conditions, such as metabolic diseases, cancers, and sepsis.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : C2C12 세포에서 lipopolysaccharide에 의해 유도된 근육위축증에 대한 butyrate의 개선효과: JNK 신호전달 억제와 미토콘드리아의 기능 개선

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대사성질환, 암, 손상, 및 패혈증 등에 의해 유도되는 염증은 산화스트레스를 통해 세포의 미토콘드리아의 기능을 감퇴시켜 신경증과 근육위축증 등을 야기한다. 본 연구에서는 lipopolysaccharide (LPS)에 의해 유도된 미토콘드리아의 기능감퇴와 근육위측증에 대한 butyrate의 억제효과를 확인하고자 하였다. LPS의 처리는 C2C12세포에서 MAPK의 활성을 통해 미토콘드리아 분열을 촉진하는 DRP1 (Ser616) 인산화와 Atrogin-1의 발현을 증가시켰다. 그러나 butyrate를 처리한 C2C12세포에서는 LPS 처리에 의한 염증 효과가 유의적으로 감소하며, 미토콘드리아 분열을 억제하는 DRP1 (Ser637)의 인산화와 mitofugin2 (Mfn2)의 발현을 증가를 유도하는 것을 확인하였다. 또한 butyrate를 처리한 세포에서 대사성질환을 유발하는 pyruvate dehydrogenase kinase 4 (PDK4)의 발현을 억제함이 관찰되었다. 이는 butyrate가 포도당 대사에서 염증에 의해 유도되는 Warburg 효과를 억제하여 산화스트레스를 개선함으로써, JNK의 활성을 억제하는 것으로 확인되었다. 이러한 결과들은 butyrate가 항산화효과를 통해 패혈증과 대사성질환과 같은 염증에 의해 유도되는 미토콘드리아의 기능 감퇴와 이에 따른 근육위축증을 개선할 수 있는 후보물질로 활용될 가능성이 있을 것으로 기대된다.