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Establishment of a novel mouse model for pioglitazone-induced skeletal muscle injury

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ABSTRACT

Skeletal muscle (SKM) injury is one of the major safety concerns in risk assessment for drug development. However, no appropriate pre-clinical animal model exists to evaluate drug-induced SKM injury except that caused by fibrates and statins. Thiazolidinedione, a PPARγ agonistic drug for type 2 diabetes mellitus, is widely used clinically but can induce adverse effects such as hepatotoxicity and SKM injury, as has been reported in recent decades. Moreover, thiazolidinedione-induced SKM injury has only been reported in humans, and no evidence of SKM injury has been observed in rodents. To establish a drug-induced SKM injury mouse model, we administered pioglitazone with a glutathione biosynthesis inhibitor, L-buthionine-*S,R*-sulfoximine, to C57BL/6J mice for 2 days and subsequently observed prominent increases in plasma aspartate aminotransferase and creatinine phosphokinase, which were associated with SKM lesions. Furthermore, plasma miR-206 (SKM-specific microRNA) level was significantly increased, whereas plasma miR-208 (heart-specific microRNA) was not detected, indicating that pioglitazone specifically caused SKM, not cardiac, injury. Furthermore, we revealed that pioglitazone-induced SKM injury was caused by oxidative stress that was independent of the PPARγ agonistic effect. This study demonstrated for the first time that the glutathione-depleted C57BL/6J mouse is a novel model for assessing drug-induced SKM injury in drug development.

1. Introduction

Drug-induced skeletal muscle (SKM) injury is observed following treatment with various drugs, although it is difficult to estimate whether SKM injury will occur in humans based on current pre-clinical safety assessment using conventional experimental animals. In addition, there is no appropriate animal model for the evaluation of drug-induced SKM injury. Recently, a collaboration between the Critical Path Institute and the Predictive Safety Testing Consortium Skeletal Muscle Working Group identified novel translational SKM biomarkers that overcome the disadvantage of creatinine phosphokinase (CPK) and aspartate aminotransferase (AST) (Burch et al., 2016), indicating that not only useful animal models but also sensitive biomarkers are needed in pre-clinical and clinical drug development.

In general, 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors (statins) and peroxisome proliferator-activated receptor alpha (PPARα) agonists (fibrates) have been known to cause SKM injury such as rhabdomyolysis and myopathy, and these changes were thought to be mediated by on-target effects (Levak-Frank et al., 1995; Osaki et al., 2015; Peraza et al., 2006). For example, cerivastatin, one of the most famous statins withdrawn from the market due to a high incidence of rhabdomyolysis, causes SKM injury in rats (Schaefer et al., 2004). However, long-term administration is needed to detect cerivastatin-induced SKM injury in normal rodents and histopathological analysis is crucial because changes in biomarkers such as CPK were not potent and specific.

A previous study reported that SKM results in low levels of catalase and superoxide dismutase compared with other tissues (Martensson and Meister, 1989), which might be expected given the dependence on glutathione (GSH) for reactive oxygen species

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSO, L-buthionine-*S,R*-sulfoximine; CD36, fatty acid translocase; CPK, creatinine phosphokinase; CRE, creatinine; DAMPs, damage-associated molecular pattern molecules; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione; HO-1, heme oxygenase 1; IL-6, interleukin 6; MIP-2, macrophage inflammatory protein-2; miRNA, microRNA; Nrf2, NF-E2-related factor 2; PIO, pioglitazone; PPAR, peroxisome proliferator-activated receptor; RT, reverse transcription; SKM, skeletal muscle; S100A8, S100 calcium binding protein A8; S100A9, S100 calcium binding protein A9; TNF, tumor necrosis factor.

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detoxication. Therefore, we speculated that an animal model with GSH depletion in SKM would be a powerful tool for detecting drug-induced SKM injury, which is caused by drug-induced oxidative stress. Actually, GSH-depleted rodents model have been widely used to elucidate mechanisms of drug-induced liver injury (Akai et al., 2016; Shimizu et al., 2009) because GSH-depletion is highly susceptible to reactive metabolite-mediated toxicity. Based on this information, SKM would also be a target organ for drug-induced injury if the drug induces oxidative stress.

Thiazolidinedione, a PPARγ agonistic drug for type 2 diabetes mellitus, is widely used in clinical practice because of its apparent efficacy, however, adverse effects have also been reported last few decades. Troglitazone has a thiazolidinedione structure but has been withdrawn from the market because of serious idiosyncratic hepatotoxicity (Gale, 2001). Recently, rosiglitazone has been rarely prescribed because it increased the risk of myocardial infarction, heart failure and total mortality in humans compared with pioglitazone (PIO) (Chen et al., 2012; Hsu et al., 2015). Conversely, PIO is currently prescribed although it is also associated with an increased risk of bladder cancer (Tuccori et al., 2016). In addition, thiazolidinediones, such as troglitazone, rosiglitazone and PIO, have caused SKM injury in patients (Kennie et al., 2007; Slim et al., 2009; Yokoyama et al., 2000), and the U.S. Food and Drug Administration has identified a potential safety issue given the correlation between PIO administration and rhabdomyolysis occurrence in humans (U.S. Food and Drug Administration, 2013). However, there is no evidence of thiazolidindione-induced SKM injury in rodents. In this study, we focused on PIO because of the high incidence of rhabdomyolysis among thiazolidinediones. PIO and L-buthionine-*S,R*-sulfoximine (BSO) were co-administered to C57BL/6J mice for 2 days, and drastic elevations in AST and CPK associated with histopathological lesions in SKM. We demonstrated for the first time that the GSH-depleted C57BL/6J mouse is a novel model for assessing drug-induced SKM injury.

2. Materials and methods

2.1. Materials

PIO was purchased from the Tokyo Chemical Industry (Tokyo, Japan). BSO and a "Tissue" ATP assay Kit were purchased from Wako Pure Chemical Industries (Osaka, Japan). Biphenol-A-diglicydyl ether (BADGE, a PPARγ antagonist) was purchased from Cayman Chemical (Ann Arbor, MI). The miRNeasy Serum/Plasma Kit was purchased from Qiagen (Valencia, CA). RNAiso Plus and SYBR Premix Ex Taq (Tli RNaseH Plus) were obtained from Takara (Otsu, Japan). The ReverTra Ace qPCR RT Kit was obtained from Toyobo (Osaka, Japan). The TaqMan® MicroRNA Reverse Transcription Kit and TaqMan® miRNA Assays including the microRNA (miRNA)-specific primer (ath-miR159a-3p, assay ID 000338; mmu-miR-133b-3p, assay ID 002247; mmu-miR-206-3p, assay ID 000510; and mmu-miR-208a-3p, assay ID 000511) and TaqMan® Universal Master Mix without UNG were obtained from Applied Biosystems (Foster City, CA). All primers for SYBR green real-time RT-PCR and ath-miR159a were commercially synthesized at Hokkaido System Sciences (Sapporo, Japan). Fuji DRI-CHEM slides of GPT/ALT-PIII, GOT/AST-PIII, CPK-PIII and CRE-PIII were from Fujifilm (Tokyo, Japan). Other chemicals used in this study were of analytical grade or were the highest grade commercially available.

2.2. Animals

Seven- to nine-week-old female ICR, BALB/c and C57BL/6J mice were obtained from Japan SLC (Hamamatsu, Japan). The animals were kept under a 12-h light/dark cycle (lights on 9:00–21:00) in a controlled environment (temperature 23 ± 2 °C and humidity 55 ± 10%) in the institutional animal facility. All animals were allowed free access to food and water, except when fasting was being conducted. An overnight fasting was conducted before PIO administration and necropsy. After PIO administration, the mice were again allowed access to food *ad libitum*. The animals were acclimatized before use in the experiments. All procedures performed on animals were approved by the Institutional Animal Care and Use Committee of Nagoya University Graduate School of Medicine (No. 27042). All animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Nagoya University Graduate School of Medicine.

2.3. PIO treatment

Co-administration of PIO and BSO was performed for 2 days to establish an SKM injury model. BSO was dissolved in saline (70 mg/mL) and intraperitoneally administered at a dose of 700 mg/kg to mice once daily 1 h before the PIO administration. PIO was mixed with corn oil (6, 20 and 60 mg/mL suspension) and subsequently orally administered to mice once daily at doses of 60, 200 and 600 mg/kg (10 mL/kg). To exclude PIO-induced PPARγ agonistic effects, BADGE was intraperitoneally administered at 120 mg/kg once daily from 2 days before PIO administration and for 2 days during PIO administration. BADGE was given between the BSO and PIO administrations. This dose regimen was selected because it has previously been shown that rosiglitazone-mediated anti-inflammatory effects were blocked by BADGE administration at a dose of 120 mg/kg (Dworzanski et al., 2010). BADGE was dissolved in DMSO and diluted with PBS to a final concentration of 10% DMSO just before administration as previously described (Naveiras et al., 2009).

2.4. Blood chemistry and pathological examination

At the necropsy, the animals were killed by isoflurane anesthesia, and blood was collected from the inferior vena cava and tissue samples were collected 24 h after the last drug administration. Plasma alanine aminotransferase (ALT), AST, CPK and creatinine (CRE) levels were measured using the Dri-Chem 4000 (Fujifilm, Tokyo, Japan) according to the manufacturer's instructions. Tissue samples from the liver, kidney, heart and biceps muscle were excised and fixed in 10% neutral-buffered formalin. The fixed samples were dehydrated with alcohols and embedded in paraffin. Serial sections were stained with hematoxylin and eosin for histopathological examination.

2.5. Quantitative measurement of PIO in plasma

To measure the plasma concentration of PIO, blood was collected from the jugular vein at 0.5, 1, 2, 4, 8, 24 h and from the inferior vena cava at 48 h (necropsy point) after the first PIO administration. Plasma samples were diluted 10-fold with Milli-Q water and 10 μL of the diluted sample was transferred into a tube that contained 280 μL Milli-Q water. Celecoxib (500 μg/mL in DMSO, 10 μL) was added as an internal standard (IS) to each sample. Then, the samples were applied onto an Oasis HLB cartridge (Waters, Milford, MA) for clean-up and washed with 5% methanol and subsequently extracted using 1000 μL of 2% formic acid/98% acetonitrile. The eluted samples were then transferred into injection vials for subsequent analysis by a Waters Acquity ultra-performance liquid chromatography system consisting of a TQ detector, binary solvent manager, sample manager and column oven. The injection volume was 5 μL. Liquid chromatographic separations were performed using the Waters Acquity ultra-performance liquid chromatography system with a Cadenza CD-