Archivum Immunologiae et Therapiae Experimentalis (2018) 66:453–461
https://doi.org/10.1007/s00005-018-0518-6

ORIGINAL ARTICLE

Baicalin Attenuates Joint Pain and Muscle Dysfunction by Inhibiting Muscular Oxidative Stress in an Experimental Osteoarthritis Rat Model

De-sheng Chen[1] · Jian-gang Cao[1] · Bo Zhu[1] · Zeng-liang Wang[1] · Tong-fu Wang[1] · Jian-jun Tang[1]

Received: 22 December 2017 / Accepted: 4 June 2018 / Published online: 3 August 2018

Abstract
Osteoarthritis (OA) is the most common degenerative joint disease, and causes major pain and disability in adults. It has been reported that muscle weakness and inflammation contribute to osteoarthritis development and progression. Oxidative stress plays important roles in muscle dysfunction and inflammation in osteomyelitis. Baicalin, the major active constituent of the isolated root of *Scutellarialateriflora Georgi*, has been shown to have anti-oxidative and anti-inflammatory effects. In this study, we evaluated the potential effects of baicalin on osteoarthritis. We established experimental osteoarthritis rat model, applied baicalin to the rats, and then explored the potential protective effect of baicalin on osteoarthritis severity, muscle dysfunction, and oxidative stress. Baicalin alleviated severity of OA in rats. Baicalin application attenuated muscle dysfunction in OA rats by increasing citrate synthase activity, myosin heavy chain IIa expression, and decreasing interleukin 6 production. Baicalin decreased muscular reactive oxygen species generation in OA rats. Baicalin inhibited nuclear factor erythroid-derived 2-like 2 expression in OA rats. Baicalin attenuated osteoarthritis in rat by inhibiting oxidative stress.

Keywords Baicalin · Osteoarthritis · Joint pain · Muscle · Oxidative stress

Introduction

Osteoarthritis (OA) is a progressive and dynamic process in joint tissues including muscle, cartilage, and synovium, which affects more than 25% the population over 18 years old (Chen et al. 2017). It has been predicted that around 67 million people in the US will be diagnosed with OA (Hootman and Helmick 2006). Therefore, OA is considered as one of the major public health problems worldwide (Velasquez and Katz 2010).

Various factors are associated with pathogenesis of OA. It has been described that oxidative stress participates in quadriceps muscle dysfunction during the initiation of OA in rats model (Hsu et al. 2015). Tonge et al. (2013) reported that the initiation of OA in the guinea pig was associated with changes in quadriceps skeletal muscles and improving quadriceps weakness was a strategy for preventing OA development. With more studies in depth, some mechanisms involved in OA-associated quadriceps muscle weakness have been elucidated. Myosin heavy chain (MHC) alteration played a dominant role in muscle strength and decreased MHC IIa mRNA and protein expressions had been reported to be associated with muscle weakness in OA patients (Callahan et al. 2014). Cytokines and citrate synthase have been also reported to be involved in the muscle mass and strength loss.

Oxidative stress is also reported to be involved in OA development and progression (Hsu et al. 2016) and is one of inflammation mediators responsible for muscle dysfunction in OA (Hsu et al. 2015) and other diseases (Pereira et al. 2015; Spassov et al. 2011). Increased oxidant generation and decreased antioxidant protection are the results once oxidative stress is imposed on cells. Under the oxidative stress, reactive oxygen species (ROS) including speroxide anion and peroxynitrite are produced and both of which are important mediators of lipid peroxidation (LPO). During oxidative stress, nuclear factor erythroid-derived 2-like 2 (Nrf2), a redox-sensitive transcriptional factor, was upregulated (Ma 2013), while the potent-free radical scavenger glutathione (GSH) which was synthesized by glutathione peroxidase

De-sheng Chen and Jian-gang Cao have contributed equally to this work.

✉ De-sheng Chen
chendesheng0901@126.com

[1] Department of Sports Injury and Arthroscopy, Tianjin Hospital, Tianjin 300222, People's Republic of China

(GPx) played important roles in attenuating oxidative stress (Kaplowitz et al. 1985). Thus, LPO, Nrf2, GSH, and GPx are all critical biomarkers of oxidative stress (Lei et al. 2017).

Baicalin is the predominant flavonoid isolated from the roots of *Scutellarialateriflora Georgi* (Huang Qin) and is well known for its anti-oxidative (Gao et al. 2001), anti-inflammation (Shen et al. 2003), anti-tumor (Ikezoe et al. 2001), and anti-virus (Xu et al. 2010) activities. The protective effects of baicalin in OA had been already described (Xing et al. 2017). However, the underlying mechanisms are not fully elucidated. In this study, we tested the potential role of baicalin on muscle dysfunction and oxidative stress in OA rats. We reported that baicalin attenuated muscle dysfunction in OA rats by increasing citrate synthase activity, MHC IIa expression, and decreasing interleukin (IL)-6 production. Baicalin decreased muscular ROS generation and inhibited Nrf2 expression in OA rats. Thus, we proposed a new molecular mechanism underlying baicalin therapy of OA.

Materials and Methods

Animals

The use of rats conformed to the Guiding Principles in the Care and Use of Animals approved by the Council of the American Physiology Society. Male Sprague–Dawley rats weighing 200–210 g were used ($n = 180$). Animals were housed 5 per cage, provided with distilled water and food ad libitum, and kept under a 12-h light/dark cycle at constant temperature (22.5 °C) and humidity (55%). The protocol was approved by the Committee on the Ethics of Animal Experiments of Tianjin Hospital (#TJH0563R).

Induction of Osteoarthritis

Meniscectomy (MNX) was used to induce osteoarthritis as described before (Lei et al. 2017). The rats were inhalational anesthetized under 3.5% isoflurane (Sigma, MO, USA). OA was induced in rats of the right knee and the left knee was not treated. Then, a small incision was made longitudinally down the medial side of the knee and a cauterizer was used to work through both the connective tissue and the muscle layers until the medial collateral ligament, anchoring the medial meniscus to the tibial plateau, was identified. The ligament was grasped at the tibial end and cut until fully transected. The ligament was then transected again at the femoral end to remove the portion overlying the meniscus. The meniscus was freed from the fine connective tissue, allowing a full-thickness MNX. The connective tissue layer and skin were closed with coated Vicryl 8–0 and 4–0 sutures, respectively. Rats were received cephalexin (Ceporex oral drops, Sigma, USA) 1 h before and 12, 24, and 36 h after surgery (30-μL/100-g body weight) as described previously (Lei et al. 2017). The rats were carefully monitored for infections and other complications for 14 days after surgery. All surgical sites were observed daily for signs of abnormal healing including, but not limited to swelling, redness, drainage, bleeding, and dehiscence. OA pathology and pain behavior were allowed to develop for a period of 14 days after model induction (Mapp et al. 2013).

Experimental Design

Rats were randomly divided into five groups, the sham ($n = 9$), osteoarthritis without treatment (OA + vehicle; $n = 10$), osteoarthritis (OA) with 50-mg/kg baicalin treatment ($n = 9$), osteoarthritis with 100-mg/kg baicalin treatment ($n = 9$), and osteoarthritis with 200-mg/kg baicalin treatment ($n = 11$). These baicalin doses were used in other rat models as previously described (Wang et al. 2014). Sham animals underwent the same surgical procedure with the omission of MNX. For baicalin-treated groups, rats were injected with different amount of baicalin daily for 14 days right after MNX. Fresh phosphate-buffered saline was used as vehicle.

Secondary Mechanical Allodynia

The secondary mechanical allodynia was assessed with von Frey Filaments (North Coast Medical, Inc. Morgan Hill, CA, USA) as described previously (Wen et al. 2016). The filament diameters corresponded to a logarithmic scale of the force exerted, and thus, the perceived intensity was measured on linear and interval scales. The withdrawal threshold was determined by Chaplan's up–down method involving the use of alternate large and small fibers to determine the 50% withdrawal threshold. Each von Frey filament was applied to the plantar surface of the paw for 5 s. Briefly, when the rat lifted its paw in response to pressure, the filament size was recorded, and a weaker filament was then tested. The von Frey filament was applied to each paw for five trials at approximately 3-min intervals.

Weight-Bearing Distribution T Test

The change in hind paw weight distribution was used as an index of joint discomfort and was determined as described previously (Wen et al. 2016). The effect of joint damage on weight distribution through knees was measured using a dual channel weight averager (Singa Technology Corporation, Taipei, Taiwan), which measures weight bearing by each hind paw independently. Briefly, rats were placed in an angled plexiglass chamber positioned, so that each hind paw rested on a separate force plate. The force exerted by each hind limb (measured in grams) was averaged over a

5-s period. Each data point is the mean of three 5-s readings. The hind paw weight distribution is expressed as the difference in weight bearing between the contralateral and ipsilateral limbs.

Muscle Harvest

Muscle harvest was performed at 14 days after the assessment of nociception as described before (Lei et al. 2017). Whole bilateral quadricep muscle samples, inclusive of the rectus femoris, were dissected, weighed, and immediately snap frozen in isopentane cooled with liquid nitrogen. Care was taken to avoid inclusion of any adipose tissue or additional muscle, most importantly the tensor fasciae latae and sartorius that are located within the dissected area. Then, the muscles from each mouse were divided into several parts for RT-PCR, ELISA, and other experiments.

RT-PCR

The total RNA from muscular tissue was isolated using an RNeasy Mini kit (Qiagen, CA, USA) according to the manufacturer's instructions with an additional DNase I (Fermentas)-treatment step to eliminate residual genomic DNA. Reverse transcription was performed using a reverse transcription kit (Applied Biosystem, Waltham, MA, USA). Real-time quantitative PCR reactions were set up in triplicate with SYBR® Green Master Mix (Bio-Rad, CA, USA) and run on a LightCycler 480 (Roche, Penzberg, Upper Bavaria, Germany). The following primers were used in the current study: MHC IIa forward: MHC IIa forward: 5′-AGA CCTGGATCGTCTCGTG-3′, MHC IIa reverse: 5′-AGT GCATGATTTGAGCGTCTC-3′. GPx forward: 5′-CCA CCGTGTATGCCTTCTCC-3′, GPx reverse: 5′-AGAGAG ACGCGAC ATTCTCAAT-3′. GAPDH forward: 5′-TGG CCTTCCGTGTTCCTAC-3′, GAPDH reverse: 5′-GAGTTG CTGTTGAAGTCGCA-3′. The amount of MHC II mRNA expression was normalized with GAPDH mRNA value.

ELISA

Serum and muscular level of LPO, and muscular production of IL-6 were detected using commercial ELISA kits from R&D systems (Minneapolis, USA), following the manufacturer's instructions. The tissue homogenate protein concentrations were determined using protein assay dye (Bio-Rad Laboratory, USA).

Citrate Synthase Activity Assay

The muscular citrate synthase activity was detected using Citrate Synthase Assay Kit purchased from Sigma (St. Louis, USA), following the manufacture's instructions.

ROS-Level Detection

The detection of ROS was performed as described before (Lei et al. 2017). Superoxide and peroxynitrite were measured using a high-performance chemiluminescence (CL) analyzer (CLA-2100; Tohoku Electronic Industrial Co., Japan). Briefly, 400 µL of tissue homogenate was mixed with 200 µL of phosphate buffer solution in a stainless dish, and then, the background CL count was read for 60 s. 100 µL of lucigenin orindoxyl b-D-glucuronide was injected into the machine, and the CL was counted. The data were analyzed using Chemiluminescence Analyzer Data Acquisition Software (Tohoku Electronic Industrial Co., Japan).

Glutathione-Level Detection

Muscular tissues were homogenized and the glutathione level was measured using Glutathione Colorimetric Detection Kit (Thermo Fisher, MA, USA), following the manufacturer's protocol.

Western Blotting

Muscular proteins were extracted using Total Protein Extraction Kit (MilliporeSigma, MA, USA) following the manufacturer's protocol. 20 µg protein was loaded on SDS-PAGE, and then transferred to polyvinylidene fluoride membrane (Bio-Rad, CA, USA). After blocking, the blots were incubated with Nrf2 or actin antibody (dilution 1:1000) in 5% non-fat skim milk. The membranes were washed and probed with appropriate horseradish peroxidase conjugated secondary antibody. Chemiluminescent substrate (Thermo Fisher Scientific, Waltham, MA) was used to detect the bands. All antibodies were purchased from Abcam (Cambridge, MA, USA), including anti-Nrf2 antibody (ab62352) and anti-β-actin antibody (ab8227). The western blot results were quantitated and analyzed using GS-900™ Calibrated Densitometer and software Image Lab (Bio-Rad, USA) following the manufacturer's instructions.

Statistical Analysis

One-way ANOVA analysis, followed by a Tukey's post hoc test, was used to determine the related protein levels. Statistical difference was considered as significant only if $p < 0.05$.

Results

Baicalin Alleviated Secondary Mechanical Allodynia in OA Nociception Pain Mode

First, we explored the effects of baicalin on secondary mechanical allodynia in OA rats. As shown in Fig. 1, paw withdrawal latency decreased in OA rats. In contrast, rats treated with baicalin displayed increased paw withdrawal latency, which started from 4-day post-OA induction and in a dose-dependent manner when compared to OA rats. The highest dose (200 mg/kg) baicalin treatment showed best anti-allodynic effect. Thus, our data indicated that baicalin alleviated secondary mechanical allodynia in OA rats.

Baicalin Attenuated Joint Pain in OA Rats

In experimental OA model, weight-bearing asymmetry was supposed to be due to the pain induced by destruction of cartilage (Mihara et al. 2007). So next, we assessed the weight distribution of the OA and contralateral hind paws. As shown in Fig. 2, OA rats showed weight-bearing asymmetry. In contrast, the weight-distribution differential between the OA and contralateral hind paws in baicalin (50 mg/kg) treated OA rats was lower than that of OA rats at 4–14 days after surgery. We observed the same trend in higher dose baicalin-treated rats (100 and 200 mg/kg), indicating that baicalin decreased the weight-distribution differential. Thus, our data suggested that baicalin attenuates joint pain in OA rats.

Baicalin Relieved Quadriceps Muscle Dysfunction in OA Rats

It had been reported that IL-6, MHC II, and citrate synthase activity were indicators of quadriceps muscle dysfunction in rat model of OA (Lei et al. 2017). Thus, we further assessed the effects of baicalin on muscle dysfunction by monitoring muscular IL-6 production, citrate synthase activity, and MHC IIa mRNA level, which is shown in Fig. 3. Muscular IL-6 production was significantly higher in OA rats when compared to rats in sham group (sham: 100 ± 8.79; OA: 188.8 ± 7.89; OA + 50 mg/kg baicalin: 167.5 ± 6.46;

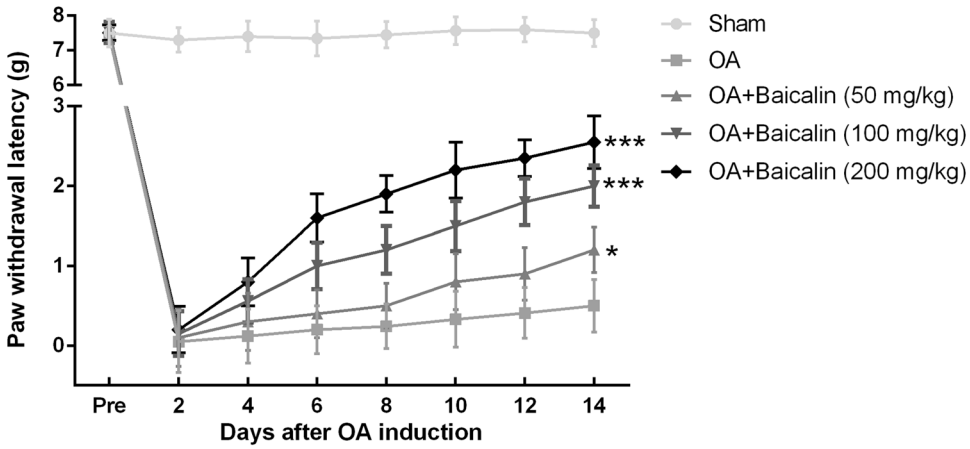

Fig. 1 Time course of the anti-allodynic effect of baicalin treatment in MNx-induced OA nociception pain model. Data are the mean ± SEM of the ipsilateral hind paw of each group. *$p < 0.05$, ***$p < 0.001$ compared to OA group

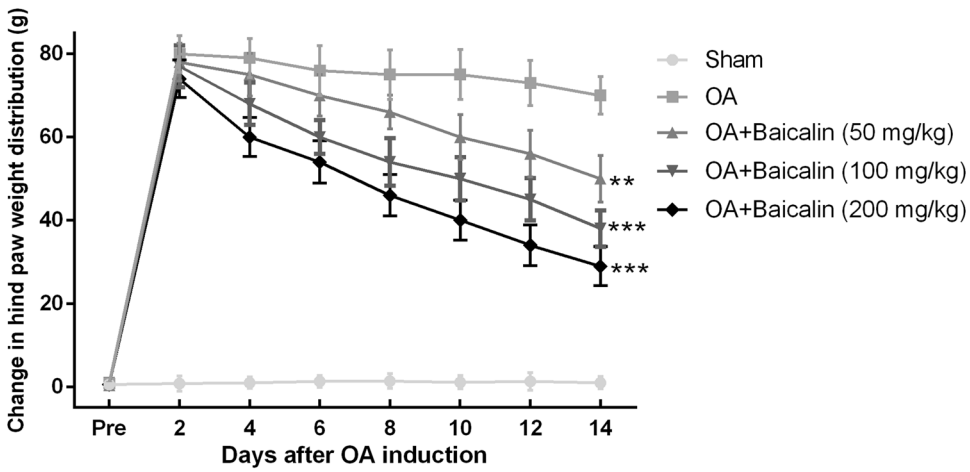

Fig. 2 Time course of the baicalin treatment on weight bearing of the contralateral (left) and ipsilateral (right) OA hind paws for 14 days after MNx surgery. The baseline was set as the weight-bearing differential prior to surgery. Data are the mean ± SEM of each group. **$p < 0.01$ and ***$p < 0.001$ compared to OA group

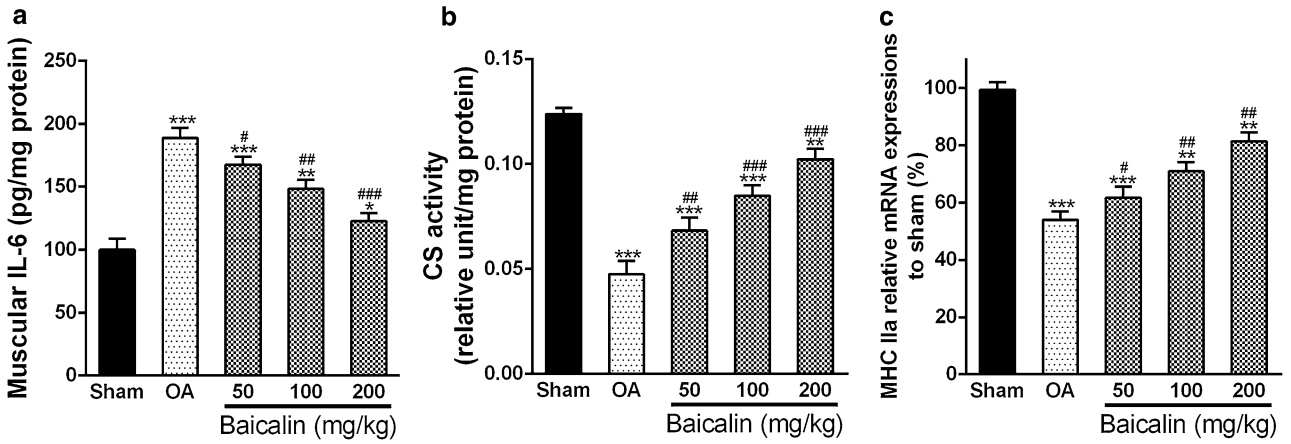

Fig. 3 Baicalin attenuates muscle dysfunction in MNx-induced OA rats. ELISA of muscular IL-6 production (**a**), citrate synthase (CS) activity (**b**), and qPCR of MHC IIa mRNA expression (**c**) levels were tested on 14 days after OA induction. Data are the mean ± SEM of each group. $N = 11$ in each group. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to sham group; $\#p < 0.05$, $\#\#p < 0.01$ and $\#\#\#p < 0.001$ compared to OA group

OA + 100 mg/kg baicalin: 148.3 ± 7.14; OA + 200 mg/kg baicalin: 122.5 ± 6.45). Baicalin significantly decreased muscular IL-6 production in a dose-dependent manner (Fig. 3a). As shown in Fig. 3b, OA caused decreased citrate synthase activity, while the activity deficiency was rescued by baicalin treatment (sham: 0.124 ± 0.003; OA: 0.047 ± 0.006; OA + 50 mg/kg baicalin: 0.068 ± 0.006; OA + 100 mg/kg baicalin: 0.085 ± 0.005; OA + 200 mg/kg baicalin: 0.102 ± 0.005). Similarly, OA also induced decreasing of MHC IIa mRNA levels and baicalin recovered MHC IIa expression in OA rats (Fig. 3c). Thus, our data indicated that baicalin relieved muscle dysfunction in OA.

Baicalin Reduced Muscular LPO Level in OA Rats

Oxidative stress were involved in the development and progress of OA (Hsu et al. 2016). Therefore, we continued to explore the effect of baicalin on serum and muscular LPO levels in OA rats. OA did not affect LPO level in serum and baicalin treatments did not affect serum level of LPO neither (Fig. 4a), as there was no difference among OA group and groups treated with different amount of baicalin (sham: 19.75 ± 2.22; OA: 19.25 ± 2.63; OA + 50 mg/kg baicalin: 19.22 ± 1.26; OA + 100 mg/kg baicalin: 19.77 ± 2.21; OA + 200 mg/kg baicalin: 18.25 ± 1.89). In contrast, OA rats showed significantly increased muscular LPO level and the increased LPO level was alleviated by baicalin treatment in a dose-dependent manner (Fig. 4b) (sham: 23.75 ± 4.79; OA: 53.77 ± 5.80; OA + 50 mg/kg baicalin: 45.50 ± 7.77; OA + 100 mg/kg baicalin: 42.25 ± 3.94; OA + 200 mg/kg baicalin: 34.33 ± 7.06). Thus, our data indicated that baicalin reduced muscular LPO level and inhibited muscular oxidative stress.

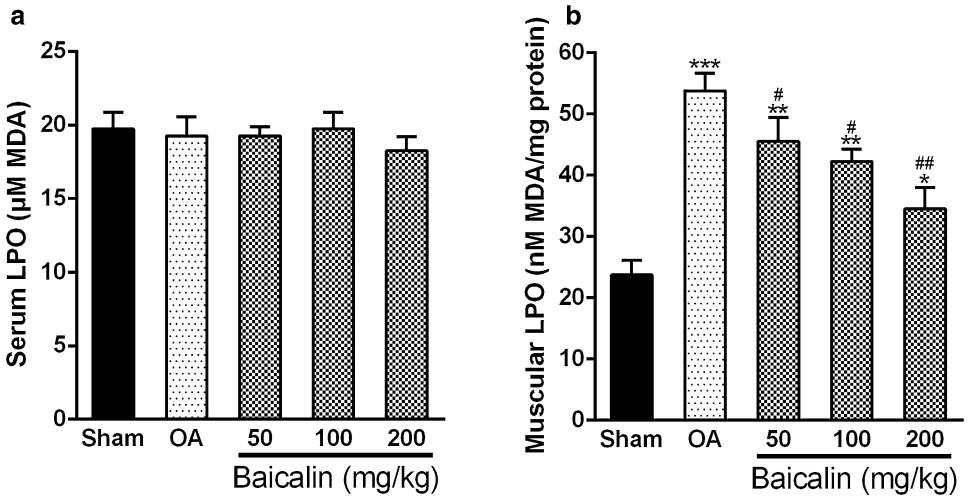

Fig. 4 Effects of baicalin on serum and muscular LPO levels in MNx-induced OA rats. Serum (**a**) and muscular (**b**) LPO levels were assessed 14 days after OA-induced. Data are the mean ± SEM of each group. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to sham group; $\#p < 0.05$, $\#\#p < 0.01$ compared to OA group

Baicalin Reduced Muscular ROS Generation and Increased Muscular Antioxidant Level in OA Rats

We continued to monitor the effect of baicalin on oxidative stress-related products. As shown in Fig. 5, OA significantly increased muscular level of superoxide anion (sham: 385.2 ± 23.18; OA: 723.8 ± 27.21; OA + 50 mg/kg baicalin: 663.8 ± 25.14; OA + 100 mg/kg baicalin: 551.3 ± 24.24; OA + 200 mg/kg baicalin: 474.9 ± 28.32) (Fig. 5a), and peroxynitrite (sham: 235.8 ± 32.21; OA: 481.3 ± 28.98; OA + 50 mg/kg baicalin: 446.3 ± 23.64; OA + 100 mg/kg baicalin: 367.3 ± 28.77; OA + 200 mg/kg baicalin: 306.2 ± 30.71) (Fig. 5b). In contrast, baicalin treatment significantly decreased both muscular superoxide anion and peroxynitrite levels in OA rat in a dose-dependent manner. We detected decreased muscular level of antioxidants GSH (sham: 26.5 ± 1.29; OA: 15.2 ± 2.26; OA + 50 mg/kg baicalin: 18.1 ± 1.83; OA + 100 mg/kg baicalin: 19.5 ± 1.57; OA + 200 mg/kg baicalin: 21.6 ± 1.71) (Fig. 5c) and GPx (sham: 99.5 ± 2.66; OA: 34.7 ± 4.21; OA + 50 mg/kg baicalin: 50.8 ± 5.13; OA + 100 mg/kg baicalin: 54.9 ± 6.19; OA + 200 mg/kg baicalin: 64.7 ± 6.02) (Fig. 5d) in OA mice, while baicalin treatment rescued the GSH and GPx expression. Thus, our data further suggested that baicalin attenuated oxidative stress in OA rats.

Baicalin Inhibited OA-Induced Nrf2 Expression

It has been reported that Nrf2 played a critical role in oxidative stress (Hsu et al. 2015). Consistent to the previous report, we confirmed that muscular expression of Nrf2 was increased in OA rats (Fig. 6a). Baicalin treatment significantly inhibited OA-induced Nrf2 expression in a dose-dependent manner (Fig. 6a, b). Thus, our data indicated the baicalin prevented oxidative stress in OA.

Discussion

Osteoarthritis, a common form of arthritis, is an age-related degenerative disease characterized by the chronic joint pain, inflammation, and the damage of joint cartilage which would affect more than 25% of adult population. Currently, there is no effective therapeutic treatment for OA, it is necessary to develop drugs to treat OA and elucidate underlying molecular mechanisms.

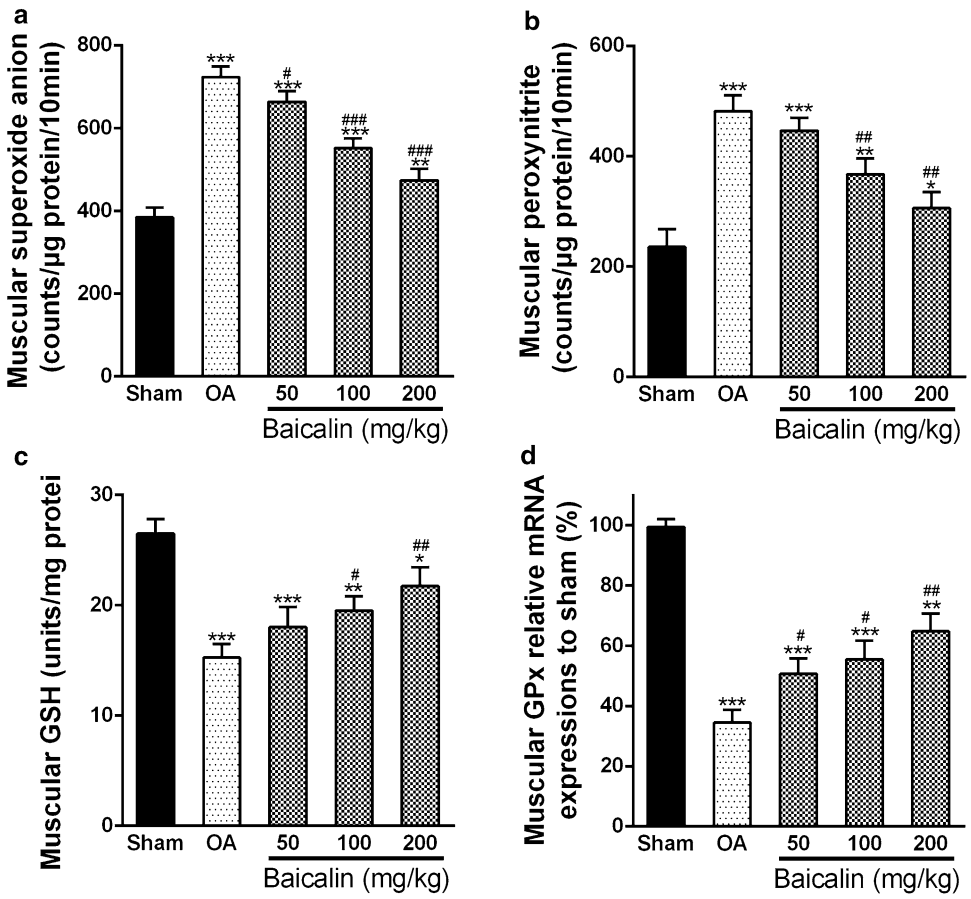

Fig. 5 Baicalin attenuates muscular ROS generation in MNx-induced osteoarthritis rats. Muscular superoxide anion (**a**), peroxynitrite (**b**), GSH (**c**), and qPCR of GPx expression compared with GAPDH (**d**) were tested on 14 days after OA-induced. Data are the mean ± SEM of each group. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to sham group; #$p < 0.05$, ##$p < 0.01$ and ###$p < 0.001$ compared to OA group

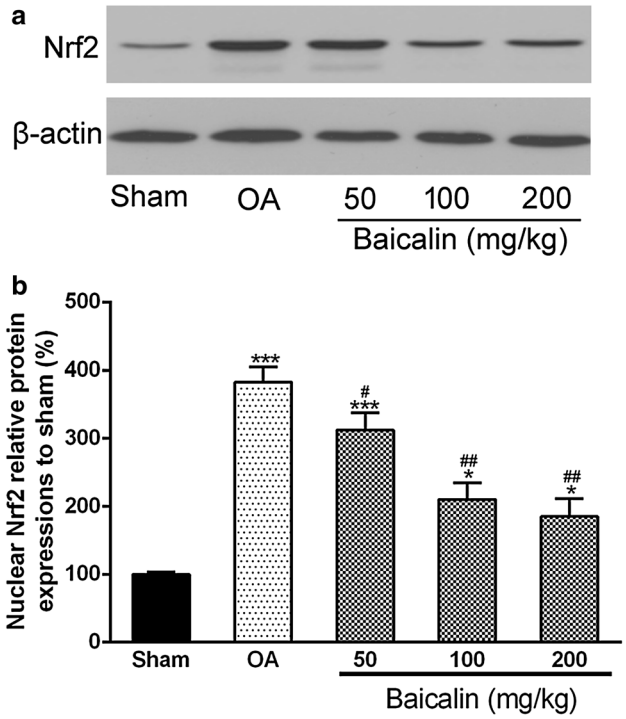

Fig. 6 Baicalin attenuates joint pain and muscle dysfunction by inhibiting the expression of muscular Nrf2 level in MNx-induced osteoarthritis rats. Western blotting was used to assay the protein expression, β-actin was used as a loading control (**a**) and relative expressions from the western blotting (**b**). Data are presented as mean ± SEM. $*p < 0.05$ and $***p < 0.001$ compared to sham group. $\#p < 0.05$, $\#\#p < 0.01$ compared to OA group

In the current study, we found that baicalin, which is the predominant flavonoid isolated from the roots of *Scutellaria-alateriflora Georgi* (Huang Qin) and has anti-inflammatory and anti-oxidative effect, attenuated joint pain and muscle dysfunction, and played a protective role in OA. Actually, the protective role of baicalin on OA has been described before. Xing et al. (2017) reported that baicalin inhibited the inflammatory response to IL-1β on human chondrocytes and reduced synovitis scores and IL-6, TNF, CXCL1, CXCL10, and MMP3 gene expression in OA mice. Their studies described the essential role of inflammation in OA development and the protective role of baicalin in OA by inhibiting inflammation. As anti-inflammatory effects of baicalin has been described in several disease model (Dong et al. 2015; Liu et al. 2016; Lixuan et al. 2010; Yang et al. 2013), it is not surprised that baicalin may also play role in inflammatory response in OA model.

Besides anti-inflammatory effect, the anti-oxidative effects of baicalin have been also reported. Gong and Zhu (2018) described that baicalin alleviates the oxidative stress damage in trabecular meshwork cell by decreasing iROS production. Lin et al. (2014) reported that baicalin ameliorates H_2O_2-induced cytotoxicity in HK-2 cells by inhibiting oxidative stress and Nrf2 signaling.

In this study, we investigated the therapeutic effects of baicalin on OA and confirmed the protective effect of baicalin on OA in the rat model. As oxidative stress and muscle dysfunction have been implicated in contributing to OA development (Hsu et al. 2016), we further investigated the effects of baicalin on muscle dysfunction and oxidative stress. We demonstrated that baicalin potently attenuated the joint pain in OA rats and oxidative stress by decreasing muscular LPO, muscular superoxide anion and peroxynitrite generations, and decreasing Nrf2 expression, increasing GSH, and GPx expression. Thus, we elucidated another mechanism of baicalin to treat OA by attenuating muscular oxidative stress in rats.

The activity of baicalin in attenuating pain has been described previously. Cherng et al. (2014) reported that baicalin ameliorated neuropathic pain in rat model by suppressing histone deacetylase expression and following inflammatory cytokines expression. Thus, both our and their results suggested the pain relief activity of baicalin. During OA, the increased oxidative stress and ROS resulted in upregulation of NF-κB and activation of MEK/ERK pathway, which induced production of proinflammatory cytokines including IL-8, IL-1β, and TNF-α (Lepetsos and Papavassiliou 2016). On the other hand, inflammatory cytokine can also stimulate the production of ROS and promote the generation of peroxides and hydroxylated radicals (Wojdasiewicz et al. 2014). The anti-inflammation and anti-oxidative activities of baicalin contributed to the protective effect on OA.

Muscle dysfunction was detected in the early stage of OA. Decreased MHC IIa mRNA expression was found in OA and decreased MHC IIa fiber was associated with muscle weakness in the quadriceps in OA patients (Hsu et al. 2015). Our data demonstrated that baicalin increased mRNA level of MHC IIa in OA rats, which could be one of the mechanisms to attenuate muscle dysfunction. Protein oxidation could also play an important role in muscle dysfunction as oxidative stress modified the structure and function of proteins. It had been suggested that oxidation resulted in a structure perturbation in myosin, which caused impaired myosin function (Yamada et al. 2006). The anti-oxidative effect of baicalin could protect myosin normal function.

Nrf2 is a redox-sensitive transcriptional factor and the major emerging function of Nrf2 is the role in resistance to oxidative stress. Consistent to the previous finding (Lei et al. 2017), we found that Nrf2 is upregulated in OA rats, indicating that these rats suffered from oxidative stress. Baicalin treatment resulted in decreased Nrf2 level in OA rats, indicating downregulated oxidative stress. As baicalin prevented oxidative stress and ROS production, it is not surprised that baicalin-treated OA rats got less Nrf2 expression.

The correlation between muscular dysfunction and OA pathogenesis had been established. For example, at the early stage of OA, the muscle weakness in patients was detected. More and more evidences have shown that muscle dysfunction participated in pathogenesis and development of OA (Ikeda et al. 2005). In contrast, strengthening of quadriceps muscles during the initial stages of OA had an important physiotherapeutic effect against OA (O'Reilly et al. 1999). Thus, improving quadriceps muscle dysfunction could be a therapeutic target in preventing OA. As baicalin attenuated the oxidative stress and muscle dysfunction, it would be a reliable therapeutic agent to treat OA. However, more studies need to be done to identify the efficient delivery method and elucidate other underlying mechanisms.

In conclusion, we demonstrated that baicalin alleviated joint pain and muscle dysfunction in OA by inhibiting muscular oxidative stress.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Research Involving Human Participants and/or Animals All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed Consent Not applicable.

References

Callahan DM, Miller MS, Sweeny AP et al (2014) Muscle disuse alters skeletal muscle contractile function at the molecular and cellular levels in older adult humans in a sex-specific manner. J Physiol 592:4555–4573

Chen D, Shen J, Zhao W et al (2017) Osteoarthritis: toward a comprehensive understanding of pathological mechanism. Bone Res 5:16044

Cherng CH, Lee KC, Chien CC et al (2014) Baicalin ameliorates neuropathic pain by suppressing HDAC1 expression in the spinal cord of spinal nerve ligation rats. J Formos Med Assoc 113:513–520

Dong SJ, Zhong YQ, Lu WT et al (2015) Baicalin inhibits lipopolysaccharide-induced inflammation through signaling NF-kappaB pathway in HBE16 airway epithelial cells. Inflammation 38:1493–1501

Gao Z, Huang K, Xu H (2001) Protective effects of flavonoids in the roots of Scutellaria baicalensis Georgi against hydrogen peroxide-induced oxidative stress in HS-SY5Y cells. Pharmacol Res 43:173–178

Gong L, Zhu J (2018) Baicalin alleviates oxidative stress damage in trabecular meshwork cells in vitro. Naunyn Schmiedebergs Arch Pharmacol 391:51–58

Hootman JM, Helmick CG (2006) Projections of US prevalence of arthritis and associated activity limitations. Arthritis Rheum 54:226–229

Hsu DZ, Chu PY, Wu PT et al (2015) Oxidative stress participates in quadriceps muscle dysfunction during the initiation of osteoarthritis in rats. Int J Clin Exp Pathol 8:12491–12499

Hsu DZ, Chu PY, Jou IM (2016) Daily sesame oil supplement attenuates joint pain by inhibiting muscular oxidative stress in osteoarthritis rat model. J Nutr Biochem 29:36–40

Ikeda S, Tsumura H, Torisu T (2005) Age-related quadriceps-dominant muscle atrophy and incident radiographic knee osteoarthritis. J Orthop Sci 10:121–126

Ikezoe T, Chen SS, Heber D et al (2001) Baicalin is a major component of PC-SPES which inhibits the proliferation of human cancer cells via apoptosis and cell cycle arrest. Prostate 49:285–292

Kaplowitz N, Aw TY, Ookhtens M (1985) The regulation of hepatic glutathione. Annu Rev Pharmacol Toxicol 25:715–744

Lei M, Guo C, Hua L et al (2017) Crocin attenuates joint pain and muscle dysfunction in osteoarthritis rat. Inflammation 40:2086–2093

Lepetsos P, Papavassiliou AG (2016) ROS/oxidative stress signaling in osteoarthritis. Biochim Biophys Acta 1862:576–591

Lin M, Li L, Zhang Y et al (2014) Baicalin ameliorates H2O2 induced cytotoxicity in HK-2 cells through the inhibition of ER stress and the activation of Nrf2 signaling. Int J Mol Sci 15:12507–12522

Liu J, Wei Y, Luo Q et al (2016) Baicalin attenuates inflammation in mice with OVA-induced asthma by inhibiting NF-kappaB and suppressing CCR7/CCL19/CCL21. Int J Mol Med 38:1541–1548

Lixuan Z, Jingcheng D, Wenqin Y et al (2010) Baicalin attenuates inflammation by inhibiting NF-kappaB activation in cigarette smoke induced inflammatory models. Pulm Pharmacol Ther 23:411–419

Ma Q (2013) Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53:401–426

Mapp PI, Sagar DR, Ashraf S et al (2013) Differences in structural and pain phenotypes in the sodium monoiodoacetate and meniscal transection models of osteoarthritis. Osteoarthritis Cartilage 21:1336–1345

Mihara M, Higo S, Uchiyama Y et al (2007) Different effects of high molecular weight sodium hyaluronate and NSAID on the progression of the cartilage degeneration in rabbit OA model. Osteoarthritis Cartilage 15:543–549

O'Reilly SC, Muir KR, Doherty M (1999) Effectiveness of home exercise on pain and disability from osteoarthritis of the knee: a randomised controlled trial. Ann Rheum Dis 58:15–19

Pereira YC, do Nascimento GC, Iyomasa DM et al (2015) Muscle characterization of reactive oxygen species in oral diseases. Acta Odontol Scand 73:81–86

Shen YC, Chiou WF, Chou YC et al (2003) Mechanisms in mediating the anti-inflammatory effects of baicalin and baicalein in human leukocytes. Eur J Pharmacol 465:171–181

Spassov A, Gredes T, Gedrange T et al (2011) Increased oxidative stress in dystrophin deficient (mdx) mice masticatory muscles. Exp Toxicol Pathol 63:549–552

Tonge DP, Bardsley RG, Parr T et al (2013) Evidence of changes to skeletal muscle contractile properties during the initiation of disease in the ageing guinea pig model of osteoarthritis. Longev Healthspan 2:15

Velasquez MT, Katz JD (2010) Osteoarthritis: another component of metabolic syndrome? Metab Syndr Relat Disord 8:295–305

Wang HZ, Wang HH, Huang SS et al (2014) Inhibitory effect of baicalin on collagen-induced arthritis in rats through the nuclear factor-kappaB pathway. J Pharmacol Exp Ther 350:435–443

Wen ZH, Tang CC, Chang YC et al (2016) Calcitonin attenuates cartilage degeneration and nociception in an experimental rat model of osteoarthritis: role of TGF-beta in chondrocytes. Sci Rep 6:28862

Wojdasiewicz P, Poniatowski LA, Szukiewicz D (2014) The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm 2014:561459

Xing D, Gao H, Liu Z et al (2017) Baicalin inhibits inflammatory responses to interleukin-1beta stimulation in human chondrocytes. J Interferon Cytokine Res 37:398–405

Xu G, Dou J, Zhang L et al (2010) Inhibitory effects of baicalein on the influenza virus in vivo is determined by baicalin in the serum. Biol Pharm Bull 33:238–243

Yamada T, Mishima T, Sakamoto M et al (2006) Oxidation of myosin heavy chain and reduction in force production in hyperthyroid rat soleus. J Appl Physiol (1985) 100:1520–1526

Yang X, Yang J, Zou H (2013) Baicalin inhibits IL-17-mediated joint inflammation in murine adjuvant-induced arthritis. Clin Dev Immunol 2013:268065