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Ibuprofen Ameliorates Fatigue- And Depressive-Like Behavior in Tumor-Bearing Mice

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Abstract

Aims: Cancer-related fatigue (CRF) is often accompanied by depressed mood, both of which reduce functional status and quality of life. Research suggests that increased expression of pro-inflammatory cytokines is associated with skeletal muscle wasting and depressive- and fatigue-like behaviors in rodents and cancer patients. We have previously shown that treatment with ibuprofen, a nonsteroidal anti-inflammatory drug, preserved muscle mass in tumor-bearing mice. Therefore, the purpose of the present study was to determine the behavioral effects of ibuprofen in a mouse model of CRF.

Main methods: Mice were injected with colon-26 adenocarcinoma cells and treated with ibuprofen (10 mg/kg) in the drinking water. Depressive-like behavior was determined using the forced swim test (FST). Fatigue-like behaviors were determined using voluntary wheel running activity (VWRA) and grip strength. The hippocampus, gastrocnemius muscle, and serum were collected for cytokine analysis.

Key findings: Tumor-bearing mice showed depressive-like behavior in the FST, which was not observed in mice treated with ibuprofen. VWRA and grip strength declined in tumor-bearing mice, and ibuprofen attenuated this decline. Tumor-bearing mice had decreased gastrocnemius muscle mass and increased expression of IL-6, MAFBx and MuRF mRNA, biomarkers of protein degradation, in the muscle. Expression of IL-1β and IL-6 was also increased in the hippocampus. Treatment with ibuprofen improved muscle mass and reduced cytokine expression in both the muscle and hippocampus of tumor-bearing mice.

Significance: Ibuprofen treatment reduced skeletal muscle wasting, inflammation in the brain, and fatigue- and depressive-like behavior in tumor-bearing mice. Therefore, ibuprofen warrants evaluation as an adjuvant treatment for CRF.

Keywords: Fatigue, Depression, Cancer, Neuroinflammation, Ibuprofen

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1. Introduction

Fatigue is a common and disabling side effect of cancer and cancer therapies. Cancer related fatigue (CRF) significantly reduces quality of life[1,2,3,4] and is frequently associated with depressed mood.[5,6,7] The mechanism for this association is unclear and is relevant for development of effective treatments to reduce fatigue and depression in cancer survivors.[1]

In response to tumor growth, increased expression of immune mediators leads to systemic inflammation throughout the host.[8] In skeletal muscle, pro-inflammatory cytokines activate NF-κB, which increases expression of proteins involved in muscle degradation, including MAFbx and MuRF1.[9,10] Tumor-bearing mice develop extensive loss of muscle mass and show functional impairments such as decreased grip strength[11,12,13] and reduced voluntary wheel running activity (VWRA).[11] Fatigue is often the presenting complaint of newly diagnosed cancer patients and sarcopenia can be present at the time of diagnosis.[14]

Increased expression of pro-inflammatory cytokines is also associated with depressed mood.[15] In animal models of CRF, tumor growth has been shown to increase inflammation within the brain.[11,16] This is important because increases in brain interleukin (IL)-1β are linked to both muscle atrophy[17] and depressed mood[11,15] in mice. In addition, evidence from rodent models indicates that inflammatory cytokines within the CNS are associated with behavioral symptoms of fatigue, such as decreased VWRA activity.[11,18,19]

Inflammatory cytokines increase prostaglandin synthesis, an important mediator of the inflammatory response. Common nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, decrease prostaglandin synthesis by inhibiting the cyclo-oxygenase (COX) enzymes and nitric oxide (NO).[20] Ibuprofen has been shown to decrease depressive-like behavior in a mouse model of bacterial infection,[21] and a recent clinical report showed decreased depression scores in osteoarthritis patients treated with ibuprofen.[22] Treatment with NSAIDs has been shown to reduce skeletal muscle wasting in tumor-bearing mice[23,24,25] and to improve grip strength and performance status in cancer patients treated with ibuprofen.[26]

However, the effects of ibuprofen or other NSAIDs on CRF have not been studied. Here, we examined the effects of ibuprofen on depressive and fatigue-like behaviors in a mouse model of CRF. Using this model, we have previously shown that tumor growth is associated with increased muscle and brain expression of IL-1β and IL-6 mRNA, increased serum levels of IL-6, skeletal muscle wasting and weakness, and the development of fatigue- and depressive-like behaviors.[11,27,28]

2. Materials and methods

2.1. Mice

Adult (10 weeks) female BALB/c x DBA/2F1 (CD2F1) mice weighing 20–22 g were obtained from Charles River Laboratories. Female mice were used because we and others have shown that tumor-bearing females maintain their food intake and lose a smaller percent of body mass than male mice[29] and male mice tend to gnaw and bite at the tumor site, causing local inflammation.[30] Mice were singly housed and maintained at 25 °C under a 12 h light cycle with ad libitum access to water and rodent chow. All procedures were performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by The Ohio State University Institutional Animal Care and Use Committee.

2.2. Mouse model of tumor-growth

The colon-26 adenocarcinoma (C26) cell line was maintained in culture and prepared for injection as previously described.[27,31] Half the mice were injected subcutaneously between the scapulae with 5×10^5 cells in 0.2 ml of PBS, and half served as healthy controls with PBS injection alone. This tumor cell line is syngeneic for CD2F1 mice and secretes IL-6 and TNF-α[25] and does not metastasize when injected subcutaneously.[32] Tumor growth is usually palpable by day 7, weight loss, splenomegaly, and muscle wasting are evident after day 14, and mice become moribund by day 24 of tumor-growth. In the present study, all data collection was completed by day 21 of tumor growth. Body weight was monitored three times a week for the first two weeks, and daily during the third week. Mice were euthanized by

inhalation of CO_2 gas on day 21 of tumor growth. Gastrocnemius muscles, spleen, and tumor were dissected and weighed; the brain was quickly dissected and hippocampus brain tissue was snap frozen in liquid nitrogen. Gastrocnemius weights were averaged and were normalized to body weight. At the time of sacrifice, 3 tumor-vehicle and 3 tumor-ibuprofen mice were removed from the study because there was no tumor growth established.

2.3. Oral ibuprofen administration

Ibuprofen sodium salt (Sigma, St. Louis) was dissolved at 50 mg per liter (0.05 mg/mL) of filtered drinking water. Ibuprofen was then administered in the drinking water of half the tumor-bearing and half the healthy control mice starting three days after injection of PBS or tumor cells. Based on water consumption of 4 mL per day, this gives a dose of 10 mg/kg/day ibuprofen. A dose of 5 mg/kg has previously been shown to decrease muscle wasting in C26 tumor-bearing mice[23] and a dose of 40 mg/kg has been shown to suppress depressive-like behavior in mice inoculated with Bacillus Calmette–Guerin (BCG).[21] All other animals received water only (vehicle). Water bottles were changed weekly throughout the study.

2.4. Voluntary wheel running activity

Fatigue-like behavior was modeled as decreased voluntary wheel running activity (VWRA).[33] Mice were singly housed and acclimated to a four inch diameter running wheel in the cage for one week, and baseline measures (week 0) of VWRA were recorded overnight prior to injection with tumor cells or PBS. Wheels were again placed in the home cages of all mice overnight (6 p.m. to 8 a.m.) on days 7 (week 1), 14 (week 2) and 20 (week 3) of tumor growth and the total number of turns each night was digitally recorded (Columbus Instruments, model 0297-004M). Data are expressed as a percentage of baseline.

2.5. Grip strength measurements

Forelimb grip strength was determined as previously described.[34] In brief, each mouse was allowed to grasp a platform with

both forelimbs and was pulled by the tail until it released itself from the platform (Columbus Instruments, model 1027DSM). Peak force measurements (N) were recorded in five trials and the average was calculated. Because smaller mice have smaller grip strength, peak force was normalized to body weight of the animal.

2.6. Depressive-like behavior

Depressive-like behavior was determined on day 13 using the forced swim test (FST) as described previously.[35] In the FST, mice were placed in an inescapable cylinder (diameter 16 cm, height 30 cm) containing 15 cm of water and behavior was recorded for 5 min. The latency to become immobile and the duration of immobility were determined.

2.7. RNA isolation and RT-PCR analysis

Total RNA was isolated from hippocampus brain sections using the Tri-Reagent protocol (Sigma) and reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantitative PCR was performed using the Applied Biosystems Assay-on-Demand Gene Expression protocol. In brief, experimental cDNA was amplified with an ABI PRISM 7300-sequence detection system (Applied Biosystems) by real-time PCR and normalized based on reference cDNA (GAPDH). RNA was isolated from the gastrocnemius muscle using a TissueLyser system in TRIzol Reagent. RNA was extracted and purified using RNeasy spin column purification (Qiagen, Valencia, CA.). RNA was reverse transcribed using iScript Reverse Transcription Supermix (Bio-Rad, 175 Hercules, CA) and quantitative PCR was performed using a three-step protocol on the CFX96 Real 176 Time System (Bio-Rad), as described previously.[27] Briefly, cDNA was amplified using real time qPCR and was normalized based on reference cDNA (GAPDH). All data were analyzed with the comparative threshold cycle method. Data is expressed as fold change from control-vehicle.

2.8. Statistical analyses

Data were subjected to a Shapiro–Wilk test using Statistical Analysis Systems (SAS) software (Cary, NC). Data were analyzed using two-way ANOVA (tumor, drug) using the General Linear Model procedures of SAS. When significant main or interaction effects were found, post hoc analyses for differences between group means were evaluated with the Least-Significant Difference procedure of SAS. All data are expressed as treatment means ± standard error of the mean (SEM).

3. Results

3.1. Ibuprofen ameliorated fatigue- and depressive-like behaviors in tumor-bearing mice

Half the tumor-bearing and half the healthy control mice received water (vehicle) or water containing ibuprofen starting three days after tumor cell inoculation until study completion. Depressive-like behavior was determined at 13 days after tumor cell inoculation using the forced swim test (FST). There was a main effect of ibuprofen on time immobile ($F_{1,38} = 7.64$, $p < 0.01$), but no main effect of tumor. As shown in Fig. 1A, tumor-bearing mice given water only (vehicle) had increased total time immobile in the FST ($p < 0.02$), compared to healthy control mice. Tumor-bearing mice treated with ibuprofen, however, did not differ from control mice in total time immobile during the FST. These data suggest ibuprofen mitigated depressive-like behavior in the tumor-bearing mice.

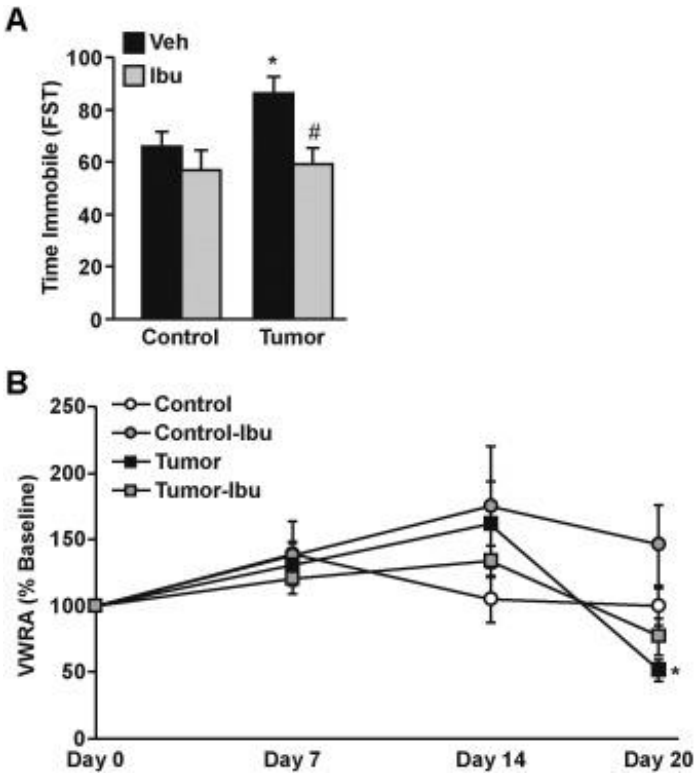

Fig. 1. Ibuprofen ameliorated fatigue- and depressive-like behaviors in tumor-bearing mice. Control and tumor-bearing mice were treated with ibuprofen (10 mg/kg/day) in the drinking water. A) Immobility in the forced swim test (FST) was determined on day 13 of tumor growth. B) Voluntary wheel running activity (VWRA) was determined before tumor cell injection and again at days 7, 14, and 20. Data are expressed as percentage of baseline. Data were analyzed using two-way ANOVA and post hoc t-tests for significant main effects: $^{*}p < 0.05$ from control-vehicle, $^{\#}p < 0.05$ from tumor-vehicle.

Fatigue-like behavior was determined using voluntary wheel running activity (VWRA)[33] prior to tumor cell inoculation and again at 7, 14, and 20 days of tumor growth. There was a main effect of tumor, but not ibuprofen, on VWRA on day 20 ($F_{1,43} = 13.78$, $p < 0.01$) (Fig. 1B). Post hoc tests revealed that VWRA of vehicle treated tumor-bearing mice was significantly decreased compared to vehicle-treated control mice ($p < 0.02$). VWRA of tumor-bearing mice treated with ibuprofen, however, was not significantly different from vehicle-treated control mice ($p = 0.09$). These data suggest that ibuprofen blunted the decline in VWRA in tumor-bearing mice.

3.2. Ibuprofen improved grip strength in tumor-bearing mice

Forelimb grip strength was determined prior to tumor cell inoculation (week 0) and at 12 days and 19 days of tumor growth. Fig. 2A shows the absolute grip strength and Fig. 2B shows the grip strength normalized to body weight. There was a main effect of tumor growth on absolute grip strength at 19 days ($F_{1,38} = 48.99$, $p < 0.01$) and a main effect of ibuprofen ($F_{1,38} = 16.3$, $p < 0.01$). Grip strength of both vehicle and ibuprofen treated tumor-bearing mice was decreased compared to control mice ($p < 0.02$). Grip strength in tumor-bearing mice treated with ibuprofen, however, was greater than vehicle treated tumor mice ($p < 0.001$). As shown in Fig. 2B, normalized grip strength of vehicle treated tumor-bearing mice was decreased compared to control mice ($p < 0.001$). Normalized grip strength in tumor-bearing mice treated with ibuprofen, however was not different from control mice ($p = 0.11$), indicating that ibuprofen blunted the decline in grip strength in tumor-bearing mice.

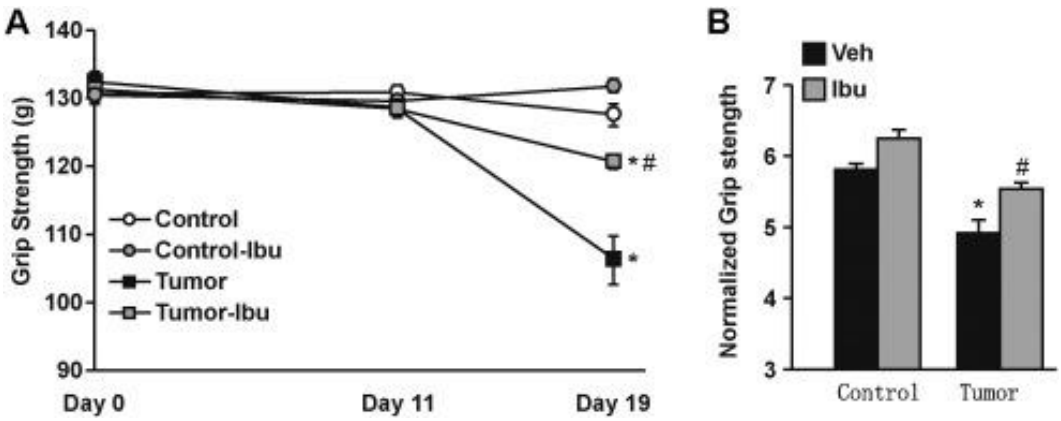

Fig. 2. Ibuprofen restored grip strength in tumor-bearing mice. Control and tumor-bearing mice were treated with ibuprofen (10 mg/kg/day) in the drinking water. A) Absolute grip strength was determined before tumor cell injection and again at day 11 and 19. B) At day 20, grip strength was normalized to body weight. Data were analyzed using two-way ANOVA and post hoc t-tests for significant main effects: $^{*}p < 0.05$ from control-vehicle, $^{\#}p < 0.05$ from tumor-vehicle.

3.3. Ibuprofen decreased systemic inflammation in tumor-bearing mice

After completion of behavioral testing on day 21, mice were weighed, euthanized, and tumor, gastrocnemius, spleen, and brain were collected. Tumor-bearing mice had increased spleen weight ($F_{1,38}$ = 87.49, $p < 0.01$), a nonspecific measure of systemic inflammation induced by tumor growth.[36] There was an interaction between tumor and ibuprofen on spleen weight ($F_{1,38}$ = 5.85, $p < 0.02$). As shown in Fig. 3A, ibuprofen treatment reduced spleen weight in the tumor-bearing mice ($p < 0.003$), although it remained significantly greater than in control animals ($p < 0.001$). Tumor-bearing mice also had increased plasma levels of IL-6 compared to control animals ($F_{1,25}$ = 14.37, $p < 0.002$). Ibuprofen tended to reduce plasma IL-6 in tumor-bearing mice, though the difference between tumor groups was not significant (p = 0.1) (Fig. 3B). As shown in Fig. 3C, tumor weight tended to be lower in ibuprofen treated mice, although the difference was not significant (p = 0.3).

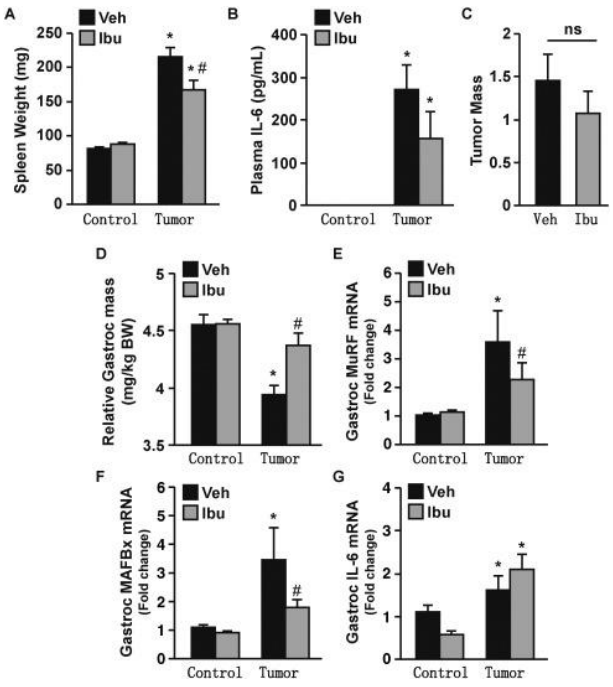

Fig. 3. Ibuprofen decreased systemic inflammation and muscle wasting in tumor-bearing mice. Control and tumor-bearing mice were treated with ibuprofen (10 mg/kg/day) in the drinking water. A) Spleen weight, B) plasma IL-6, C) tumor mass, and D) gastrocnemius muscle mass was determined at day 21. Gastrocnemius mRNA expression of E) MuRF, F) MAFBx, and G) IL-6 mRNA expression was

determined at day 21. Data were analyzed using two-way ANOVA and post hoc t-tests for significant main effects: $^{*}p < 0.05$ from control-vehicle, $^{\#}p < 0.05$ from tumor-vehicle.

3.4. Ibuprofen reduced muscle wasting in tumor-bearing mice

There was a main effect of tumor growth on the relative gastrocnemius muscle mass ($F_{1,38} = 18.40$, $p < 0.001$), and an interaction effect of ibuprofen and tumor on muscle mass ($F_{1,38} = 5.16$, $p < 0.03$). Post hoc analyses demonstrated that relative muscle mass was decreased in tumor-bearing mice ($p < 0.01$) but that muscle mass of tumor-bearing mice treated with ibuprofen was not different from controls, consistent with our previous study[23] (Fig. 3D). Because ibuprofen preserved gastrocnemius muscle mass in the tumor-bearing mice, we next determined mRNA expression of MuRF and MAFBx, biomarkers of protein degradation, in the muscle. There was a main effect of tumor on mRNA expression of MuRF ($F_{1,38} = 6.94$, $p < 0.01$). However, post hoc analyses showed that MuRF expression in tumor-bearing mice treated with ibuprofen was not different from control mice (Fig. 3E). Similar to MuRF, there was a main effect of tumor growth on mRNA expression of MAFBx ($F_{1,38} = 6.28$, $p < 0.02$). Post hoc analyses showed that only the tumor-vehicle treated group, and not the tumor-ibuprofen treated group, was significantly different from controls ($p < 0.006$) (Fig. 3F). Thus, ibuprofen mitigated the effect of tumor growth on muscle expression of MuRF and MAFBx, as previously reported.[23,24,25] Similarly, IL-6 mRNA expression was increased in muscle of tumor-bearing mice ($F_{1,38} = 13.97$, $p < 0.001$), but there was no significant effect of ibuprofen treatment (Fig. 3G). These data indicate that ibuprofen treatment abrogated expression of MAFBx and MuRF, biomarkers of muscle degradation in gastrocnemius muscle of tumor-bearing mice, and this was associated with decreased muscle wasting.

3.5. Ibuprofen attenuated neuroinflammation in tumor-bearing mice

We have previously shown that tumor growth increased IL-1β and IL-6 mRNA expression in the brain at 2 weeks of tumor growth and that expression levels were further increased by 3 weeks.[11] In the

present study, tumor-bearing mice had increased expression of IL-1β ($F_{1,45}$ = 13.06, p < 0.01) and IL-6 ($F_{1,45}$ = 5.83, p < 0.02) mRNA in the hippocampus compared to controls at day 21 (Fig. 4A & B). There was also a main effect of ibuprofen on IL-1β expression ($F_{1,45}$ = 4.68, p < 0.04), but there was no main effect of ibuprofen on IL-6 expression (p = 0.08). However, there was an interaction effect of ibuprofen on IL-6 mRNA expression in the hippocampus of tumor-bearing animals ($F_{1,45}$ = 8.1, p < 0.01). These data suggest that ibuprofen reduced expression of IL-1β in both tumor-bearing and control mice, but selectively decreased IL-6 expression in the tumor-bearing animals. Post hoc tests revealed that mRNA expression of IL-1β in the tumor-vehicle mice was greater than in healthy controls, while expression levels in the tumor-ibuprofen mice was not different from controls and was significantly lower than the tumor-vehicle mice (p < 0.02). Similarly, expression of IL-6 mRNA in the untreated tumor mice was greater than in controls, and expression in the ibuprofen treated tumor mice was not different from controls. Thus, ibuprofen reduced neuroinflammation in the tumor-bearing mice.

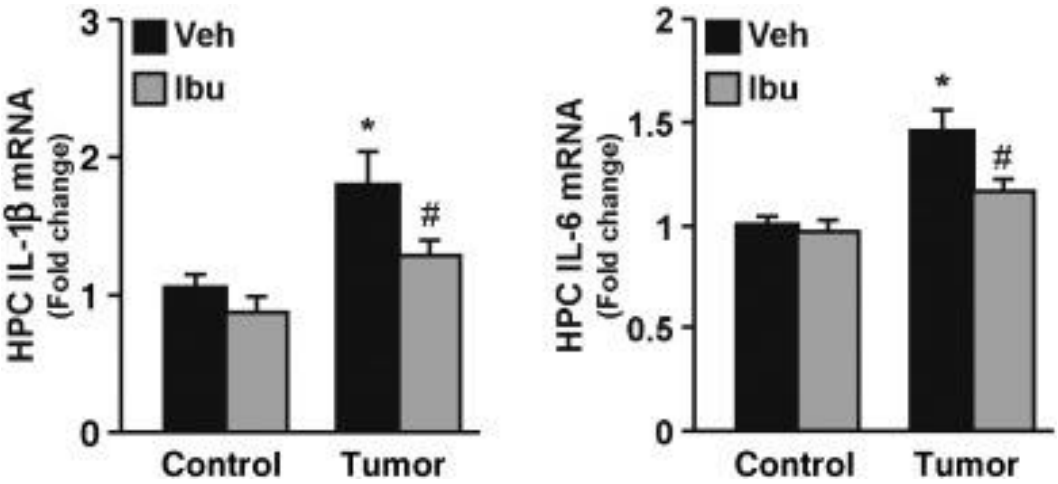

Fig. 4. Ibuprofen attenuated neuroinflammation in tumor-bearing mice. Control and tumor-bearing mice were treated with ibuprofen (10 mg/kg/day) in the drinking water. On day 21, the brain was collected and the hippocampus was dissected. A) IL-1β and B) IL-6 mRNA expression was determined. Data were analyzed using two-way ANOVA and post hoc t-tests for significant main effects: *p < 0.05 from control-vehicle, $^{\#}p$ < 0.05 from tumor-vehicle.

4. Discussion

Many cancer patients suffer with a constellation of symptoms termed cancer-related fatigue (CRF) which significantly impacts their quality of life.[37,38] To date, there are no effective therapies for CRF. Moreover, CRF is often associated with depressed mood which also lowers quality of life and increases morbidity and mortality in cancer survivors.[39,40] Both conditions are associated with increased expression of pro-inflammatory cytokines.[1,8,15,16] In the present study, we used a mouse model of CRF in which tumor-bearing mice had increased plasma levels of IL-6, depressive-like behaviors in the forced swim test, decreased VWRA, and decreased muscle mass and grip strength. Ibuprofen treatment reduced muscle wasting and inflammation within the hippocampus of tumor-bearing mice. This is significant as the hippocampus is a brain region involved in both mood and cognition.[41] As a result, both fatigue- and depressive-like behaviors were attenuated in the tumor-bearing mice treated with ibuprofen.

In our study, tumor-bearing mice had elevated levels of IL-6 in the plasma. Mice treated with ibuprofen tended to have decreased levels of IL-6 compared to vehicle treated mice, although IL-6 levels remained elevated compared to control mice. These data suggest that ibuprofen attenuated, but did not prevent, systemic inflammation, compared to control mice. This observation is supported by the increased spleen mass in tumor-bearing animals that was reduced by treatment with ibuprofen, although spleen mass remained greater than healthy control animals. It is possible that a higher dose of ibuprofen, or different NSAIDs might further reduce systemic inflammation in the tumor-bearing mice.

Pro-inflammatory cytokines increase expression of biomarkers of autophagy and muscle protein degradation within muscle tissue, which likely contributes to the loss of muscle mass and grip strength in the tumor-bearing mice.[9,42] We have previously shown that NSAID treatment can reduce expression of mediators of degradation, such as MAFBx and MuRF1, and reduce muscle wasting in tumor-bearing mice.[23,24,25] Here, we confirm that ibuprofen treatment abrogated expression of MAFBx and MuRF mRNA in the muscle of in tumor-bearing mice. However, we did not find that ibuprofen reduced

expression of IL-6 in muscle tissue, again raising the question of dose or alternative anti-inflammatory agents on tumor-induced muscle wasting. It is also possible that muscle IL-6 does not play a major role in muscle wasting. More importantly, the preservation of muscle mass was associated with improved grip strength and a smaller decline in VWRA in the tumor-bearing mice treated with ibuprofen.

In addition to their peripheral effects, tumor-induced cytokines can activate the brain resident immune cells, microglia, which can further propagate these inflammatory signals throughout the central nervous system (CNS). This is critical as we have previously shown that increased inflammation in the brains of tumor-bearing mice is associated with both fatigue- and depressive like behaviors.[19] Administration of minocycline, a broad spectrum antibiotic with anti-inflammatory capacity, inhibited microglial activation in tumor-bearing mice which resulted in reduced neuroinflammation and reduced depressive-like behavior in tumor-bearing mice.[11] Furthermore, increased neuroinflammation is thought to induce depressive-like behavior via increased activity of IDO and KMO, which may reduce serotonin synthesis and availability in the brain.[43,44] For example, we have previously shown that treatment with the SSRI Fluoxetine ameliorated depressive-like behavior in tumor bearing mice.[19] Here we show that ibuprofen decreased IL-1β and IL-6 mRNA expression in the hippocampus of tumor-bearing mice to levels seen in healthy control mice. Further research using methods such as microdialysis is needed to determine if ibuprofen also restored serotonin levels in the cerebrospinal fluid of treated versus untreated tumor-bearing mice. Overall, the reduction in inflammation within the brains of tumor-bearing mice treated with ibuprofen was associated with reduced depressive-like behaviors determined using the forced swim test. These data support other studies demonstrating that ibuprofen reduces depressive mood associated with inflammatory conditions.[21,22]

In the present study, fatigue was modeled as reduced VWRA.[33,45] We demonstrated that ibuprofen treatment tended to decrease this fatigue behavior in tumor-bearing mice. CRF may also involve muscle weakness, which was modeled as decreased grip strength. However, we have previously shown that grip strength can be improved without improvement in muscle mass.[11] Therefore, in the present study, it is possible that improvement in these fatigue

behaviors may have been influenced by both the reduction in depressed mood and the rescue in muscle mass with ibuprofen treatment.

In conclusion, we show beneficial effects of ibuprofen treatment on skeletal muscle mass and fatigue- and depressive-like behaviors in this mouse model of CRF. Previous clinical studies have shown prolonged survival[46] and improved grip strength and performance status in cancer patients treated with NSAIDs.[26] Our findings presented here suggest that NSAID treatment could have beneficial effects on muscle mass, physical performance, and mood of persons with CRF.

Acknowledgments

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