JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

The antinociceptive effect of resveratrol in bone cancer pain is inhibited by the Silent Information Regulator 1 inhibitor selisistat

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Keywords
cancer pain; mice; resveratrol; selisistat; Silent Information Regulator 1

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Received July 26, 2018
Accepted December 2, 2018

doi: 10.1111/jphp.13064

Abstract

Objectives To study the antinociceptive effect of single and repeated doses of resveratrol in a bone cancer pain model, and whether this effect is prevented by the Silent Information Regulator 1 (SIRT1) inhibitor selisistat.

Methods The femoral intercondylar bone of BALB/c mice was injected with 1 000 000 BJ3Z cancer cells. Bone resorption and tumour mass growth (measured by *in vivo* X-ray and fluorescence imaging), as well as mechanical nociceptive thresholds (von Frey device) and dynamic functionality (rotarod machine), were evaluated during the following 4 weeks. Acute resveratrol (100 mg/kg i.p.) and/or selisistat (10 mg/kg s.c.) were administered on day 14. Chronic resveratrol (100 mg/kg i.p., daily) and/or selisistat (0.5 μg/h s.c., Alzet pump) were administered between days 14 and 20.

Key findings Tumour growth gradually incremented until day 31, while mechanical hyperalgesia started on day 3 after cancer cell injection. Acute resveratrol increased the mechanical threshold of pain (peaking at 1.5 h), while the dynamic functionality decreased. Chronic resveratrol produced a sustained antinociceptive effect on mechanical hyperalgesia and improved the loss of dynamic functionality induced by the bone cancer tumour. Selisistat prevented all the effects of resveratrol.

Conclusions Acute and chronic resveratrol induces antinociceptive effect in the model of metastatic osseous oncological pain, an effect that would be mediated by SIRT1 molecular signalling.

Introduction

Pain is one of the most frequent manifestations in patients with cancer, and this can be triggered by direct infiltration of the tumour into the tissue, the generation of metastasis, the surgery to remove cancer, the chemotherapy or the radiation received as treatment.[1] Among the different types of cancer, those localized in bone marrow exhibit the highest frequency in patients experiencing intense pain (75–90%).[2] Bone cancer is caused by multiple myeloma or metastasis arising from primary cancer in the lung, breast, prostate or colon.[2] Although the mechanisms underlying the pain elicited during bone cancer are increasingly understood, for example local tumour expansion, infiltration of nerve plexuses, secretion of inflammatory molecules,[3,4] the analgesic drugs available for treating the pain associated to bone cancer still have low effectiveness. Current drug treatment for advanced bone cancer pain includes classic painkillers such as strong opioids (morphine, methadone, oxycodone, hydromorphone, fentanyl

and buprenorphine). However, the analgesic effects of opioids can be less effective in cancer-induced bone pain compared with other pain states, and effective analgesia is achieved only with the use of high doses that can also cause frequent side effects.[5] Furthermore, recent findings did not support the usefulness of additional adjuvant drugs in combination with different analgesics, even if in clinical practice are commonly used, although agents that inhibit osteoclast activity, such as bisphosphonates, could play an important role in delaying the onset and progression of pain.[5] Therefore, translational studies on the neurobiological mechanisms associated with the perception of pain aimed to identify drugs that may target some crucial step in the pain transduction pathway in bone cancer are today a key factor for the design of new suitable therapies for pain relief.

One of these drugs with high therapeutic potential could be resveratrol, a phytoalexin that is present in fruits such as grapes and wine, generated in response to some conditions such as stress, excessive sunlight, ultraviolet radiation or a fungal infection.[6] There is evidence suggesting that resveratrol has several beneficial biological effects, such as antioxidant, neuroprotective, antitumour, cardioprotective and anti-inflammatory, together with antihyperalgesic activity.[6] The antinociceptive effect of resveratrol has been demonstrated in animals with diverse types of experimental pain, arising from tissue inflammation,[7,8] diabetic neuropathy,[9,10] neuropathy due to spinal nerve constriction,[11] surgery-induced postpain,[12] as well as from bone cancer.[13] Thus, resveratrol may have antinociceptive actions both in acute and in chronic pain models, including cancer pain. The antinociceptive effects of resveratrol have been reported to be caused mainly by inhibition of pro-inflammatory cytokines and chemokines and by promotion of the anti-inflammatory cytokine IL-10,[14,15] inhibition of COX-1 and COX-2 activity,[8,16,17] and modulation of some ligand-gated receptors in spinal cord such as glutamatergic NMDA,[18,19] purinergic P2X7,[20] and serotonergic 5-HT_3[21] and 5-HT_7 receptors.[22] Besides, resveratrol has also been found to exert antinociceptive activity incision-induced acute and chronic pain by targeting the AMPK-dependent molecular cascade, thereby inhibiting ERK and mTOR signalling,[12,23] two pathways that converge onto the cap-dependent translational machinery that regulates essential genes for the development of nociceptive sensitization.[24] Thus, it seems important to re-evaluate the issue of the antinociceptive effect of resveratrol in bone cancer pain to contribute to precise some key step underlying analgesic mechanism.

The mechanism of action of resveratrol in cancer pain models has recently been reported as related to the AMPK pathway because resveratrol activation of this pathway resulted in amelioration of bone cancer pain.[25] The fact that resveratrol has been found to attenuate inflammatory hyperalgesia[26] and burn injury pain[27] by increasing the activity of the Silent Information Regulator 1 (SIRT1), a type of histone deacetylase responding to NAD^+ concentration which is sited downstream to the AMPK cascade[28] suggests the involvement of the AMPK/SIRT1 pathway in bone cancer pain. The AMPK/SIRT1 molecular cascade has been reported to be engaged in the alleviation of spinal cord injury via neuronal apoptosis in the spinal cord[29,30] and in the ageing process in general,[31] but the involvement of this pathway in cancer pain relief has not still been studied. On these bases, the present study was addressed to reevaluate the efficacy of resveratrol as an antinociceptive agent in bone cancer pain, together with testing the possibility of involvement of the SIRT1 pathway in the antinociceptive action of resveratrol, by utilizing the SIRT1 inhibitor selisistat (formerly known as EX527) as a pharmacological tool. To this end, we propose to utilize a modification of one of the first intraosseous tumour injection model, originally performed using osteolytic sarcoma NCTC 2472 cell lines introduced into the femoral bone marrow of C3H/HeJ mice,[32] a technique that allows tumour development without the possibility of generating extraosseous dissemination during the initial phase of tumour growth.

Materials and Methods

Animals

Male BALB/c mice (20–30 g) from the facility of the Faculty of Medicine of the University of Chile were used. The mice were maintained with controlled temperature (21 ± 1°C) and light conditions (12 : 12 h light-dark cycle, lights on at 8:00 a.m.), and with *ad libitum* access to food and water. All behavioural tests were performed between 9:00 and 13:00 h, and mice were allowed to habituate to the housing facility for 1 h before the beginning of experiments. The experimental procedures were approved by the Bioethics Committee of the Faculty of Medicine, University of Chile, (protocol CBA0721 from January 2015) and were in agreement with recently published animal welfare norms on pain management[33] and with the Guide for the Care and Use of Laboratory Animals of NIH.[34] To determine the number of required mice in each experimental group, a sample size power analysis was conducted by using the G*Power 3 Software (Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany).[35] Briefly, $n = 6$ mice per group was computed from pilot studies carried out in our laboratory assaying the antinociceptive effect of resveratrol on mechanical allodynia of mice with bone cancer pain (see below), where a significance level $\alpha = 0.05$ and a power level $1-\beta = 0.80$ were prespecified.

All the experimental measurements were performed in blinded condition. Each mouse was sacrificed at the end of the experiment by a carbon dioxide overdose.

Induction of bone cancer

Cell line (BJ3Z)

The tumour cells used to generate the bone cancer model were obtained from transformed murine stromal mammary cells (BJ3Z), which express the green fluorescent protein ZsGreen.[36]

Intraosseous injection

The mice were injected with 1 000 000 BJ3Z cells or culture medium (sham experiments) through a femoral intercondylar perforation. For this, the mice were anesthetized intraperitoneally (i.p.) with ketamine (50 mg/kg) and xylazine (10 mg/kg). A skin cut of 4 mm was made at the level of the left knee, the quadriceps femoris muscle and the patella were displaced laterally, and an incision of 2 mm was made above the intercondylar region. Once the intercondylar region of the femur was visible, a perforation was made by drilling with a 1 mm round bur, generating a notch. Immediately, a 27G needle was inserted into the medullary space, and 15 μl of BJ3Z cells (equivalent to 1 000 000 cells) or culture medium (sham controls) was injected with a 29G needle. Finally, the orifice was covered with glass ionomer, the patellar ligament was repositioned and the musculature and skin were sutured.[37] Ketoprofen 50 mg/kg i.p. was administered after the procedure for achieving postsurgical analgesia.

Tumour development

The bone resorption (osteolysis) on days 3, 10, 17, 24 and 31 after surgery and tumour growth on day 31 after surgery was evaluated using the In-Vivo FX PRO imaging system (Bruker Corporation, Billerica, MA, USA). The mice were evaluated under anaesthesia (ketamine 50 mg/kg and xylazine 10 mg/kg). They were introduced into the imaging equipment in a prone position with the hips in semiflexion, external rotation and with the knees in 90 degrees of flexion. The bone resorption was studied by radiographs, and tumour lesions in the femoral were assigned scores of 0–5[37]: (0) normal bone with no signs of destruction; (1) small radiolucent lesions indicative of bone destruction (one to three lesions); (2) increased number of lesions (three to six lesions) and loss of medullary bone; (3) loss of medullary bone and erosion of cortical bone; (4) full-thickness unicortical bone loss; and (5) full-thickness bicortical bone loss and displaced skeletal fracture. At the end of the experiment (on day 31), the mice were euthanized and the femurs were extracted to evaluate the progression of bone damage produced by the tumour, in accordance with the scale mentioned above. The growth of the tumour mass was evaluated by the fluorescence emitted by BJ3Z cell over both femurs, using the Molecular Imaging Software MI 7.5. The excitation was adjusted to an emission of 490–510 nm, and the image was obtained with 5 min of exposure.

Behavioural evaluation

Mechanical allodynia

The evaluation of mechanical allodynia was performed with an electronic von Frey device (Ugo Basile), with the mice positioned in a platform over a metal mesh. Before each experiment, the mouse was maintained 1:30 h over the metal mesh, for acclimation. Once the mouse maintained its four legs over the metal mesh, the analgesiometer was positioned below the left plantar surface, and the force required to trigger a withdrawal reflex was recording. For each mouse, this measurement was performed five times with 60 s of rest interval.

Dynamic functionality

Assessment of the dynamic functionality was made on a digital rotarod machine. Briefly, the mice were placed on a rotating roller at 20 cycles per minute at day zero. Previously, the mice were trained in at least two previous sessions before day zero. In this experiment, the functionality of the operated leg is evaluated on days 3, 7, 10, 14, 17, 21, 24 and 28 after neoplastic cells injection, three times a day, in the following way: 0: the mouse moves the leg normally; 1: minimal lameness in the affected leg; 2: substantial lameness; 3: substantial lameness and the mouse partially hides the leg: 4: substantial lameness and the mouse completely hides the leg. 5: the mouse does not use the affected hindlimb.[37]

Pharmacotherapy

Resveratrol and selisistat were dissolved in DMSO 10% *v*/*v* as vehicle. Resveratrol was always injected i.p. while selisistat was administered s.c. DMSO 10% alone (as control) was administered either i.p. or s.c. Therefore, four groups of treated animals were formed as follows: (a) vehicle i.p. plus vehicle s.c. (control group), (b) resveratrol i.p. plus vehicle s.c. (c) vehicle i.p. plus selisistat s.c. and (d) resveratrol i.p. plus selisistat s.c. In the acute experiments, mice received on day 14 after cancer cells inoculation one of the four pharmacological treatments described above, using 100 mg/kg i.p. of resveratrol, 10 mg/kg of selisistat s.c., or similar volume of vehicle. For the chronic experiments, the mice were given for 7 days (between days 14 and 20 after surgery) with any of

the four treatments described, by using a daily 100 mg/kg i.p. injection of resveratrol (or vehicle) and/or 0.5 µg/h s.c. of selisistat (or vehicle) administered with an Alzet minipump. The Alzet minipumps used (model 1007D) deliver 0.5 µl/h over a 1-week period and were subcutaneously implanted on the back of mice, slightly posterior to the scapulae, under ketamine/xylazine anaesthesia.

Statistic analysis

A sample size of $n = 6$ was calculated according to G*Power 3 Software.[35] In the initial experiments, it was considered a 25% of failure in the tumour implantation; therefore, the sample size was increased to $n = 8$ in the mice with the injection of tumour cells. The data were presented as mean ± SEM. For comparing significant differences between two values, the unpaired two-tailed Student's *t*-test was used; for more than two values, one-way ANOVA followed by the Bonferroni *post hoc* test was used. A value of $P < 0.05$ was accepted as statistically significant. Statistical analyses and graphs were performed using the statistical software GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA).

Results

Development of bone cancer pain model and behavioural evaluation

Intraosseous femoral intercondylar injection of neoplastic BJ3Z cells resulted in bone resorption (Figure 1a), evaluable by X-rays from day 10 after the injection of neoplastic cells, with a progressive increase in bone damage up to day 31 (Figure 1b). The tumour formed by BJ3Z cells was visualized by fluorescence at day 31 in postmortem naked femur (Figure 1c). It was found that there was a significant increase in the ipsilateral fluorescence of each group compared to its control (*$P < 0.05$, unpaired two-tailed Student's *t*-test) in the selected area (Figure 1d).

Intraosseous injection of neoplastic BJ3Z cells generated measurable and persistent nociception over time. The mice group injected with neoplastic cells injection showed a decrease in the mechanical threshold 3 days after the injection, which was maintained at least for 31 days as compared to mechanical threshold values obtained before injection, while the sham group recovered the initial mechanical threshold at day 17 (Figure 2a, *$P < 0.05$,

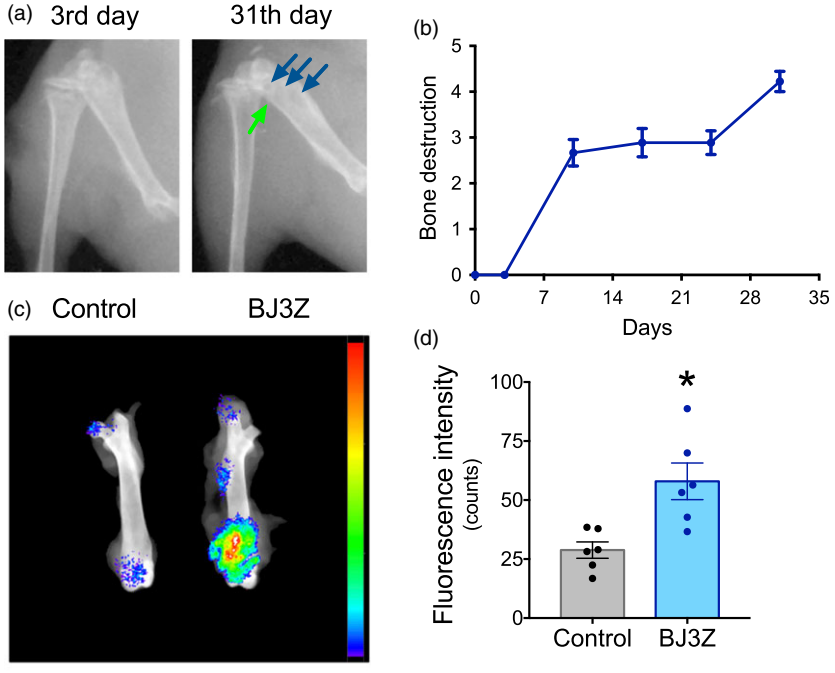

Figure 1 Physical changes in femur of Balb/c mice after inoculation of BJ3Z-106 cancer cells on day zero: (a) representative *in vivo* X-ray image on days 3 and 31 after inoculation, (green arrow shows decreased thickness of the cortical bone; blue arrows show radiolucent lesions, indicating area with bone resorption); (b) bone resorption scores (0–5 scale), as measured from X-ray photographs on days 3, 10, 17, 24 and 31 following BJ3Z cancer cell inoculation; (c) representative *ex vivo* fluorescence image merged with X-ray image from sham and BJ3Z cells inoculated mice, at day 31 after inoculation (fluorescence scale bar is shown on the right side of the figure); (d) Fluorescence counts in sham and BJ3Z cells injected mice, at day 31 after inoculation; data of each animal are depicted by dots. Columns with vertical bars are means ± SEM, $n = 6$ mice per group. *$P < 0.05$ (unpaired two-tailed Student's *t*-test). [Colour figure can be viewed at wileyonlinelibrary.com]

repeated-measures ANOVA followed by Bonferroni *post hoc* test). Calculation of the area under the time-course curves (AUC), as a measure of the averaged pain threshold during the complete period of observation, showed that the allodynia induced by bone cancer was statistically significant as compared with naïve and sham groups (Figure 2b, ****$P < 0.0001$, one-way ANOVA followed by Bonferroni *post hoc* test). Concomitantly, the forced gait test assessing the degree of limping of the affected leg showed that mice with cancer had significantly less functionality than control mice from day 3 and that limping is constant throughout the observation period, as opposed to the sham group that recovers at day 17 (Figure 2c, *$P < 0.05$, repeated-measures ANOVA followed by Bonferroni *post hoc* test; Figure 2d, ****$P < 0.0001$, one-way ANOVA followed by Bonferroni *post hoc* test).

Effect of acute resveratrol in bone cancer pain and its prevention by selisistat

The antinociceptive effect of acute resveratrol 100 mg/kg or vehicle was evaluated in mice on day 14 postsurgery for 3 h. The time-course curve for resveratrol shows that this drug had a transient antiallodynic effect in mice with bone cancer that peaked at 1.5 h and that the mechanical nociceptive threshold returned to the initial allodynic score in around 2.5–3 h (Figure 3a, *$P < 0.05$, repeated-measures ANOVA followed by Bonferroni *post hoc* test). While selisistat alone did not elicit any change in the mechanical withdrawal threshold of mice with bone cancer, co-administration of selisistat completely prevented the antiallodynic effect of resveratrol as the values of mechanical threshold in this group of animals did not differ from those of vehicle controls at any time. Calculation of the AUC, as a measure of the effect of the drugs during the complete period of observation, showed that the antiallodynic effect of resveratrol was statistically significant (Figure 3b, *$P < 0.05$, one-way ANOVA followed by Bonferroni *post hoc* test). Also, acute resveratrol transiently improved the functionality of the hindlimb with bone cancer, exhibiting a maximum effect at 1.5 h after injection (Figure 3c, *$P < 0.05$, repeated-measures ANOVA followed by Bonferroni *post hoc* test). This effect was statistically significant and fully reverted by co-administration of selisistat (Figure 3d, *$P < 0.05$, 1-way ANOVA followed by Bonferroni *post hoc* test).

Effect of repeated administration of resveratrol in bone cancer pain and its prevention by selisistat

The chronic administration of resveratrol 100 mg/kg daily from day 14–20 after bioplastic cells inoculation increased

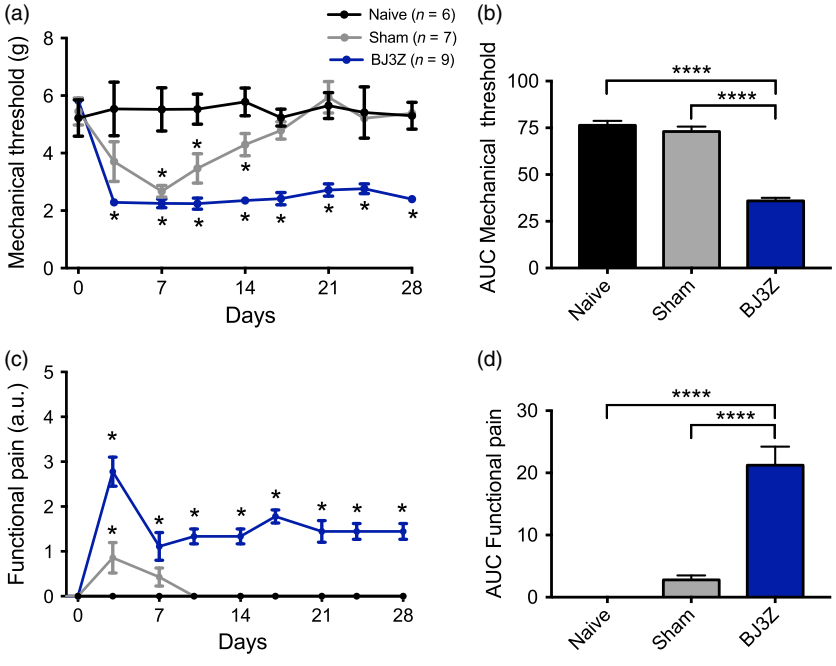

Figure 2 Mechanical allodynia and hindlimb functionality in Balb/c mice after inoculation of BJ3Z-106 cancer cells on day zero: (a) withdrawal threshold (in g) to paw pressure on the 4-week period that follows cancer cell inoculation; (b) area under the curve (AUC) of withdrawal threshold time-course; (c) functional pain scores on rotarod (0–5 scale); (d) AUC of functional pain time-course. Data are means ± SEM, $n = 6$ mice per group. For A and C, *$P < 0.05$ (repeated-measures ANOVA followed by Bonferroni *post hoc* test). For B and D, ****$P < 0.0001$ (one-way ANOVA followed by Bonferroni multiple comparisons test). [Colour figure can be viewed at wileyonlinelibrary.com]

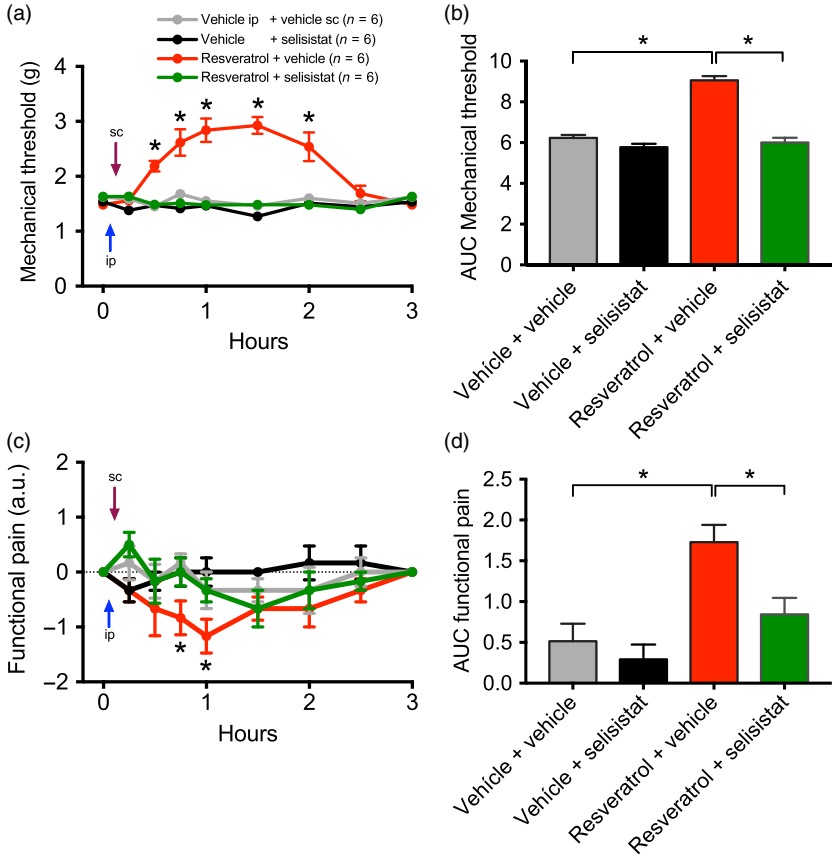

Figure 3 Effects of acute administration of i.p. resveratrol (100 mg/kg) or vehicle, co-administered with s.c. selisistat (10 mg/kg) or vehicle, on mechanical allodynia and hindlimb functionality of mice, on day 14 after inoculation of cancer cells: (a) time-course of mechanical withdrawal threshold; (b) area under the curve (AUC) of withdrawal threshold time-course; (c) time-course of functional pain scores on rotarod; (d) AUC of functional pain time-course. Data are means ± SEM, $n = 6$ mice per group. For (a) and (c), $^*P < 0.05$ (repeated-measures ANOVA followed by Bonferroni *post hoc* test. For (b) and (d), $^*P < 0.05$ (one-way ANOVA followed by Bonferroni multiple comparisons test). [Colour figure can be viewed at wileyonlinelibrary.com]

the mechanical threshold in the von Frey test, reaching maximum effect on day 17 and 21 ($^*P < 0.05$, repeated-measures ANOVA followed by Bonferroni *post hoc* test), and after that decaying until disappear at day 28 (Figure 4a). In the same figure, it can also be observed that selisistat prevented the antiallodynic effect of resveratrol. To assess the total antinociceptive effect of resveratrol, the area under the curve (AUC) between days 14 and 28 postadministration was determined. Comparison of AUC obtained from resveratrol-injected mice against AUC from mice receiving selisistat or vehicle demonstrated a significant antiallodynic effect of resveratrol upon the complete period of testing (Figure 4b, $^*P < 0.05$, one-way ANOVA followed by Bonferroni *post hoc* test). The effect of resveratrol on the function of the affected leg in the forced gait test was also evaluated. Results demonstrated that repeated administration of resveratrol starting on day 17 after cancer cells injection led to an improvement in the functionality of the affected leg, being the effect maximum at day 21 and persisting until day 24 (Figure 4c). This effect of resveratrol was prevented by selisistat. The total effect of resveratrol upon the complete period of assessment of the functionality of the leg affected with cancer, as assessed by AUC, demonstrated that resveratrol produced an enduring improvement of the hindlimb functionality, with respect to similar measures in vehicle or selisistat-treated mice (Figure 4d, $^*P < 0.05$, one-way ANOVA followed by Bonferroni *post hoc* test).

Discussion

The main findings of this study are that both acute and chronic resveratrol treatments reversed pain behaviour and hindlimb functionality in mice with cancer in the femur and that these effects were prevented by administration of the SIRT1 inhibitor selisistat. These results are valid and potentially important for future applications to the extent that the bone cancer experimental pain model originally

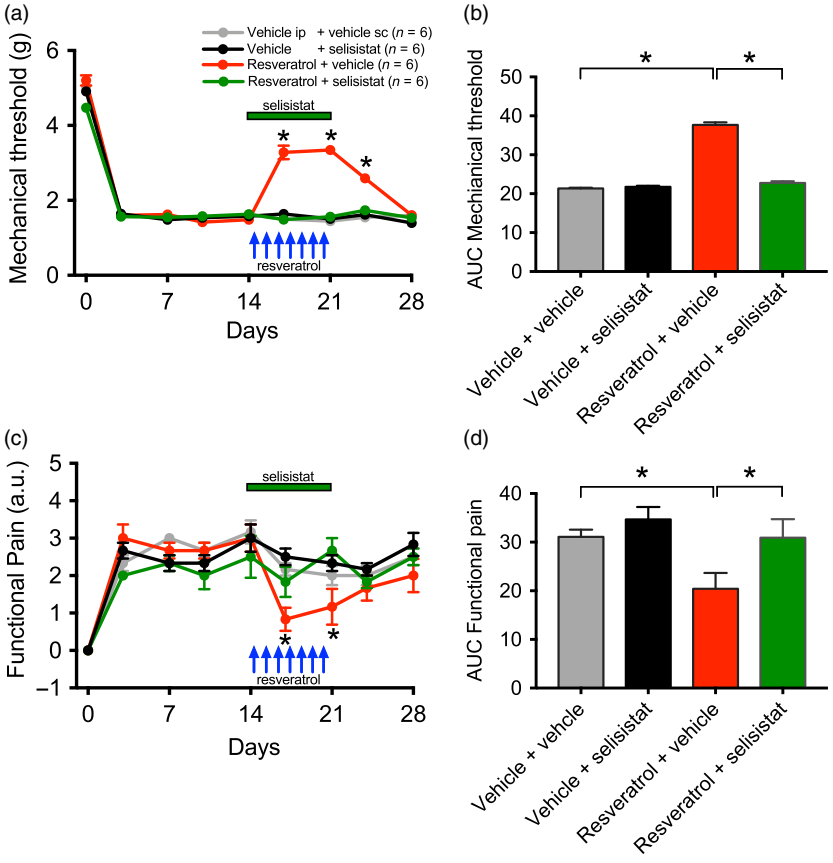

Figure 4 Effects of chronic administration of resveratrol (100 mg/kg daily) or vehicle for 7 days (between days 14 and 21 after inoculation of cancer cells), co-administered with s.c. selisistat (0.5 μg/h with Alzet minipump) or vehicle, on mechanical allodynia and hindlimb functionality of mice: (a) time-course of mechanical withdrawal threshold; (b) area under the curve (AUC) of withdrawal threshold time-course; (c) time-course of functional pain scores on rotarod; (d) AUC of functional pain time-course. Data are means ± SEM, $n = 6$ mice per group. For (a) and (c), $^*P < 0.05$ (repeated-measures ANOVA followed by Bonferroni *post hoc* test). For (b) and (d), $^*P < 0.05$ (one-way ANOVA followed by Bonferroni multiple comparisons test). [Colour figure can be viewed at wileyonlinelibrary.com]

reported in mice had been successfully replicated in the present study. In this regard, we evaluated and confirmed the tumour presence and growth in the femur *in vivo* through bone resorption X-rays imaging, and *ex vivo* through fluorescence emitted by cancer cells. Together with the structural damage of the bone, we confirmed the existence of algesic behaviours that developed in the 4-week time-frame that followed cancer cells inoculation, in terms of mechanical allodynia and dynamic functionality of the affected leg.

In humans, primary bone cancer and bone metastasis often manifest with pain and most cancer patients will present pain at some point during their illness.[2] In practice, cancer pain is generally managed in agreement to the WHO pain scale,[38,39] but the drugs used according to these guidelines have been associated with poor effectiveness and various adverse effects. Examples are nonsteroidal anti-inflammatories, opioids and adjuvants.[2] In the last 20 years, the bone cancer mice model replicated herein has been useful to evaluate the analgesic effect of a variety of molecules, such as osteoprotegerin,[37] denosumab,[40] bisphosphonates,[41,42] sulfas,[43] TRPV1 antagonist,[2] gabapentin,[44] anti-NGF[45] and TRK inhibitors,[46] in searching for an alternative to classical drugs, but with limited success. However, the use of several of these drugs has been useful for the understanding of the mechanisms that explain cancer pain with elements in common with inflammatory and neuropathic pain, but with unique characteristics.[2]

The limited success of current treatments for cancer pain, in particular, bone tumours, is thought to arise from the incomplete knowledge of the mechanisms that underlie the induction and maintenance of cancer-related pain. Sensory neurons are known to innervate the periosteum and marrow cavity, but little is known about the physiology of these neurons. Several pro-inflammatory cytokines (IL-1β,

TNFα, IL-6 and TGFβ) are increased in the DRG in response to bone cancer, altogether with increased DRG expression of several membrane receptors/channels (TRPV1, P2X3, ASIC1a/1b, Nav 1.8, and Nav 1.9) which are known to be involved in the transduction of nociceptive stimuli and/or in the excitability of nociceptors, but evidence that these mediators and ion channels directly activate or sensitize bone nociceptors is still lacking.[4] It is also clear that spinal dorsal horn neurons can be activated by noxious stimuli applied to bone, but at present, there is only limited evidence that peripheral bone afferent neurons can be sensitized.[47,48] In the present study, we observed that resveratrol has antiallodynic effect in the von Frey test, both after repeated and single regimens of administration of the drug. We also showed that there was an improvement of the functionality in the rotary cylinder test. We further showed that when the specific inhibitor of SIRT1 selisistat was administered together with resveratrol, both under acute and continuous administration regimens, the analgesic effect of resveratrol was suppressed. Two conclusions could arise from these results: (a) SIRT1 is a direct or indirect molecular target of resveratrol, and (b) SIRT1 activation is coupled to some antinociceptive mechanism operating in bone cancer pain. The first issue is clearly supported by recent data indicating that, on the one hand, resveratrol is a direct allosteric activator of SIRT1 *in vivo* and *in vitro* through binding to the amino-terminal activation domain of SIRT1[49] and, on the other hand, resveratrol results in AMPK activation which in turn would lead to indirect downstream activation of SIRT1.[50] The second conclusion remains more elusive. Mechanistic coupling between SIRT1 activation and reduced nociceptive responses in bone cancer is likely to depend on downstream SIRT1 signalling, including regulation of gene expression at the transcriptional level by influencing chromatin remodelling, together with modulation of protein activity via the removal of acetyl functional groups.[51] Likely substrates for SIRT1-mediated deacetylation could be some pronociceptive mediators such as NFkB, IL-6, TNF-α and iNOS, because their spinal cord expression levels have been found to be reduced after activation of SIRT1 in neuropathic rats, together with a parallel decrease in the mechanical allodynia and the thermal hyperalgesia.[52] Another possible downstream target for SIRT1 could be the Grm1/5 promoter region of genes encoding transcription of the metabotropic glutamatergic receptors mGluR1/5 (which are known to play a key role in central sensitization and neuropathic pain), because the spinal expression level of these receptors has been found to be decreased after SIRT1 activation, altogether with the existence of reduced H3 acetylation levels at the Grm1/5 promoter regions in the spinal cord of diabetic neuropathic animals.[53] Nevertheless, whether similar substrates to those reported for neuropathic pain are also targeted by SIRT1 activation in bone cancer pain is yet unexplored.

Finally, it seems worthy to mention that in the last decade, some human intervention studies using resveratrol as anticarcinogenic agent have been published, aimed to help determine whether resveratrol may be useful in cancer management (see Gescher *et al.*[54] for review). Although these studies did not assess the painkiller ability of resveratrol in cancer pain, they brought useful information for future clinical studies that may address this particular topic. The overall evidence from these studies suggests that resveratrol at oral daily doses of up to 1 g is safe, and that the molecule can reach remote tissues from the site of absorption at concentrations that may have therapeutic usefulness, thus making attractive to test resveratrol for future clinical applications in bone cancer pain.

Conclusion

In the present study, we showed that acute and repeated administration of resveratrol decreased mechanical allodynia in a bone cancer pain model while improving functionality and gait. It should be noted that those antinociceptive effects of chronic administration of resveratrol were measured 3 days after the administration of resveratrol and lasted for at least 3 days after the last dose, thereby showing an important postdrug effect not described previously in the literature. Additionally, we demonstrated that inhibiting SIRT1 with the competitive antagonist selisistat completely suppressed the analgesic effect induced by resveratrol, demonstrating that resveratrol acted on SIRT1 or upstream this molecule. Pain in cancer patients, especially patients with advanced bone cancer, contributes considerably to deterioration in the quality of life, and SIRT1 activation with resveratrol could be useful and safe for the treatment of bone cancer pain in clinical settings.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported by the Centers of Excellence with Basal/Conicyt financing, CEDENNA (grant FB0807), Fondecyt Project (grant 1181622), Fondap Project (grant 15130011) and Conicyt Fellowship (grant 21140171).

References

1. Christo PJ, Mazloomdoost D. Cancer pain and analgesia. *Ann N Y Acad Sci* 2008; 1138: 278–298.
2. Mantyh P. Bone cancer pain: causes, consequences, and therapeutic opportunities. *Pain* 2013; 154(Suppl. 1): s54–s62.
3. Manthy PW. Bone cancer pain: from mechanism to therapy. *Curr Opin Support Palliat Care* 2014; 8: 83–90.
4. Nencini S, Ivanusic JJ. The physiology of bone pain. How much do we really know?. *Front Physiol* 2016;7:157.
5. Lucchesi M *et al.* Developing drugs in cancer-related bone pain. *Crit Rev Oncol Hematol* 2017; 119: 66–74.
6. Singh C *et al.* Resveratrol-based combinatorial strategies for cancer management. *Ann NY Acad Sci* 2013; 1290: 113–121.
7. Gentilli M *et al.* Resveratrol decreases hyperalgesia induced by carrageenan in the rat hind paw. *Life Sci* 2001; 68: 1317–1321.
8. Pham-Marcou TA *et al.* Antinociceptive effect of resveratrol in carrageenan-evoked hyperalgesia in rats: prolonged effect related to COX-2 expression impairment. *Pain* 2008; 140: 274–283.
9. Sharma S *et al.* Resveratrol, a polyphenolic phytoalexin attenuates thermal hyperalgesia and cold allodynia in STZ-induced diabetic rats. *Indian J Exp Biol* 2006; 44: 566–569.
10. Kumar A *et al.* Effects of resveratrol on nerve functions, oxidative stress and DNA fragmentation in experimental diabetic neuropathy. *Life Sci* 2007; 80: 1236–1244.
11. Yin Q *et al.* Resveratrol facilitates pain attenuation in a rat model of neuropathic pain through the activation of spinal Sirt1. *Reg Anesth Pain Med* 2013; 38: 93–99.
12. Tillu DV *et al.* Resveratrol engages AMPK to attenuate ERK and mTOR signaling in sensory neurons and inhibits incision-induced acute and chronic pain. *Mol Pain* 2012; 8: 5.
13. Cheng W *et al.* Resveratrol attenuates bone cancer pain through the inhibition of spinal glial activation and CX3CR1 upregulation. *Fundam Clin Pharmacol* 2014; 28: 661–670.
14. Singh AK, Vinayak M. Resveratrol alleviates inflammatory hyperalgesia by modulation of reactive oxygen species (ROS), antioxidant enzymes and ERK activation. *Inflamm Res* 2017; 66: 911–921.
15. Tao L *et al.* Resveratrol attenuates neuropathic pain through balancing pro-inflammatory and anti-inflammatory cytokines release in mice. *Int Immunopharmacol* 2016; 34: 165–172.
16. Shakibaei M *et al.* Resveratrol suppresses interleukin-1beta-induced inflammatory signaling and apoptosis in human articular chondrocytes: potential for use as a novel nutraceutical for the treatment of osteoarthritis. *Biochem Pharmacol* 2008; 76: 1426–1439.
17. Torres-López JE *et al.* Comparison of the antinociceptive effect of celecoxib, diclofenac and resveratrol in the formalin test. *Life Sci* 2002; 70: 1669–1676.
18. Tsai RY *et al.* Resveratrol regulates N-methyl-D-aspartate receptor expression and suppresses neuroinflammation in morphine-tolerant rats. *Anesth Analg* 2012; 115: 944–952.
19. Singh AK *et al.* Recent development in antihyperalgesic effect of phytochemicals: anti-inflammatory and neuro-modulatory actions. *Inflamm Res* 2018; 67: 633–654.
20. Wu B *et al.* Resveratrol-decreased hyperalgesia mediated by the $P2X_7$ receptor in gp120-treated rats. *Mol Pain* 2017; 13: 1744806917707667.
21. Lee BH *et al.* Resveratrol enhances 5-hydroxytryptamine type 3A receptor-mediated ion currents: the role of arginine 222 residue in pre-transmembrane domain I. *Biol Pharm Bull* 2011; 34: 523–527.
22. Zhao X *et al.* Chronic resveratrol treatment exerts antihyperalgesic effect and corrects co-morbid depressive like behaviors in mice with mononeuropathy: involvement of serotonergic system. *Neuropharmacology* 2014; 85: 131–141.
23. Burton MD *et al.* Pharmacological activation of AMPK inhibits incision-evoked mechanical hypersensitivity and the development of hyperalgesic priming in mice. *Neuroscience* 2017; 359: 119–129.
24. Melemedjian OK, Khoutorsky A. Translational control of chronic pain. *Prog Mol Biol Transl Sci* 2015; 131: 185–213.
25. Song H *et al.* Activation of adenosine monophosphate-activated protein kinase suppresses neuroinflammation and ameliorates bone cancer pain: involvement of inhibition on mitogen-activated protein kinase. *Anesthesiology* 2015; 123: 1170–1185.
26. Wang LL *et al.* Resveratrol attenuates inflammatory hyperalgesia by inhibiting glial activation in mice spinal cords. *Mol Med Rep* 2016; 13: 4051–4057.
27. Cheng W *et al.* Intrathecal injection of resveratrol attenuates burn injury pain by activating spinal sirtuin 1. *Pharmacogn Mag* 2016; 12(Suppl 2): S201–S205.
28. Cantó C *et al.* AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* 2009; 458: 1056–1060.
29. Zhao H *et al.* Resveratrol protects against spinal cord injury by activating autophagy and inhibiting apoptosis mediated by the SIRT1/AMPK signaling pathway. *Neuroscience* 2017; 348: 241–251.
30. Yan P *et al.* Regulation of autophagy by AMP-activated protein kinase/sirtuin 1 pathway reduces spinal cord neurons damage. *Iran J Basic Med Sci* 2017; 20: 1029–1036.
31. Salminen A, Kaarniranta K. AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. *Ageing Res Rev* 2012; 11: 230–241.
32. Schwei MJ *et al.* Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. *J Neurosci* 1999; 19: 10886–10897.
33. Carbone L, Austin J. Pain and laboratory animals: publication practices for better data reproducibility and better animal welfare. *PLoS One* 2016; 11: e0155001.

34. National Research Council. *Guide for the Care and Use of Laboratory Animals*, 8th edn. Washington, DC: National Academies Press, 2011.
35. Faul F *et al.* G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; 39: 175–191.
36. Pinto MP *et al.* Vascular endothelial growth factor secreted by activated stroma enhances angiogenesis and hormone-independent growth of estrogen receptor-positive breast cancer. *Cancer Res* 2010; 70: 2655–2664.
37. Honore P *et al.* Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord. *Nat Med* 2000; 6: 521–528.
38. World Health Organization. *Cancer Pain Relief*. Geneva, Switzerland: World Health Organization, 1986.
39. Jadad AR, Browman GP. The WHO analgesic ladder for cancer pain management: stepping up the quality of its evaluation. *JAMA* 1995; 274: 1870–1873.
40. Yuasa T *et al.* Denosumab: a new option in the treatment of bone metastases from urological cancers. *Onco Targets Ther* 2012; 5: 221–229.
41. Halvorson KG *et al.* Intravenous ibandronate rapidly reduces pain, neurochemical indices of central sensitization, tumor burden, and skeletal destruction in a mouse model of bone cancer. *J Pain Symptom Manage* 2008; 36: 289–303.
42. Sevcik MA *et al.* Bone cancer pain: the effects of the bisphosphonate alendronate on pain, skeletal remodeling, tumor growth and tumor necrosis. *Pain* 2004; 111: 169–180.
43. Ungard RG *et al.* Inhibition of breast cancer-cell glutamate release with sulfasalazine limits cancer-induced bone pain. *Pain* 2014; 155: 28–36.
44. Colvin L, Fallon M. Challenges in cancer pain management - bone pain. *Eur J Cancer* 2008; 44: 1083–1090.
45. Sevcik MA *et al.* Anti-NGF therapy profoundly reduces bone cancer pain and the accompanying increase in markers of peripheral and central sensitization. *Pain* 2005; 115: 128–141.
46. Ghilardi JR *et al.* Administration of a tropomyosin receptor kinase inhibitor attenuates sarcoma-induced nerve sprouting, neuroma formation and bone cancer pain. *Mol Pain* 2010; 6: 87.
47. Cain DM *et al.* Functional interactions between tumor and peripheral nerve in a model of cancer pain in the mouse. *Pain Med* 2001; 2: 15–23.
48. Uhelski ML *et al.* The non-selective cannabinoid receptor agonist WIN 55,212-2 attenuates responses of C-fiber nociceptors in a murine model of cancer pain. *Neuroscience* 2013; 247: 84–94.
49. Sinclair DA, Guarente L. Small-molecule allosteric activators of airtuins. *Annu Rev Pharmacol Toxicol* 2014; 54: 363–380.
50. Kulkarni SS, Cantó C. The molecular targets of resveratrol. *Biochim Biophys Acta* 2015; 1852: 1114–1123.
51. Zhang T, Kraus WL. SIRT1-dependent regulation of chromatin and transcription: linking NAD(+) metabolism and signaling to the control of cellular functions. *Biochim Biophys Acta* 2010; 1804: 1666–1675.
52. Gui Y *et al.* Involvement of AMPK/SIRT1 pathway in anti-allodynic effect of troxerutin in CCI-induced neuropathic pain. *Eur J Pharmacol* 2015; 769: 234–241.
53. Yang Y *et al.* Resveratrol suppresses glial activation and alleviates trigeminal neuralgia via activation of AMPK. *J Neuroinflammation* 2016; 13: 84.
54. Gescher A *et al.* Resveratrol in the management of human cancer: how strong is the clinical evidence? *Ann N Y Acad Sci* 2013; 1290: 12–20.