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ORIGINAL ARTICLE

Analgesic effect and possible mechanism of SCH772984 intrathecal injection on rats with bone cancer pain☆

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KEYWORDS

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p90RSK;
Fos protein;
Bone cancer pain;
Intrathecal injection;
Spinal cord

Abstract This study is to establish a model of rat tibial osteocarcinoma pain, intrathecally inject specific ERK1/2 inhibitors SCH772984, observe the analgesic effect, and discuss the influence of ERK-P90RSK-Fos signal path in bone cancer pain. Forty female SD rats were randomly divided into 5 groups. Establish a bone cancer pain model after putting the intrathecal tube 5d and determine the rats' mechanical withdrawal threshold (MWT) after tube 5d; 40 SD rats with intrathecal tube back 5d were randomly divided into 5 groups. Sham Group receives no medication, the other four respectively receive 5% DMSO 10 μl, SCH 0.1, 1.0, 10 μg (SCH dissolved in 10 μl 5% DMSO) intrathecally. Determine the rats' mechanical withdrawal threshold (MWT) before and after giving medication 1, 3, 6, 9, 12, 15, 18, 24 h, and 2 min spontaneous paw withdrawal. Western blot and immuno-fluorescence determine the expression condition of spinal cord dorsal horn of p-ERK, p-p90RSK and Fos protein. Intrathecal injection of SCH772984 has analgesic effects on rats with bone cancer pain, and the effects enhance with increasing dose; intrathecal injection of SCH772984 10 μg could greatly reduce the expression of spinal dorsal horn Fos protein. Injecting walker 256 tumor cells into rats' tibia could cause behavior changes, such as idiopathic pain sensitivity and pain; the intrathecal tube almost has no effect on motor function of rats; ERK1/2 is involved in bone cancer pain, and intrathecal injection of ERK1/2 specific inhibitors SCH772984 10 μg may effectively relieve bone cancer pain.

☆ This work was done in Jiangsu Provincial Key Laboratory of Anesthesiology, Xuzhou Medical College.

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1. Introduction

World Cancer Report 2014 published by the World Health Organization (WHO) notes that the number of cancer patients and death cases in the world are on the rise, and it shows more severe situations of cancer in developing countries particularly. About 50% of cancer patients feel varying degrees of pain, and the incidence of pain among people with advanced cancer is as high as over 80% (Porter et al., 2005). As the medicare level of modern medicine is constantly improving, lives of cancer patients have been prolonged, while pain has become the vital issue that affects their quality of life. Bone cancer pain is the most common pain in cancer patients, about one-third of advanced cancer patients would have bone metastasis. Bone cancer pain is a common symptom of bone metastases in patients with advanced cancer. Bone cancer pain is chronic pain, and its mechanism is still not entirely clear. Acidosis caused by oppression from tumor cells and destruction to sclerot in the tumor pathological changes and products released from tumor cells can both cause pain. A large number of studies have shown that, in central and peripheral nervous systems, bone cancer pain activates mitogen-activated protein kinases (MAPKs) family, and extracellular signal-regulated kinase (ERK) is an important member of the MAPK family, which mediates many cell signal transduction. Past studies show that phosphorylation of spinal dorsal horn ERK expression has significantly increased in a variety of pain models, and the upstream kinases MEK inhibitors can reduce pain sensitivity, suggesting activation of ERK relates to neural sensitization of pain (Rygh et al., 2002; Ji et al., 2001). This study is to observe the effect of intrathecal injection of new specific ERK1/2 inhibitor SCH772984 on behaviors in rats with bone cancer pain.

2. Materials and methods

2.1. Experimental animal

Adult female Sprague–Dawley rats, 200–220 g, were provided by Laboratory Animal Center of Xuzhou Medical College. Experiments would be carried out after rats' one-week adaption to the environment. Light time in the laboratory is from 8:00 to 20:00, temperature 20 to 25 °C, and humidity 40 to 60%. Rats are free to get food and water.

2.2. Main reagents and apparatus

Walker256 (Shanghai Rongbai biological technology Co., Ltd.); SCH772984, produced by AbMole, China, and solvents are 5% dimethyl sulfoxide (DMSO), rabbit antibodies p-ERK1/2, p-p90RSK and (Cell Signaling Technology, Inc, CST), c-fos antibodies (abcam), Western Blot Kit (Beyotime Technology); electronic mechanical device (IITC Life Science Inc., CA), ELISA (Thermo Fisher Scientific Oy); speed centrifuge (Beckman Coulter, Inc.), electrophoresis, transfer film instrument (BIO-RAD, PowerPacTM HC); frozen machine (LEICA CM1950), optical confocal microscope (OLYMPUS).

2.3. Methods

2.3.1. Spinal cord subarachnoid catheterization

Rats are intraperitoneally injected with 10% chloral hydrate (0.3 ml/100 g), and after being anaesthetized, subarachnoid catheter would be put according to improving Yang and others' methods (Yang et al., 1993). With animals prone, roll out a cloth under the abdomen to support the back, longitudinally cut for 1 cm at spinous process gap L6S1 after disinfection, then cut up the skin and fascia superficialis, reveal spinous process gap after muscle with blunt dissention, slowly push Catheter PE-10 of built-in guide silk, with sense of loose, and the rat tail can be seen jittering that the vertebral tube has been into it. Then take out the guide silk, slowly put Catheter PE-10 into cavum subarachnoidale, with cerebrospinal fluid clearly spilling, determining the depth which is 3 cm, then suture and fix near end catheter. Lead lateral ends of the catheter to the back of the neck through a hole under skin with epidural puncture needle, 2 cm outside, suture and fix, and seal the catheter with hot melt. Feed in a single cage after operation. Observe rats' behaviors after their waking up for 24 h, and those with paralysis, lameness, and dystropy would be given up; intrathecally inject 20 μl lidocaine, and within 30 s no one has paraplegia.

2.3.2. Establishment of bone cancer pain model

Establish rats tibia bone cancer pian model according to Medhurst (Medhurst et al., 2002) and others' methods. Select rats recovering well and meeting experimental requirements with an intrathecal tube, anesthetize them with 10% chloral hydrate (0.3 ml/100 g), make them supine, fixed, right hind leg clipped, skin disinfection, cut about 0.5 cm incision at the top of tibia, expose tibia, and make a hole with 1 ml syringe needles. A sense of breakthrough proves the needle has thorn into marrow cavity, then push microsyringe into marrow cavity while pulling out the needle, then slowly pull out the needle, and red spinal cord can be seen, which shows that the needle has thorn into marrow cavity. Slowly inject 10 μl cell suspension (containing cell number 100000) with Walker256 for 1 min, pull out microsyringe after 2 min, and quickly seal the pinhole with medical glue, then bond skin.

2.4. Experiment I grouping and observation index

2.4.1. Grouping

40 SD rats are randomly divided into 5 groups ($n = 8$): model group (Group N) which contains normal rats; sham group (Group S) which contains rats with 10 μl normal saline in their tibia; intrathecal tube group (Group IT) which contains rats with Tube PE10 intrathecally in spinal cord L6S1; bone cancer pain group (Group BCP) which contains rats with Walker256 cells in marrow cavity of right side of tibia; and intrathecal tube + bone cancer pain group (Group IT + BCP).

2.4.2. Behavioral determination index

Establish a bone cancer pain model after putting the intrathecal tube 5d, determine the rats' mechanical withdrawal threshold (MWT) after tube 5d and establish 1, 3, 6, 9, 12, 15, 18d, 4 min spontaneous paw withdrawal and Fugl–Meyer.

2.4.2.1. Determination of MWT. Mechanical hyperalgesia is determined by electronic Von Frey tenderness instrument (IITC Life Science Inc., CA). Place a plexiglass box (22 cm × 12 cm × 22 cm) on a metal sifter, put rats in for 30 min, and stimulate central area of right rear foot with Von Frey. When rats lift foot, shrink foot or even fast record numbers on electronic screen which is MWT (g). Continuously measure 5 times with 10 s for each interval, and then take average value.

2.4.2.2. Determination of times of spontaneous shrinking foot. Observe rats and get times of rats' spontaneous shrinking foot in 2 min in a quiet situation and accumulative time rats lift foot, according to Mouedden's methods [Evaluation of pain-related behavior, bone destruction and effectiveness of fentanyl, sufentanil, and morphine in a murine model of cancer pain]. Place a plexiglass box on a metal sifter, put rats in for 30 min, and the temperature should be 24 ± 1 °C. During animals staying quiet for 2 min, record times of rats' spontaneous shrinking right rear foot and accumulative time rats lift foot.

2.4.2.3. Right rear foot gait scores (0–4). Rats are placed in transparent plexiglass boxes, where they can walk freely. Observe rats' gait and their usage of left rear legs. Observe for 2 min, score according to the standards [Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord]: 0 point: completely unable to use the limb; 1 point: some cannot use the limb; 2 points: obvious lameness, some stop and lift the limb; 3 points: minor lameness; and 4 points: normal.

2.4.3. Radiology study

After modeling X-ray photograph 9d and 18d respectively to verify the model and assess bone damage caused by tumors.

2.4.4. Pathology HE stain

After X-ray photograph, take 2 tibia HE to dye to further verify the model and Pathology HE stain X-ray take 2 tibia HE dyed to further verify the model and access bone damage caused by tumors.

2.5. Experiment II grouping and observation index

2.5.1. Grouping

40 SD rats with intrathecal tube are randomly divided into 5 groups ($n = 8$): sham group and bone cancer pain group (Group BCP + DMSO, Group BCP + SCH0.1 Group BCP + SCH1.0 and Group BCP + SCH10). Sham group is injected with 10 μg SCH772984 intrathecally, and bone cancer pain group is injected with 10 μl 5%DMSO, 0.1 μg SCH, 1.0 μg SCH, 10 μg SCH (SCH dissolved in 10 μl 5% DMSO) intrathecally.

2.5.2. Behavioral determination index

Before and after modeling 3, 6, 9d, determine the rats' mechanical withdrawal threshold (MWT) 1 h before medication and 1, 3, 6, 9, 12, 15, 18, 24 h after medication, as well as 2-min spontaneous paw withdrawal. (Methods for measurement of MWT and 2 min spontaneous paw withdrawal can be seen in Experiment I).

2.6. Experiment III grouping and observation index

2.6.1. Grouping

30 SD rats with intrathecal tube 5d were randomly divided into 5 groups ($n = 6$): Group B1, B2 and B3 would be intrathecally injected SCH 10 μg on the 9th day after establishing the model, then be respectively taken samples in 1, 9, and 24 h; Group M is the model control group, and it would be taken samples after intrathecally injecting 5% DMSO for 9 h; and Group S is the blank control group.

2.6.2. Observation index

With Western blot, measure rats' spinal dorsal horn p-ERK1/2, p-RSK and Fos protein expression; with immunofluorescence measure Fos expression in spinal dorsal horn.

2.6.2.1. Western blot. After rats were decapitated, quickly remove L4L5 segment of the spinal cord to ice, rats' spinal dorsal horn p-ERK1/2, p-RSK and Fos protein expression, then put into a – 80 °C freezer for later use. Put spinal cord tissue in precooled lysate RIPA (P0013B, Beyotime Technology) and enzyme inhibitor PMSF (ST506, Beyotime Technology) to get homogenate. After taking precipitate, put lysate and enzyme inhibitor again to get homogenate, then take supernatant. Confect standard proteins according to instructions of the BCA Kit (P0012, Beyotime Technology), with concentration of 0.5 mg/ml. Add samples in the porous plate, incubate at 37 °C for 30 min, and determine protein concentration (wavelength 570 nm) with ELISA. After calculations in Excel, add sample buffer, and confect the to-be-measured sample of equal volume and concentration. Take samples with equal volume and add 10% SDS–polyacrylamide gel electrophoresis with constant pressure, 80V stacking gel and 150V separation gel, transfer protein to nitrocellulose membranes with semi-dry method, add primary antibodies after sealing 5% skim milk powder for 2 h, and incubate at 4 °C overnight. Rinse with Washing Buffer (1×) for 3 × 5 min, and add goat anti-rabbit secondary antibodies with alkaline phosphatase (1:1000).

Incubate on a shaker at room temperature for 2 h, then rinse with Washing Buffer for 3 × 5 min and DDH2O for 1 × 5 min. Put it into a small box with chromogenic reagent (NBT/BCIP), then observe, after the band meets the requirements, rinse the band with water till not showing color. Semi-quantitatively analyze the band using image analysis software tools.

2.6.2.2. Immunofluorescence. After rats are intraperitoneally injected 10% chloral hydrate (0.3 ml/100 g) and get anesthetized, quickly inject 100 ml normal saline by aorta, then inject 400 ml 4% paraformaldehyde from fast to slow until the body twitches. Rats straight hind legs and tails suggest a successful injection. Take L4L5 spinal cord and 4% paraformaldehyde out, after 12 h put them in 30% sugar for 48 h. Cut spinal cord into 30 μm slices with freezing microtome. Rinse sections for 3 × 5 min with 0.01MPBS, then rinse it for 2 × 10 min with 0.4%PBS-Triton. Seal it with 10% donkey serum for 1 h (0.4%TBS), then add anti-rabbit c-fos for the night. After rinsing for 3 × 10 min with TBS, add secondary antibodies (dissolved in 0.4% TBS), and incubate at

37 °C for 1 h. Rinse it with TBS for 2 × 10 min and with PBS for 1 × 10 min, then patch and seal the sections. Observe with optical confocal microscope (OLYMPUS) and take a photograph.

2.7. Statistical analysis

All the data are presented as mean ± marked deviation, and analyzed with Graph Pad statistical analysis software. Method of pairing and testing is adopted within groups, while one-way ANOVA between groups. When $P < 0.05$, differences are considered significant.

3. Results

3.1. Experiment I results

3.1.1. MWT determination

Comparing model group with Sham group and intrathecal tube group, MWT shows no significant differences at different time ($p > 0.05$); comparing model group with BCP group and intrathecal tube + BCP group, MWT shows significant differences at different time ($p < 0.05$); comparing model group with BCP group, MWT shows significant differences ($p < 0.05$); comparing BCP group and intrathecal tube + BCP group, MWT shows no significant differences ($p > 0.05$). See Fig. 1.

3.1.2. Determination of 2 min spontaneous shrinking foot times and lifting foot time

Comparing model group with Sham group and intrathecal tube group, spontaneous shrinking foot times and lifting foot time have no significant differences at different time ($p > 0.05$); comparing model group with BCP group and intrathecal tube + BCP group, spontaneous shrinking foot times and lifting foot time of intrathecal tube group obviously increase after modeling from 6d ($p < 0.05$), then decrease from 15d ($p < 0.05$), while lifting foot time still increases from 15d ($p < 0.05$); comparing BCP group with intrathecal tube group, spontaneous shrinking foot times and lifting foot time have significant differences at different time ($p < 0.05$); comparing BCP group and intrathecal tube group, there are no significant differences ($p > 0.05$). See Figs. 2 and 3.

3.1.3. Right rear foot gait scores

Model group, Shan group and intrathecal tube group all get 3 more points on gait, and have no significant differences from each other ($P > 0.05$); BCP group and BCP + intrathecal tube group both get less than 2 points, and have no significant difference from each other ($P > 0.05$), while they have significant differences with model group ($P < 0.05$). See chart below.

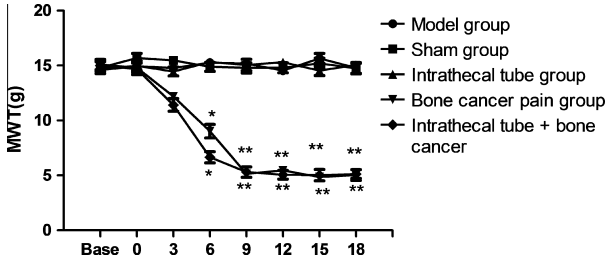

Figure 1 MWT changes before and after modeling at different time (Time (*d*)). In comparison with model group, sham group, intrathecal tube group. $^{*}p < 0.05$ and $^{**}p < 0.01$.

3.1.4. Radiological study

X-ray photograph 9d and 18d before and after modeling, seeing 9d bone partly destructed, and 18d completely destructed. See Figs. 4–6.

3.1.5. Rats' tibia pathology HE stain

Heterotypic cells are full in marrow cavity, and bone is partly destructed. From a picture magnified 10 times under a microscope, a large amount of heterotypic cells can be seen in marrow cavity, and some bones are in invasion. From a picture magnified 40 times under a microscope, a large amount of heterotypic cells can be seen in marrow cavity. See Figs. 7 and 8 below.

3.2. Experiment II results

3.2.1. MWT determination

Intrathecal injection of 10 μg SCH772984 to rats in false model group has no influence on mechanical paw threshold. Compared with false model group, mechanical paw threshold in bone cancer pain group obviously falls after operation for 6d thereafter. Intrathecal injection of 10 μg SCH772984 to rats in sham group has no influence on MWT. MWT in SCH1.0 group after medication for 6 h, SCH10 group after medication for 3 h, 6 h, 9 h, and SCH0.1 group has significant differences, which suggests intrathecally injecting 10 μg SCH772984 can relieve mechanical pain sensitivity and last for 15 h. See Fig. 9 below.

3.2.2. Two mins spontaneous shrinking foot times and lifting foot time

Intrathecal injection of 10 μg SCH772984 to rats in sham group has no influence on spontaneous shrinking foot times and lifting foot time. Compared with false model group, spontaneous shrinking foot times in bone cancer pain group obviously increase after operation for 6 h. Spontaneous shrinking foot times and lifting foot time in BCP + SCH0.1 group, BCP + SCH1.0 group and BCP + SCH10 after medication for 6 h, 9 h, 12 h caused by bone cancer pain can be increased. See Figs. 10 and 11 below.

3.3. Experiment III results

3.3.1. Immunoblotting

Intrathecal injection of ERK1/2 inhibitor SCH772984 has the effect on the protein content of L4L5 segment of spinal cord p-ERK1/2 and p-P90RSK in rats with bone cancer pain. Compared with Sham group, the protein content of rats' lumbar spinal cord p-ERK1/2 and p-P90RSK in control group was significantly increased ($p < 0.01$). Compared with the control group, after one hour's intrathecal injection of 10 μg SCH772984, there is no significant change in the protein content of rats' lumbar spinal cord p-ERK1/2 and p-P90RSK ($p > 0.05$). 9 h after administration, the protein content of rat's lumbar spinal cord p-ERK1/2 and p-P90RSK decreased

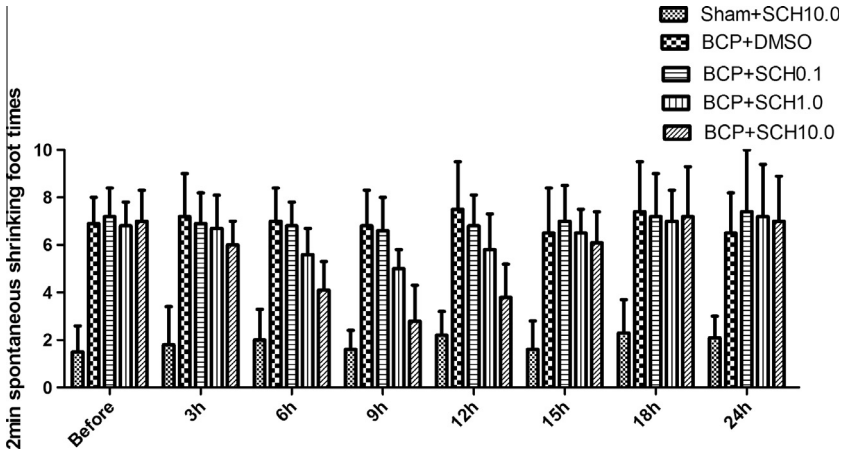

Figure 2 Graph of 2 min spontaneous shrinking foot times.

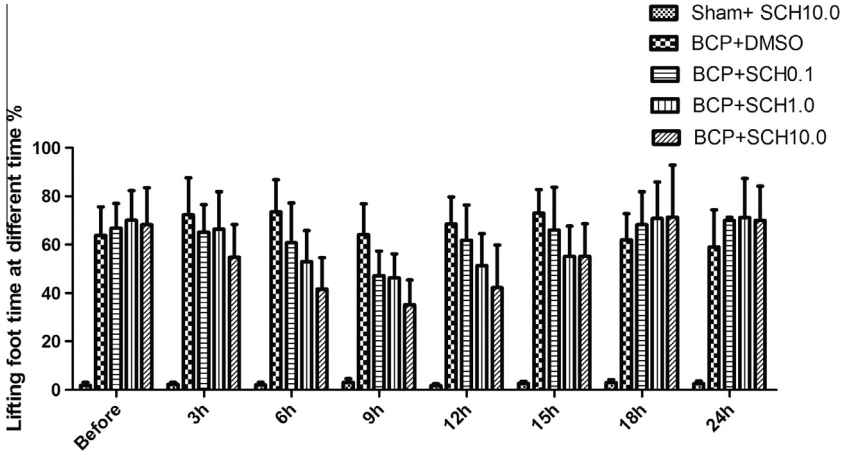

Figure 3 Percentage of lifting foot time at different time.

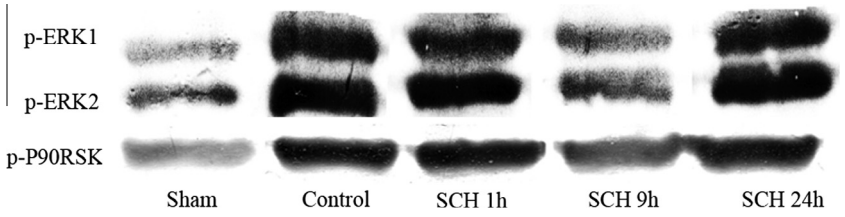

Figure 4 Specimen chart. Right is a normal tibia, while left is one inoculated with tumor for 9 days.

significantly compared with control group ($p < 0.05$). However, no significant difference appeared compared with Sham group ($p > 0.05$). There was also no statistical significance in difference between the protein content of the rats' lumbar spinal cord p-ERK1/2 and p-P90RSK and the control group 24 h after administration ($p > 0.05$) (shown in Fig. 12).

3.3.2. Immunofluorescence

The effect of intrathecal injection of ERK1/2 inhibitor SCH772984 on Fos immunoreactive neurons in L4L5 spinal dorsal horn of rats with bone cancer pain Compared with Sham group, the number of Fos immunoreactive neurons in superficial laminae of the spinal dorsal horn of rats in control group was significantly increased ($P < 0.01$). Compared with control group, after 1 hour's intrathecal injection of 10 μg SCH772984, there was no significant change in the number of Fos immunoreactive neurons in superficial laminae of the spinal dorsal horn of rats ($p > 0.05$). After administration of 9 h, the number of Fos immunoreactive neurons in superficial laminae of the spinal dorsal horn of rats compared with control group was significantly reduced, and the difference was statistically significant ($p < 0.05$). Meanwhile, there was no significant difference in the number of Fos immunoreactive neurons in superficial laminae of the spinal dorsal horn of rats compared with control group after the administration of 24 h ($p > 0.05$) (shown in Fig. 13).

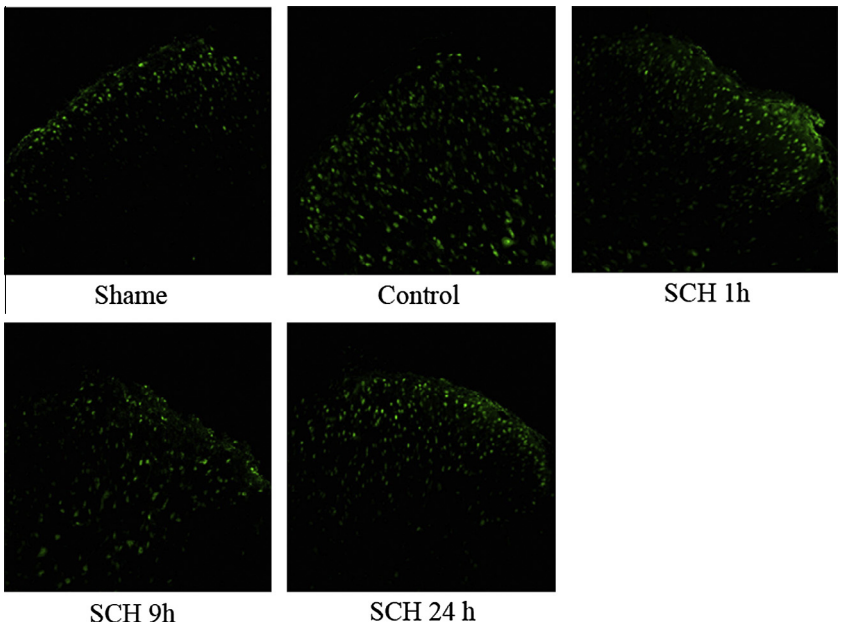

Figure 5 9th X-ray changes after inoculation. Left is a normal tibia, while bone cortex is constructed on the right.

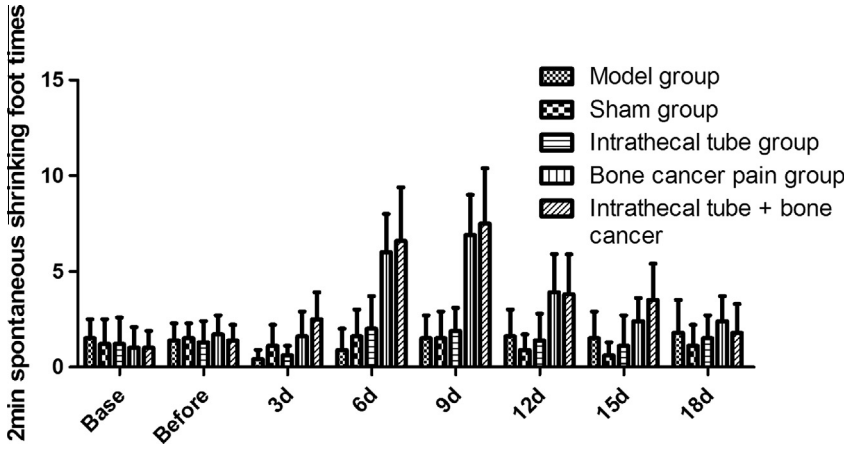

Figure 6 18th X-ray changes after inoculating tumor cells. Right is a normal tibia, while left is one with inoculation.

4. Discussion

Because of the complexity of bone cancer pain mechanism, giving potent analgesic drugs is the main treatment for bone cancer pain clinically, but side effects of opioid analgesics have greatly restricted its application, so medicine based on bone cancer pain mechanism needs to be developed. This study is to establish a bone cancer pain model, transplanting Walker256 cell subcultured by ascites cells to rats of the same strain with marrow cavity in hind tibia. Currently, pain expression model of animals with tibia cancer model resembles bone transfer symptoms, which can be seen as metastatic bone cancer pain model. The methods adopted in this model to inoculate tumors are easy to handle; moreover, observation of tumor cell growth and determination of bone damage degree, as well as method of pain measurement are simple and ripe. The influence of surgery itself does little effect on results, which is currently an relatively ideal model for bone cancer pain experimental researches. MWT changes on limbs 6d after modeling can be observed, and subsequent behavior index shows progressive changes such as mechanical and spontaneous pain sensitivity. Furthermore, X-ray photograph shows bone invaded by tumors and tumor cells seen through measurement of tibia pathology HE stain from model, which proves a successful model of bone cancer pain.

Mitogen-activated protein kinase (MAPK) is signal pathways with highly conserved evolution, which transducts extracellular signals from cell surface to nucleus, mediating signal transduction. Extracellular signal-regulated kinase (ERK) is an important member of mitogen-activated protein family, and intracellular signal transduction mediated by it plays a vital role in cell differentiation, growth and development, neuronal plasticity and many other physiological and pathological processes. In recent years, many studies have shown that ERK activation is the key factor of formation and maintenance of chronic pain (Ji et al., 1999; Mantyh, 2014). MAPK/ERK1/2

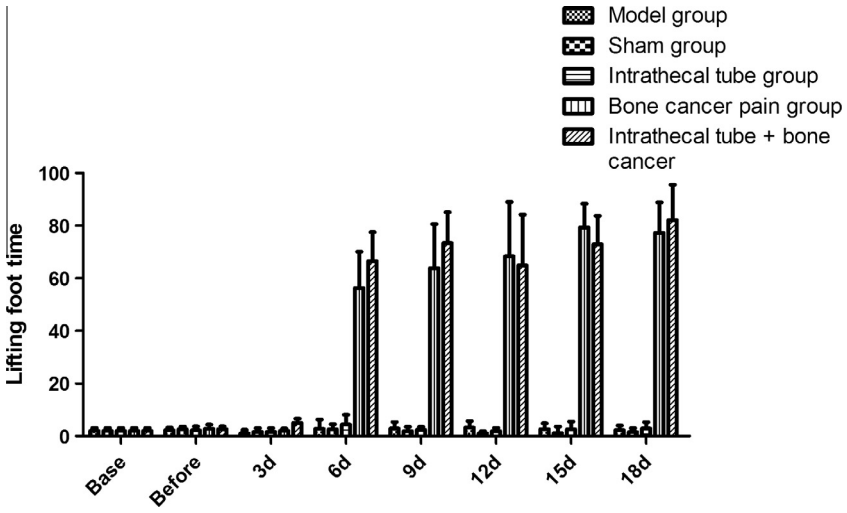

Figure 7 From a picture magnified 10 times under a microscope.

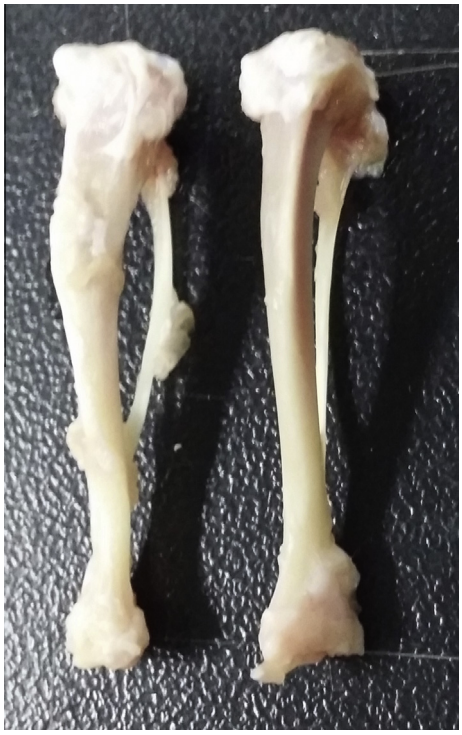

Figure 8 From a picture magnified 40 times under a microscope.

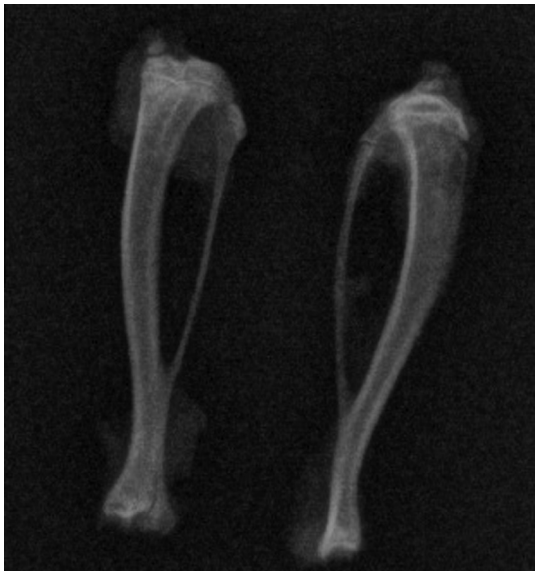

Figure 9 Comparison of MWT of rats at different time before and after medication ($n = 8$, time (h). In comparison with control group $p < 0.05$, $^{**}p < 0.01$. In comparison with sham group $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

signaling pathway plays an important role in changes of central and peripheral neurotransmitter and ion channels caused by noxious stimulation, which is a target for controlling pain (Zhuang et al., 2005). MAPK/ERK1/2 signaling pathways regulate transcription of the gene product through processing after interpretation, so as to regulate pain sensitization process of chronic pain, meanwhile adjust pain-related proteins or receptors in the spinal cord to indirectly regulate pain sensitization process. Earlier studies confirm that ERK participates in the formation and maintenance of pain sensitization in inflammatory pain and a rat model of neuropathic pain (Ji, 2004; Seino et al., 2006). Recent studies also show that in neuropathic pain, phosphorylated ERK1/2 in spinal cord dorsal horn neurons maintains pain by activating transcription factors and regulating gene transcription (Zhang et al., 2013).

This study shows that after the establishment of a rat model of bone cancer pain, p-ERK1/2, p-RSK in spinal dorsal horn in rats significantly increases, as well as its downstream signaling molecules Fos protein expression. After intrathecal injection of specific ERK1/2 inhibitors, dorsal horn of the spinal cord stimulates and activates ERK1/2-RSK-Fos signaling pathway. ERK1/2's activation activates its downstream substrate RSK to p-RSK, and pulls it into nucleus, regulating its downstream gene c-fos, increasing expression of Fos protein, which plays a role in pain sensitization. C-fos, Immediate early genes, IEG, whose expression product is Fos protein, is proved in many pain models its effectiveness of reflection of

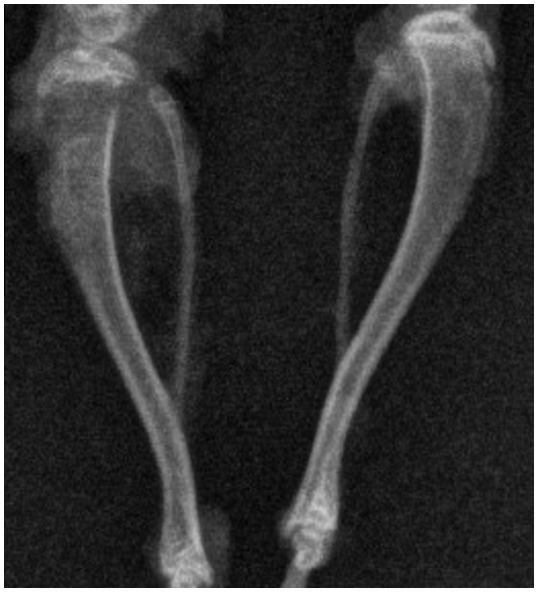

Figure 10 Graph of changes of 2 min spontaneous shrinking foot times at different time before and after medication.

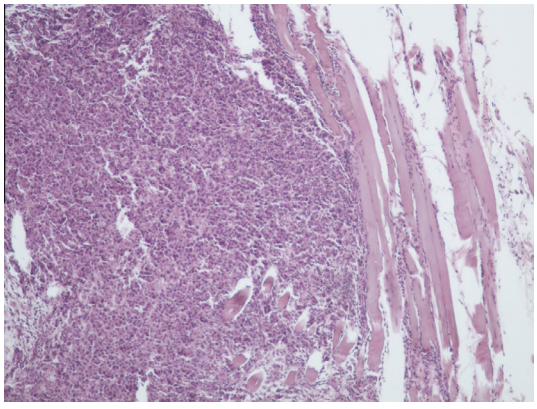

Figure 11 Graph of changes of Lifting foot time at different time before and after medication.

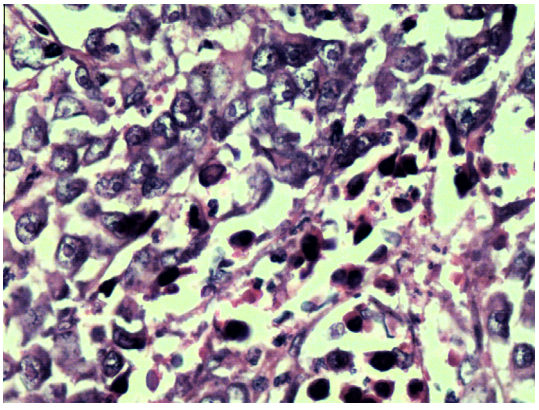

Figure 12 The results of immunoblotting.

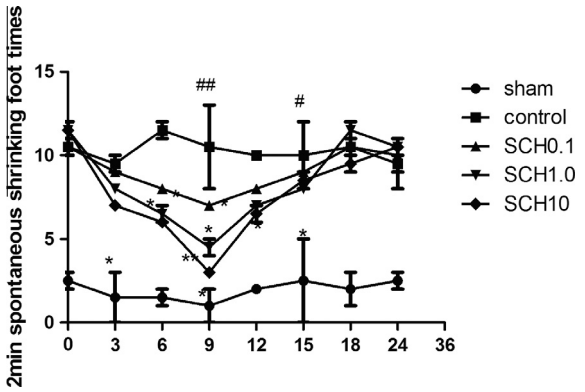

Figure 13 The results of immunofluorescence.

the extent of noxious stimuli in spinal cord level (Presley et al., 1990; Honore et al., 1996). Fos-immunoreactive neurons were concentrated in layers I, II, V afferently terminated in the dorsal horn of the spinal cord A Delta and c-fiber, while Fos-immunoreactive cells are few in layers III and IV non-nociceptive afferently terminated; therefore, noxious stimulation causes Fos expression in certain parts of spinal dorsal horn, and Fos can be seen as a marker of noxious stimuli (Presley et al., 1990; Munglani and Hunt, 1995). In this study, Fos-immunoreactive cells express mainly in superficial layer of the dorsal horn of the spinal cord (layer I and II), so take the number of immunopositive neurons into account in the dorsal horn of the spinal cord when inoculating tumor cells. From the experimental results, Fos expression in spinal dorsal horn of rats in model group after inoculation progressively increases, and obvious hyperalgesia can be seen, which means Fos protein expression relates to pain sensitivity. After the intrathecal injection of ERK1/2 inhibitor, behavioral tests show that the degree of pain in rats decreases, and immunofluorescence tests show that Fos protein expression in spinal dorsal horn reduces, which means that the analgesic effect of ERK1/2 inhibitor works by inhibiting the expression of Fos protein.

Because MAPK/ERK1/2 signaling pathways play an important role in formation and maintenance of chronic pain, some studies (Huang et al., 2000; Pezet et al., 2002) find inhibiting pain sensitivity in rats of bone cancer pain is possible by intrathecally injecting ERK's upstream material MEK's inhibitor U0126. U0126 has not been clinically applied though it has been discovered for a long time; it may be due to its quick tolerability, and the reason for quick tolerance is re-activation of ERK1/2 signaling pathway (Morris et al., 2013). ERK1/2 is the direct material of MEK, and it is known that ERK1/2 directly transmits information with more than 100 kinds of cell signaling proteins downstream, so in the target controlled infusion of ERK1/2, MAPK pathway has its unique advantages, and ERK1/2 is a key point of MAPK signaling pathway. SCH772984 is a new type of specific ERK1/2 inhibitor, the phosphorylation and of its specific inhibitor. ERK1/2's substrate P90 ribosomal S6 kinase (T359/363p-RSK) and the residue phosphorylation of activation loop in itself are dose-dependent, which is also reflected in this study.

In this study, hyperalgesia of rats in bone cancer pain group with intrathecal injection of ERK1/2 SCH772984 is relieved and Fos protein expression in spinal dorsal horn is also

significantly reduced, but it is still higher than that of Sham group; this could be that other factors such as glial cell activation (Wang et al., 2011) also participate in the formation of bone cancer pain.

5. Conclusion

To sum up, ERK1/2/RSK signaling pathways and its downstream c-fos are involved in formation and maintenance of bone cancer pain in rats, and ERK1/2 specific inhibitor SCH772984 has good prospects in development and application in treatment of bone cancer pain.

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Further reading

Ji, R.-R., Woolf, C.J., 2002. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. Neurobiol. Dis. 8, 1–10.