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ORIGINAL ARTICLE

Effect of music therapy on pain behaviors in rats with bone cancer pain

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Summary

Purpose: *To investigate the effects of music therapy on the pain behaviors and survival of rats with bone cancer pain and analyze the mediating mechanism of mitogen activated protein kinase (MAPK) signal transduction pathway.*

Methods: *Male Wistar rats aged 5-8 weeks and weighing 160-200g were collected. The rat models of colorectal cancer bone cancer pain was successfully established. Animals were divided into experimental and control group, each with 10 rats. The animals in the observation group were given Mozart K448 sonata, sound intensity of 60 db, played the sonata once every 1 hr in the daytime, stopped playing during the night, and this cycle was kept for 2 weeks. On the other hand, rats in the control group were kept under the same environment without music.*

Results: *Animals in the experimental group consumed more feed and gained significant weight in comparison to the control group. The tumor volume of the experimental group was significantly smaller than that of the control group ($p<0.05$). After 1-2 weeks of treatment, spontaneous foot withdrawal reflection caused by pain in the experimental group was significantly lower than that in the control group, heat pain threshold and free walking pain scoring in the experimental group were also significantly higher as compared with the control group ($p<0.05$). The expression of p38a and p38β in animals' spinal cord and dorsal root ganglion was significantly lower in the experimental group than in the control group ($p< 0.05$).*

Conclusion: *Music therapy may improve the pain behaviors in rats with bone cancer pain, which might be related with low expression of p38a and p38β in the MAPK signal transduction pathway.*

Key words: *MARK signal transduction pathway, music therapy, pain behavior, rat model of bone cancer pain*

Introduction

The incidence and mortality of cancer are increasing year by year, and has become the third international chronic disease, following cardiovascular and cerebrovascular diseases. Cancer can hardly be diagnosed in the early period. Besides, its progress is usually rapid and can hardly be completely cured, which not only bring serious physical and mental damage to the individual and the family, but also bring serious economic burden to the country and the society. Actively exploring the occurrence of the disease and developing effective treatments are the ultimate ways to fight cancer. Recent studies [1-3] have shown that music therapy could improve the anxiety and pains of cancer patients and also the patients' prognosis and survival. In this study, we investigated the effects of music therapy on the pain behaviors of rats having bone cancer and also analyzed the involvement of MARK signal transduction pathway in this condition.

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Methods

Experimental animal & cell lines

Male Wistar rats (N=20) aged 5-8 weeks and weighing 160-200g were purchased from Shanghai Super B&K laboratory animal Corp Ltd were included in this study. All rats were placed in dark environment with 40W lamp as artificial solar light (light instensity <0.0004 cd/m2), 45% humidity and room temperature (22°C±0.5°C) with 12 hr day and night cycle, free feeding and drinking. Noise level was kept <60db.

Mouse-derived colorectal cancer cell line CT-26 was provided by Shanghai Biomedical Engineering Research Institute. Cells were kept in 5% CO_2 incubator at 37°C, 95% humidity, and were digested with 2.5g/L trypsin. Cells collected in logarithmic growth phase were used in the study.

Establishment of rat model having colorectal cancer bone metastasis

Animal models of tibial cancer pain were created according to the method given by Medhurst et al. [4]. Animals were anesthetized by intraperitoneal injection of 8% perchlorate hydrate, and the right knee joint part was shaved and disinfected with 75% alcohol. A hole was drilled by no.7 pinhead in the peripheral part of the knee joint patellar ligament to 1 cm of the tibia pulp cavity. The needle was drawn out and changed by micro injector carried with 4 μl tumor cells, and then cancer cells were injected into the bone marrow cavity.

Experimental method

The study begun one week post-surgery. The rats were randomly divided into experimental and control group (N=10 each). Mozart K448 sonata was played in the experimental group, with sound intensity of 60 db once every 1 hr in the daytime. The music was stopped during night and the same procedure was repeated for 2 weeks. Rats in the control group were exposed to the same environment without music.

Observation index and detection technology

The weight and feed efficiency ratio as well as the tumor volume were compared and analyzed for the two groups. The weight of consumed feed was measured every day before music playing. The difference between the measured values was the total feed consumption of the rats in the cage as follows:

Feed efficiency ratio=Body weight gain during the experimental period (g)/Total amount of food intake during the experimental period (g) ×100.

Animals in both groups were sacrificed after 2 weeks and the volume of metastatic bone tumor was measured. The left rear limbs were dissected, and skin and muscle tissues were removed to expose the tibia with metastatic tumor. Tumor's long and short diameters were measured by vernier caliper and the total volume was calculated as follows:

Volume of tumor= [long diameter× (short diameter)^2]/2

The pain behavior changes after music therapy were compared for 1-2 weeks, and the results were shown as the number of spontaneous foot withdrawal reflection caused by pain, heat pain threshold and free walking pain scoring. The rats were placed into a transparent flat plastic box (40x40x40 cm) and were free to act in the box. After they adapted to the environment, the number of spontaneous foot withdrawal reflection caused by pain was observed. BME410A radiation heat meter (produced by Institute of Biomedical Engineering, Chinese Academy of Medical Sciences) was applied to detect the threshold value of rats' reflection to heat radiation; the heat pain was presented as threshold value by foot withdrawal reflection time. Then, the rats were placed into an organic glass cage. After they adapted to the environment, the pain threshold of the right paw was measured under thermal stimulus, every 5 min. The average value of three measurements was taken as the heat pain threshold value (the upper time limit of thermal stimulus was set at 20s to prevent the thermal stimulus from injuring the rats). Heat pain scoring was performed according to the following standard: Animals acting normally on the hind limbs on the operation and control side were scored as 0; in case the hind limbs on the operation side had a slight limp, this was scored as 1; in case that the degree of limp on hind limb on the operation side ranged between 1 and 3, this was scored as 2; in case that limp on hind limb on the operation side was severe, this was scored as 3; and in case that the hind limb on the operation side could hardly walk freely or touch down to the ground was scored as 4.

Immunohistochemistry

To detect the expression differences of p38α and p38β in the rats' spinal cord and dorsal root ganglion, rats were anesthetized with mebumal natrium Thoracotomy was performed to collect aorta ascendent from the left ventricle which was incubated in 100 ml normal saline for washing; then, endocardial perfusion fixation was performed with 300 ml 4% poly formaldehyde phosphate buffer, and L4-6 section of the spinal cord was taken out and frozen coronal sections of 30 μm were prepared.

The sections were rinsed in 0.01 moL/L of PBS for 10 min and then placed in 3% H_2O_2 for 10 min for incubation. Then, the sections were rinsed with PBS for 5 min, followed by addition of sheep serum for 20 min incubation. The sections were incubated in rabbit anti p38α (1:300, R&D) and sheep anti p38β (1:50, Santa Cruz) respectively and placed at 4°C overnight. Then, the sections were washed with PBS for 3 min×3 times. Subsequently, secondary antibodies goat anti-rabbit lgG p38a, 1:300 and mouse anti-sheep lgG p38b, 1:50,

Santa Cruz) was placed at room temperature for 20 min, followed by rinsing in PBS for 3 min×3 times, dropping horseradish peroxidase labeled streptavidin to incubate at room temperature for 20 min and followed by rinsing in PBS for 3 min×3 times. The color was developed by DAB and sections were dried for analysis. The gray color intensity scale of immunohistochemical reaction for p38α and p38β receptors on spinal cord dorsal horn was measured under x200 magnification by HPIAS-1000 high resolution color image analysis system, for semiquantitative analysis. For analyses, 5 sections from each rat were randomly taken for calculating the average.

Fluorescent double-labeling

The sections were rinsed with 0.01% moL/L PBS for 10 min and incubated with sheep serum for 30 min. Then they were incubated with rabbit anti-p38α antibody at 4°C overnight, rinsed with PBS for 3 min×3 times, added FITC-labeled goat anti-rabbit IgG fluorescent secondary antibody and incubated at room temperature for 1 hr. Afterwards they were rinsed with PBS for 3 min×3 times, incubated with sheep serum for 30 min, added mouse anti-NeuN (1: 500, Santa Cruz) and put at 4°C for overnight incubation. Then they were rinsed with PBS for 3 min×3 times, added TRITC-labeled goat anti-rat IgG fluorescent secondary antibody and incubated at room temperature for 1 h, and rinsed again with PBS for 3 min × 3 times. Then, the sections were sealed with non-blooming buffered glycerin and the results of the double labeling were observed under fluorescence microscope. As for double-labelling immunofluorescence on sheep anti-p38β and mouse anti-OX42 (1:50, Santa Cruz), please refer to the above method.

Statistics

SPSS 20.0 software package (SPSS Inc, Chicago, Ill) was used for statistical analyses. Assessed data are presented as means±standard deviation. Student's t-test was used for comparison between groups, and $p<0.05$ was considered as statistically significant.

Results

Comparison on the changes of body weight, feed efficiency ratio and tumor volume

Before music intervention, there was no significant difference in body weight between the two groups ($p>0.05$), whereas after music intervention the weight of the rats in the two groups decreased. Of note, in the experimental group the decrease was significantly less compared with the control group ($p<0.05$). The feed efficiency of the experimental group was significantly higher than that of the control group, and the tumor volume was significantly smaller than in the control group ($p< 0.05$) (Table 1).

Comparison on the change of pain behaviors

After music therapy for 1 and 2 weeks, the results showed that the number of spontaneous foot withdrawal reflection was less than that in the control group, and the heat pain threshold as well as free walking pain scoring were significantly higher than in the control group ($p<0.05$) (Table 2).

Table 1. Comparison on the changes of body weight, feed efficiency ratio and tumor volume

Group	*Weight before intervention (g)*	*Weight after 2 weeks of intervention (g)*	*Weight gain (g)*	*Feed efficiency ratio (%)*	*Tumor volume (mm^3)*
Control group	172.8±5.6	163.9±4.3	10.5±1.3	35.4±6.2	114.3±24.7
Experimental group	169.5±4.3	165.7±4.1	4.9±1.2	62.3±5.8	32.6±12.2
t	0.537	3.106	6.524	5.748	6.957
p	0.426	0.043	0.012	0.026	0.008

Table 2. Comparison on the changes of pain behaviors

Group		*Control group*	*Observation group*	*t*	*p*
Intervention for 1 week	Times of spontaneous foot withdrawal reflection caused by pain(time/10min)	24.6±5.3	15.5±3.4	5.857	0.028
	Paw withdrawal reflection time (s)	32.4±4.7	43.7±5.3	4.328	0.039
	Free walking pain scoring	3.2±0.7	2.9±0.5	3.526	0.037
Intervention for 2 weeks	Times of spontaneous foot withdrawal reflection caused by pain	28.7±6.2	10.4±3.2	6.659	0.011
	Paw withdrawal reflection time (s)	27.8±4.3	49.3±5.7	5.623	0.031
	Free walking pain scoring	3.6±0.6	2.5±0.3	5.574	0.033

Table 3. Comparison of expression of p38α and p38β

Group	*Control group*	*Experimental group*	*t*	*p*
p38α light absorption value	71.2±3.9	35.4±3.7	6.847	0.014
p38β light absorption value	68.5±3.3	40.2±3.5	6.632	0.018

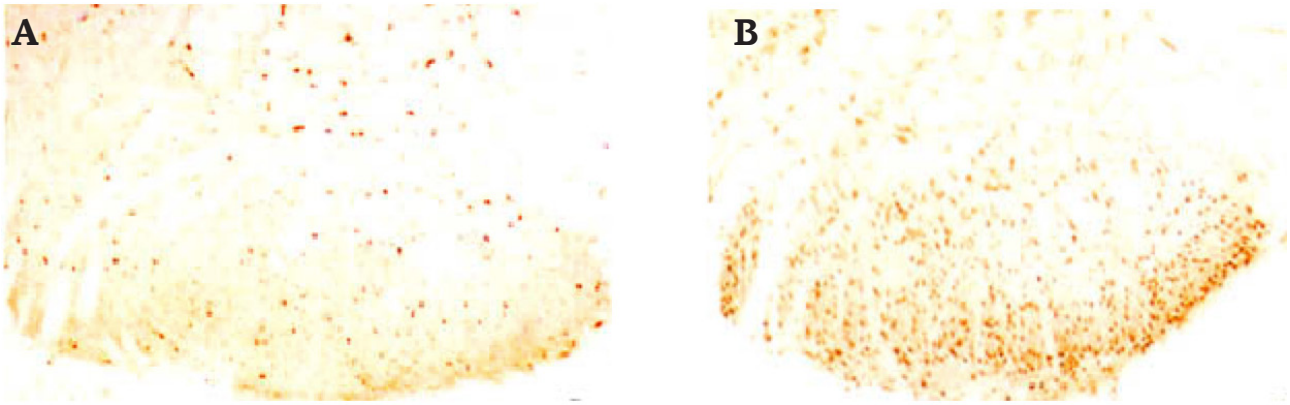

Figure 1. Immunohistochemistry of p38α of the experimental group (**A**) and the control group (**B**). The expression intensity of p38a in the dorsal horn of the spinal cord in the experimental group is significantly lower than that in the control group.

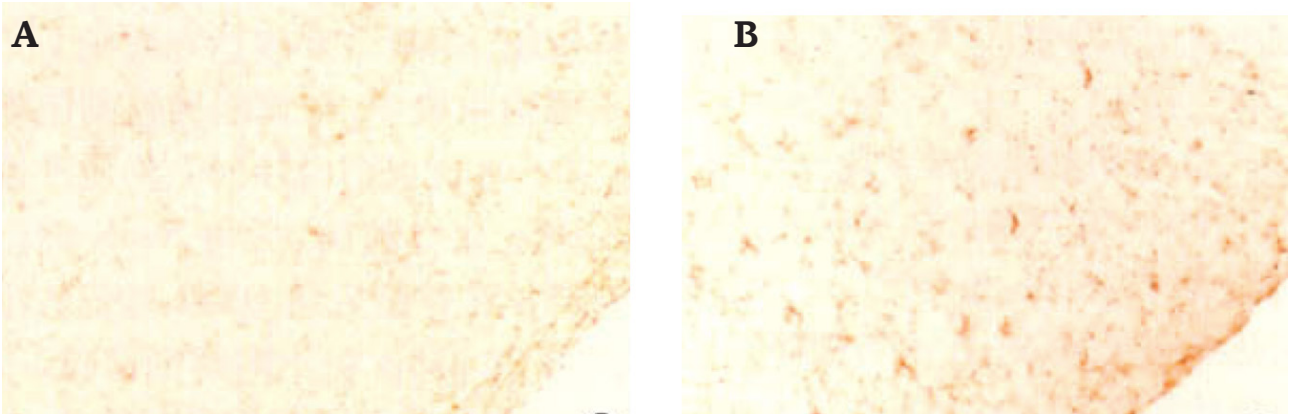

Figure 2. Immunohistochemistry of p38β in the experimental group (**A**) and the control group (**B**). The expression intensity of p38b in the dorsal horn of the spinal cord in the experimental group is significantly lower than that in the control group.

Comparison on the expression of p38a and p38β

The expression of p38α and p38 β in the experimental group were significantly lower than those in the control group (p38α light absorption value: 35.4±3.7 to 71.2±3.9, t=6.847, p=0.014; p38β light absorption value: 40.2±3.5 to 68.5±3.3, t=6.632, p=0.018) (Figures 1-4, Table 3).

Discussion

Music therapy is a new interdisciplinary subject integrating music, medicine, and psychology. Studies on cancer care have confirmed that pain treatment, vomiting control, and the patients' psychological mood such as anxiety and depression can be included into the targets of music therapy [5]. Bruscia [6] from Temple University, USA, has defined music therapy as a systematic intervention process by using the treating impetus developed from music experience and treatment process to help the treated subjects recover health. All activities related to music such as listening, singing, instrumental music, music creation, lyrics creation, dancing, art and other activities could be used as the means for music therapy.

A number of studies [7-9] has found that music therapy has a significant effect on the treatment of chronic cancer pain. Moreover, the organic combination of music therapy and other psychological intervention techniques such as muscle re-

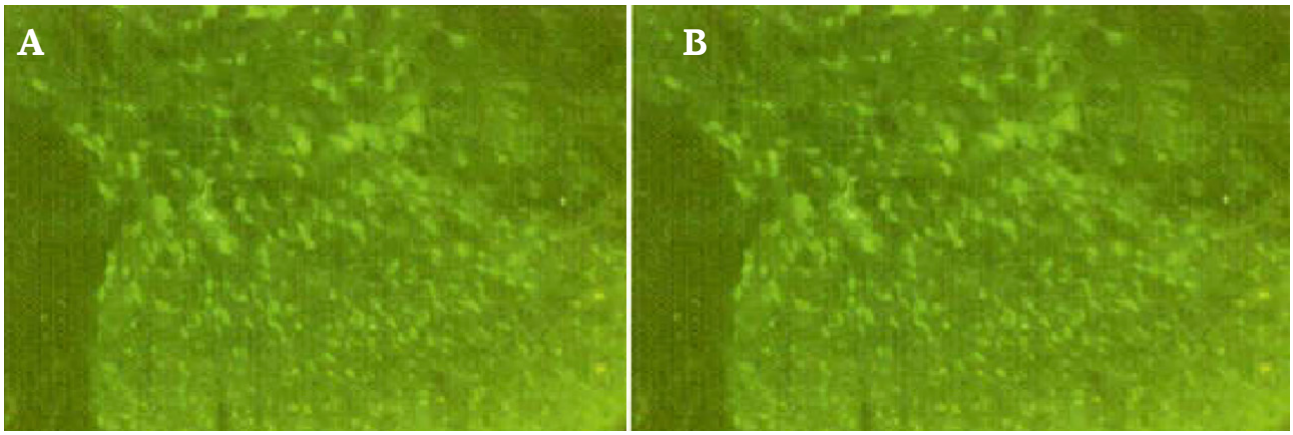

Figure 3. Double immunofluorescent labelling of p38α in the experimental group (**A**) and the control group (**B**). In the experimental group p38a in the spinal dorsal horn is mainly expressed in neurons and co expressed with the specific nuclear protein marker Neu N, the expression intentsity of which is significantly lower than that in the control group.

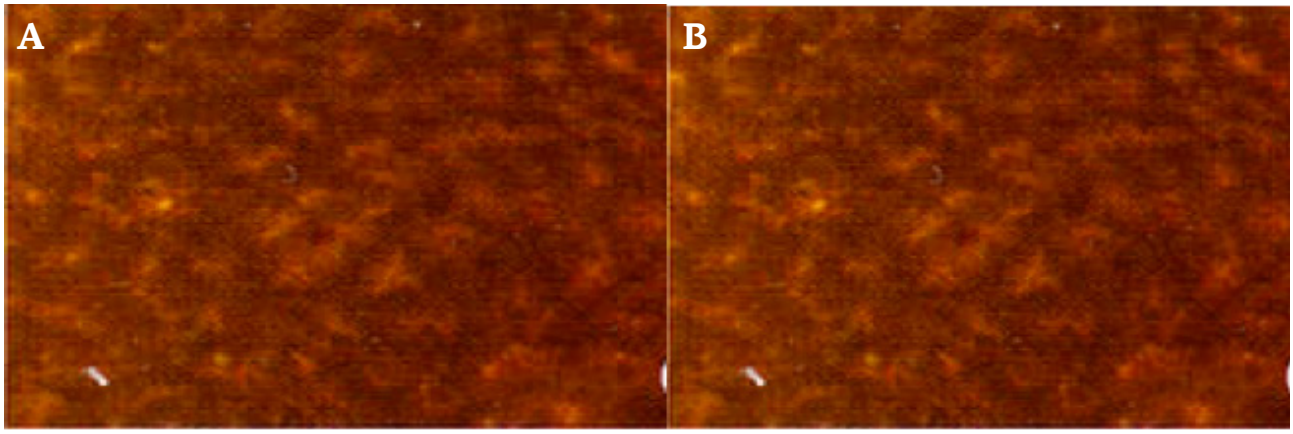

Figure 4. Double immunofluorescent labelling of p38β in the experimental group (**A**) and the control group (**B**). In the experimental group in the spinal dorsal horn p38b is mainly expressed in microglia cells and co expressed with the cell marker OX-42, the expression

laxation, guiding imagination, self-hypnosis and biofeedback can improve the therapeutic effect. But at present, related basic researches on music treatment are too few to reach a deeper interpretation of this treatment mechanism. In our study, we have established a rat model of bone cancer pain with rectal cancer cells. Compared with most of the previous models of bone cancer pain in breast cancer, our model showed higher success rate and better stability. It might be because tibial metastasis is more common in rectal cancer than in breast cancer. In this study, we have played K448 sonata of Mozart, which was suitable to the audible threshold and frequency domain of rats. To keep in pace with the daily routine of rats, we played the sonata during daytime by intervals. We have also combined the observation indexes related with the prognosis of cancer including weight, feed efficiency ratio and tumor volume with the pain behavior indexes including the number of spontaneous foot withdrawal reflection caused by pain, heat pain threshold and free walking pain scoring together, so as to evaluate the effect of music therapy on the prognosis of cancer more effectively.

We observed less reduction in body weight in the experimental group than in the control group, the effect of feed efficiency was larger, and the tumor volume was smaller (Table 1). As the experiment went on, the number of spontaneous foot withdrawal reflection caused by pain in the control group increased, thermal pain threshold decreased and free walking pain scoring increased, while the number of spontaneous foot withdrawal reflection caused by pain in the experimental group decreased, pain threshold increased and free walking pain scoring decreased. These between-groups differences were statistically significant.

MAPK belongs to the family of serine/threo-

nine protein kinases, which can transduce extracellular stimuli into intracellular transcription and translation effects [10-19]. MAPK family includes extracellular regulated kinase (ERK), p38 MAPK, c-Jun, and N-terminal kinase (JNK) [11]. A number of studies [12,13] have confirmed that in the process of inflammatory and neuropathic pains, three signaling pathways of spinal cord MAPK were activated to different degrees. Inhibiting MAPK signaling pathway could relieve allodynia, suggesting that MAPK signaling pathway was involved in the central sensitization of inflammatory and neuropathic pains. The p38MAPK family consists of p38α, p38β, p38γ, p38δ [11], and p38α, and p38β are widely present in colloid cells of the spinal cord and dorsal root ganglia [14]. Many authors [15-18] have confirmed that the p38 signaling pathway in spinal microglia cells was involved in the process of chronic pains, including neuropathic pain, inflammatory pain and cancer pain. P38 inhibitor SB203580 can significantly reduce the allodynia of chronic pain. We found that expression of p38α and p38β in the experimental group were significantly lower than those in the control group, the differences being statistically significant (Figures 1-4).

In conclusion, music therapy may improve the pain behaviors in rats with bone cancer pain, which might be related with the low expression of p38α and p38β in MAPK signal transduction pathway.

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