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Effects of intrathecally administered interferon α on chronic constriction injury model rats' mechanical pain threshold and G protein expression in the spinal cord

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Abstract

Introduction: *The aim of the study was to explore the analgesic mechanism of effects of intrathecally administered interferon α (IFN-α) on chronic constriction injury (CCI) model rats.*

Material and methods: *24 rats were divided into 6 groups, with 4 rats in each group, including the negative control group (Group N, no operation or treatment), the sham operation group (Group S, only the left sciatic nerve of the rats was exposed without ligation, 0.9% NaCl was intrathecally administered), and four experimental groups (CCI model was established first and then different drugs were intrathecally administered respectively), including 0.9% NaCl (Group C), IFN-α (Group CI), morphine (Group CM), and IFN-α combined with morphine (Group CIM). The mRNA levels of G proteins in both the spinal cord and dorsal root ganglia (DRG), as well as the content of amino acid and chemokine (C-X-C motif) ligand 6 (CXCL-6) in the cerebrospinal fluid were measured and analysed in each group.*

Results: *Intrathecal administration of IFN-α increased the mechanical pain threshold in CCI rats (33.32 ±1.36 vs. 21.08 ±1.59, $p < 0.001$), achieving the effect comparable to that of morphine (33.32 ±1.36 vs. 32.44 ±3.18, $p > 0.05$), increased the mRNA expression level of Gi protein (0.62 ±0.04 vs. 0.49 ±0.05, $p = 0.006$), and decreased the mRNA expression level of Gs protein in the spinal cord (1.80 ±0.16 vs. 2.06 ±0.15, $p = 0.035$) and DRG (2.11 ±0.10 vs. 2.79 ±0.13, $p < 0.001$). The intrathecal administration of both IFN-α and morphine can reduce the glutamate content in the cerebrospinal fluid (261.55 ±38.12 vs. 347.70 ±40.69, $p = 0.012$), but without any statistically significant difference in the content of CXCL-6 across all groups ($p > 0.05$).*

Conclusions: *Intrathecal injection of IFN-α improved the mechanical pain threshold in CCI rats, so we inferred that intrathecal administration of IFN-α had analgesic effects on neuropathic pain, possibly related to the activation of G-protein-coupled μ receptors in the spinal cord and the inhibition of glutamate release.*

Key words: *interferon α, analgesia, intrathecal administration, CCI model, rats, G protein.*

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Introduction

Interferon α (IFN-α) is a 20 kDa cytokine synthesized and secreted by immune cells, neuroglial cells, and neuronal cells, and its molecule contains amino acid sequences of corticotropin and endorphin. Interferon α is an important neuroregulator in the central nervous system as well as an important immune regulator [4]. Blalock *et al*. [3] believe that there is structural homology and functional crossover between IFN-α and endorphin, and IFN-α has endorphin-like analgesic effects. The studies of Wang and Jiang confirmed that there exist distinct domains in the IFN-α molecule, which mediate immune and analgesic effects of IFN-α, respectively [6,13,14], and the central analgesia of IFN-α is mediated by opioid receptors [10,12]. A lot of research has proved that systemic administration [11] and intraventricular injection of IFN-α has analgesic effects on neurogenic pain, while the intrathecal injection of IFN-α does not [7]. However, several clinical studies showed that IFN-α had a significant analgesic effect at the level of the spinal cord when applied to paravertebral block in patients with herpes zoster neuralgia [5] and spinal metastatic neuralgia [8]. Liu *et al*. also found that IFN-α/β receptors are present in the superficial layers of the spinal dorsal horn (layer I-II) and dorsal root ganglion [9], therefore more research is needed to investigate the analgesic mechanism of IFN-α after intrathecal administration, whether IFN-α worked at the level of the supraspinal central nervous system only or worked at the level of the spinal cord as well.

We designed this experiment to further investigate the analgesic effect of intrathecal administration of IFN-α on neuropathic pain and the underlying mechanism, and to provide theoretical basis for clinical treatment of neuropathic pain.

Material and methods

This study was approved by the Institutional Animal Care and Use Committee of Shanxi Medical University, China (SYDL2020002). The animal breeding environment is quiet, with a good ventilation and air filtration system. The room temperature is about 22°C, the humidity is about 50% and the animals can eat freely during a photoperiod of 12 hours of light and 12 hours of darkness. The cage and padding are changed every other day.

Experimental animal grouping

The experiment started 7 days after adaptive feeding of rats. 24 adult male Sprague-Dawley (SD) rats aged 6-8 weeks (200-240 g) were divided into 6 groups, with 4 animals in each group. Group N is the negative control group, receiving no operation or treatment. Group S is the sham operation group. Only the left sciatic nerve of the rats was exposed without ligation. 0.9% NaCl was intrathecally administered 7 days after the sham operation (25 µl each). For the four experimental groups, the CCI model was established as follows.

Establishment of CCI model

The rats in the experimental groups (C, CI, CM and CIM) were anesthetized with intraperitoneal injection of 1% pentobarbital sodium (40 mg/kg). The CCI model of sciatic nerve ligation was established according to the procedures reported by Bennett *et al*. [2]. 200,000 U penicillin was intraperitoneally injected in all the five surgery groups (S, C, CI, CM and CIM) immediately after surgical closure.

Intrathecal administration

After the establishment of the CCI model, different drugs were intrathecally administered: 0.9% NaCl (25 µl each) for Group C, IFN-α (100 ng each, based on the study of Liu *et al*.) for Group CI, morphine (0.03 mg/kg, according to the human intrathecal dosage) for Group CM, and IFN-α (100 ng each) combined with morphine (0.03 mg/kg) for Group CIM. The drugs in all experimental groups were diluted with 0.9% NaCl to obtain 25 µl and then intrathecally injected through intervertebral foramina of L4-L6 with a microsyringe according to the instructions.

Seven days after the modelling operation, the rats were anesthetized and their left transverse processes of L4-6 and intervertebral foramens were exposed. IFN-α (Sino Biological, 80174-R02H, Rat, Recombinant; 100 ng each in 25 µl), or morphine (Northeast Pharmaceutical Company 180202-2; 0.03 mg/kg in 25 µl), or IFN-α plus morphine (in 25 µl) were then intrathecally administered with a disposable microsyringe *via* L4-L5 intervertebral foramen to rats of Groups CI, CM and CIM. The same volume of 0.9% NaCI was intrathecally administered (25 µl each) in Group C.

Sampling of cerebrospinal fluid, dorsal root ganglion tissue and spinal cord tissue in rats

Two hours after intrathecal administration, the CCI model rats were anesthetized and fixed in the prone position. The skin was cut open along both sides of the spinous process, the muscle tissue was separated with blunt dissection, and the thoracolumbar spine was then exposed. 0.4 ml cerebrospinal fluid was drawn with a microsyringe and stored in a refrigerator at –80°C. Then we transected the spine at the upper

thoracic segment, and gently used ophthalmic scissors to open the spinal canal downward along the facet joints on both sides of the spinal canal in order to fully expose L2 ~ S1 spinal cord. The proximal end of the spinal cord was clamped with curved forceps, and bilateral nerve roots were cut gently along the spinal cord with ophthalmic scissors, so that the spinal cord and bilateral dorsal root ganglions (DRGs) can be fully exposed. The spinal cord of left L4, L5 DRG and the intumescentia lumbalis segment was carefully removed, placed in sampling tubes separately, then rapidly frozen in liquid nitrogen, and finally stored in a refrigerator at –80°C.

Determination of the paw withdrawal mechanical threshold of rats

The probe needle of pain gauge (Calvin, type: KW – CT) was used to vertically stimulate the part between the third and fourth toe of the left planta pedis at four time points of 0 h before modelling (T0), 7 d after modelling (T1), 0.5 h after intrathecal administration (T2), and 2 h after intrathecal administration (T3), respectively. The intensity of stimulation was considered proper when obvious leg withdrawal or paw licking was observed. In the process, the device can detect the withdrawal of the tested paw and record automatically the stimulus intensity, namely PWT (paw withdrawal mechanical threshold), and the results would be immediately imported into the computer.

Specimen assay

RT-qPCR of mRNA levels of Gs and Gi proteins in the spinal cord and DRG

First, we took about 100 mg of DRG or spinal cord tissue freshly frozen and stored in –80°C refrigerator, added 1 ml Trizol reagent, and then ground the mixture into pulp with a homogenizer. Dried RNA precipitate was obtained through repeated centrifugation and purification and then dissolved in 20 µl DEPC-treated water. We took 2 µl dissolved RNA and calculated the values of OD260 and OD280 with a micro-spectrophotometer to determine the purity and concentration of RNA. RNA quality was estimated based on the OD260/OD280 ratio, which ranged from 1.8 to 2.0 and met experimental requirements. According to the absorbance value, we calculated the concentration of the sample RNA using the following formula: Total RNA concentration (µg/µl) = OD260 × 40 × 10^{-3}.

Using the mRNA as the template, we adopted a random primer and reverse transcriptase to transcribe mRNA into cDNA. There the action was performed under the following conditions: 25°C 5 min, 50°C 15 min, 85°C 5 min, and 4°C 10 min. The cDNA was diluted 5 times and then detected by real-time fluorescence quantitative PCR. The PCRs were performed with the following cycle parameters: 50°C 2 min, 95°C 10 min, 95°C 30 sec, 60°C 30 sec, and 40 cycles. Fluorescence quantitative PCR was used to calculate CT (cycle CT) value, which was transformed into a linear form for statistical processing, and the final data were analysed using $2^{-\Delta\Delta Ct}$ methods. The Gs primer sequences are: forward 5'-GTGTGGCCCTGAGTGACTAT-3' and reverse 5'-AGCCGCCTCTTCATATGTGT-3'. The Gi primer sequences are: forward 5'-TGCCACACCAACTTTAGCAC-3' and reverse 5'-TTCTTCTCCTGGCCGACAAT-3'.

Determination of glutamate, γ-aminobutyric acid, and chemokine ligand 6 in the cerebrospinal fluid

0.2 ml cerebrospinal fluid was taken from each specimen stored in the refrigerator. Glutamic acid concentration (µmol/l) was calculated based on the reading of ultraviolet colorimetry using an assay kit (Nanjing Jiancheng Bioengineering Institute). Another 0.2 ml cerebrospinal fluid was subjected to ELISA assay for quantification of chemokine ligand 6 (CXCL-6; Sangon Biotech, AD3314Ra) and γ-aminobutyric acid (GABA; Sangon Biotech, AD2929Ra). The kits manufacturer's instructions were rigorously followed respectively in above assay procedures.

Statistical analyses

The software SPSS 17.0 was used for data analysis. Quantitative data were presented as mean ± standard deviation (x ±SD). One-way ANOVA was used for intergroup comparison, and LSD Post hoc test was conducted. $P < 0.05$ was considered statistically significant.

Results

Paw withdrawal mechanical threshold

As shown in Figure 1, there was no significant difference in PWT at T0 across all the groups. Compared with Group N, PWT of Group S is significantly lower at T1 (30.16 ±2.79 vs. 33.72 ±2.06; $p = 0.003$) and T3 (29.92 ±2.72 vs. 34 ±1.73; $p = 0.004$). Compared with Group S, PWT of all other groups is significantly lower at T1 ($p < 0.001$). There was no significant difference in PWT at any time point among groups CI, CM, CIM and C.

Compared with Group C, PWT of Groups CI, CM and CIM is dramatically higher at T2 and T3 ($p < 0.001$). There was no significant difference among groups CI, CM and CIM. Neither Group CI nor Group CM showed significantly different PWT from Group S. Compared with Group S, Group CIM showed a marked increase ($p = 0.003$ and $p = 0.004$, respectively).

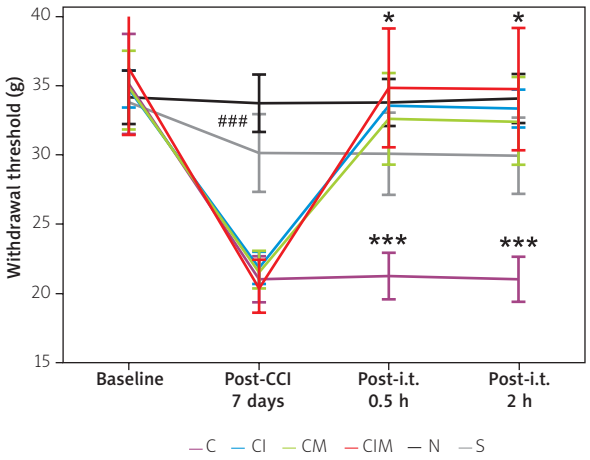

Fig. 1. Analgesic effect of intrathecally administered IFN-α on the PWT. Comparisons of PWT values between groups at 4 time points before and after surgery. At T2 and T3, the PWT value of Group CIM increased (*$p < 0.05$, vs. Group S, two-way ANOVA, $n = 4$); at T2 and T3, the PWT values increased in Groups CI, CM and CIM (***$p < 0.001$, vs. Group C, two-way ANOVA, $n = 4$); at T1, the PWT values decreased in Groups C, CI, CM and CIM (###$p < 0.001$, vs. Group S, two-way ANOVA, $n = 4$).

RT-qPCR results of mRNA of G proteins in the spinal cord and dorsal root ganglion at T3

Test results of the spinal cord of the intumescentia lumbalis segment

As shown in Figure 2, the mRNA level of Gi protein of Group C (0.49 ±0.05) is significantly lower than that of Groups S (1.05 ±0.08) and N (0.96 ±0.06) ($p < 0.001$), while Gs level of Group C (2.06 ±0.15) is significantly higher than that of Groups S (0.99 ±0.05) and N (0.95 ±0.07) ($p < 0.001$).

Compared with Group C, Groups CI, CM and CIM showed significantly higher Gi protein mRNA levels (0.49 ±0.05 vs. 0.62 ±0.04, $p = 0.006$; 0.69 ±0.03, $p < 0.001$; 0.78 ±0.05, $p < 0.001$, respectively), and significantly lower Gs protein levels (2.06 ±0.15 vs. 1.80 ±0.16, $p = 0.035$; 1.65 ±0.15, $p = 0.003$; 1.46 ±0.22, $p < 0.001$, respectively). However, there was no significant difference between Group CI and Group CM in the mRNA level of both Gi and Gs proteins. Compared with Group CI, Group CIM showed a significantly higher mRNA level of Gi protein ($p = 0.002$), and a significantly lower level of Gs protein ($p = 0.009$). Compared with Group CM, Group CIM showed a significantly higher mRNA level of Gi protein ($p = 0.049$), and no significant difference in the mRNA level of Gs protein.

Results for dorsal root ganglion

Compared with Groups S and N, Group C showed a significantly lower Gi protein mRNA level (1.04 ±0.12 vs. 0.34 ±0.04; 1.03 ±0.11 vs. 0.34 ±0.04, $p < 0.001$ for both), and a significantly higher Gs protein mRNA level (1.01 ±0.13 vs. 2.79 ±0.13; 1.03 ±0.08 vs. 2.79 ±0.13, $p < 0.001$ for both). Compared with Group C, Groups CI, CM and CIM showed significantly higher levels of Gi protein mRNA (0.34 ±0.04 vs. 0.53 ±0.08, $p = 0.013$; 0.70 ±0.06, $p < 0.001$; 0.81 ±0.07, $p < 0.001$, respectively), and significantly lower Gs protein mRNA (2.79 ±0.13 vs. 2.11 ±0.10, 2.04 ±0.13, and 1.64 ±0.15, $p < 0.001$ for all). Compared with Group CI, Group CIM showed a significantly higher expression level of Gi protein mRNA ($p = 0.001$), and a significantly lower expression level of Gs protein mRNA ($p = 0.002$). Compared with Group CM, Group CI showed a significantly lower expression level of Gi protein mRNA ($p = 0.023$), and no significant difference in the expression level of Gs protein mRNA. Compared with Group CM, Group CIM showed no significant difference in the expression level of Gi protein mRNA, and a significantly lower expression level of Gs protein mRNA ($p = 0.006$) (Fig. 2).

Contents of glutamate, γ-aminobutyric acid and chemokine ligand 6 in CSF at T3

Compared with Groups N and S, Group C showed significantly higher glutamate (Glu) content in the cerebrospinal fluid (440.00 ±66.94 vs. 126.18 ±32.35 and 135.40 ±17.39, $p < 0.001$ for both). Compared with Group C, Groups CI, CM and CIM showed significantly lower Glu content in cerebrospinal fluid (CSF) (440.00 ±66.94 vs. 347.70 ±40.69, $p = 0.008$; 304.63 ±35.33, $p < 0.001$; and 261.55 ±38.12, $p < 0.001$; respectively). Compared with Group CI, Group CIM showed significantly lower Glu content in CSF ($p = 0.012$) (Fig. 3).

Compared with Groups N (21.23 ±0.90) and S (21.46 ±0.81), Groups C, CM and CIM showed significantly higher GABA content in the cerebrospinal fluid (23.60 ±0.94, $p = 0.004$; 25.32 ±0.97, $p = 0.009$; and 23.29 ±0.65, $p < 0.001$). Compared with Group C, Group CIM showed no obvious difference in the GABA content in CSF, group CM showed significantly higher GABA content (25.32 ±0.97 vs. 23.60 ±0.94, $p = 0.026$), and Group CI showed significantly lower GABA content ($p = 0.006$). Compared with Group CI, Groups CM and CIM showed significantly higher GABA content in CSF ($p < 0.001$, $p = 0.016$). Compared with Group CM, Group CIM showed significantly lower GABA content in CSF ($p = 0.010$) (Fig. 3).

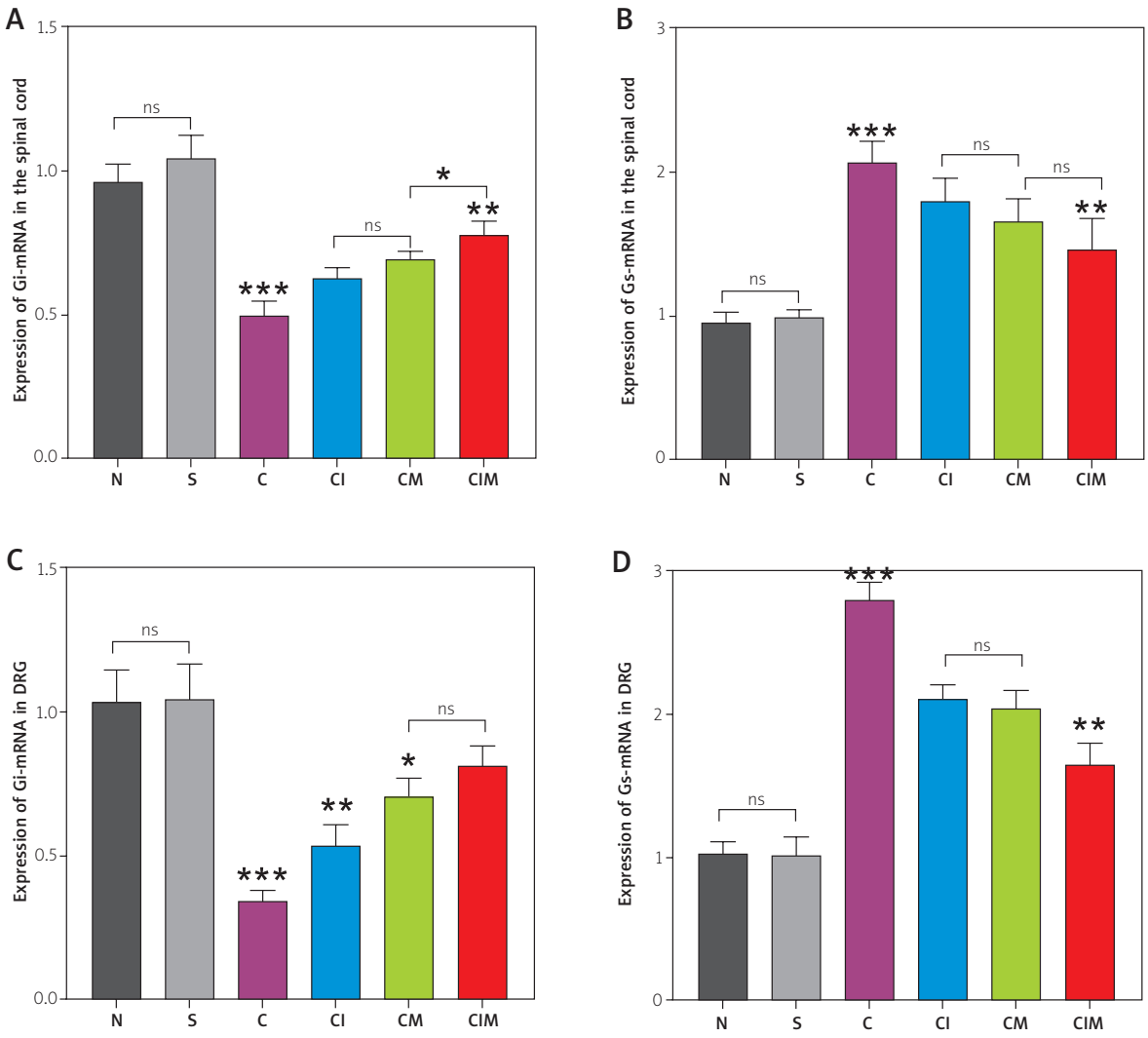

Fig. 2. Effect of intrathecal IFN-α administration on the expression level of G proteins mRNA in the rat spinal cord and DRG. Expression of G proteins mRNA in the spinal cord and DRG of rats in all 6 groups (**A-D**). Compared with Group S and Group N, Group C showed a significant decrease in the expression level of Gi protein mRNA (***$p < 0.001$, one-way ANOVA, $n = 4$), and a significant increase in the expression level of Gs protein mRNA (***$p < 0.001$, one-way ANOVA, $n = 4$). Compared with Group C, Groups CI, CM and CIM showed a significant increase in the expression level of Gi protein mRNA (***$p < 0.001$, one-way ANOVA, $n = 4$), and a significant decrease in the expression level of Gs protein mRNA (***$p < 0.001$, one-way ANOVA, $n = 4$). In the spinal cord, **A**) the expression level of Gi protein mRNA increased in Group CIM (**$p < 0.01$, vs. Group CI, *$p < 0.05$; vs. Group CM, one-way ANOVA, $n = 4$), **B**) the expression level of Gs protein mRNA decreased in Group CIM (**$p < 0.01$, vs. Group CI, one-way ANOVA, $n = 4$). In DRG, **C**) the expression level of Gi protein mRNA increased in Group CIM (**$p < 0.01$, vs. Group CI, one-way ANOVA, $n = 4$), and the expression level of Gi protein mRNA increased in Group CM (*$p < 0.05$, vs. Group CI, one-way ANOVA, $n = 4$); **D**) the expression level of Gs protein mRNA decreased in Group CIM (**$p < 0.01$, vs. Group CI and Group CM, one-way ANOVA, $n = 4$).

Compared with Groups N (129.95 ±3.86) and S (129.21 ±3.12), Groups C (129.79 ±2.30) showed no obvious difference in the CXCL-6 content in CSF of rats. There was no significant difference between Groups C (129.79 ±2.30), CI (124.55 ±3.04) and CIM (123.98 ±3.74), either. However, compared with other groups,

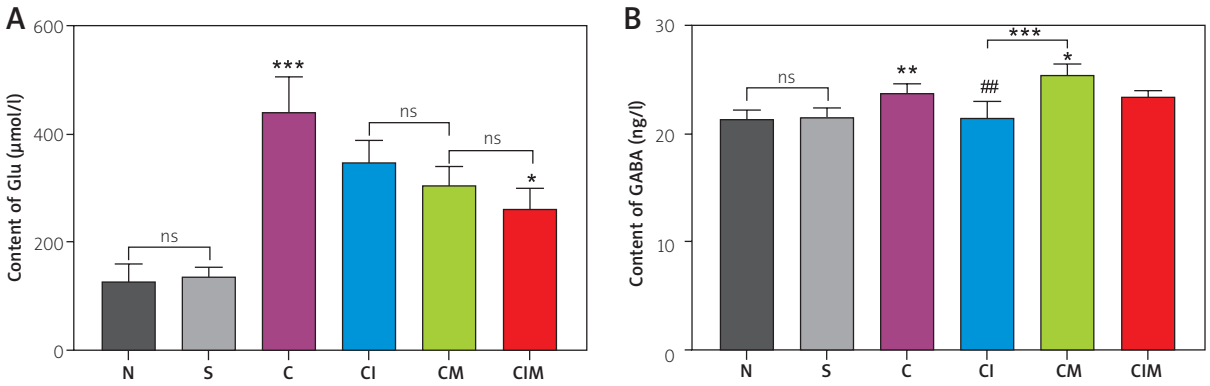

Fig. 3. Effect of intrathecal IFN-α administration on the content of Glu and GABA in the rat cerebrospinal fluid. Comparisons of amino acid content in the cerebrospinal fluid of rats between groups. **A**) The Glu content in cerebrospinal fluid of rats increased in Group C (***$p < 0.001$, vs. Groups N and S, one-way ANOVA, $n = 4$), the Glu content in the cerebrospinal fluid of rats decreased in Groups CI, CM and CIM ($p = 0.008$, $p < 0.001$, $p < 0.001$, respectively, vs. Group C, one-way ANOVA, $n = 4$), and the Glu content in the cerebrospinal fluid of rats decreased in Group CIM (**$p < 0.05$, vs. Group CI, one-way ANOVA, $n = 4$). **B**) The GABA content in the cerebrospinal fluid of rats increased in Group C (**$p < 0.01$, vs. Groups N and S, one-way ANOVA, $n = 4$), the GABA content in the cerebrospinal fluid of rats decreased in Group CI ($^{\#\#}p < 0.01$, vs. Groups C and CIM, one-way ANOVA, $n = 4$), and the GABA content in the cerebrospinal fluid of rats increased in Group CM (*$p < 0.05$, vs. Groups C and CIM, ***$p < 0.001$, vs. Group CI, one-way ANOVA, $n = 4$).

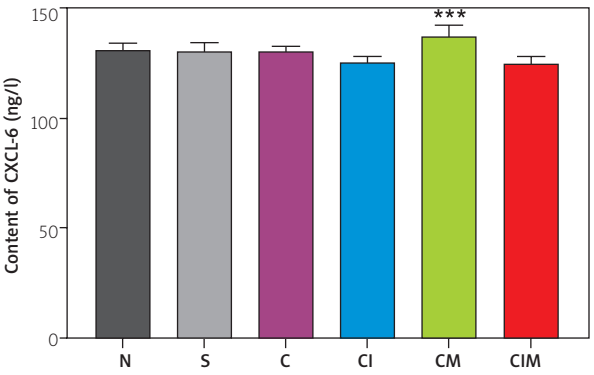

Fig. 4. Effect of intrathecal IFN-α administration on the content of CXCL-6 in the rat cerebrospinal fluid. Comparisons of CXCL-6 content in the cerebrospinal fluid of rats between groups. The CXCL-6 content in the cerebrospinal fluid of rats increased in Group CM (***$p < 0.001$, vs. Groups N, S, C, CI, and CIM, one-way ANOVA, $n = 4$).

Group CM (136.25 ±5.62) showed a significant increase ($p < 0.001$) (Fig. 4).

Discussion

We have reported that there were significant differences in the VAS score, length of disease course and incidence rate of post-herpetic neuralgia between the treatment groups of IFN-α and conventional blockers which were paravertebrally injected in patients with acute-stage herpes zoster neuralgia [5]. However, the underlying mechanism remains largely unclear, and it is uncertain whether IFN-α has a direct analgesic effect, besides its known anti-inflammatory and immunomodulatory effects. Therefore, in this study, we compared the analgesic effects of intrathecally administered IFN-α, morphine, and their combination at comparable dosages on neuropathic pain of the rat CCI model. Based on existing studies, we established the group of intrathecal administration of morphine as a control for the agonist of G-protein-coupled μ receptors. Furthermore, to provide insight into the underlying mechanism of the analgesic effect, we assayed the mRNA levels of G proteins at both the spinal cord and DRG, as well as the content of amino acid and chemokine (C-X-C motif) ligand 6 (CXCL-6) in the cerebrospinal fluid 2 h after the intrathecal administration.

We found that intrathecal administration of IFN-α increased the mechanical pain threshold in CCI rats and achieved an effect comparable to that of morphine. Interferon α took effect 0.5 h after intrathecal administration, and the analgesic effect lasted for more than 2 h. Our results are not consistent with those of Lee *et al.* [7], who found that IFN-α administered intracerebroventricularly (i.c.v.) showed an antinociceptive effect in a dose-dependent manner (0.05-5 μg), but IFN-α administered intrathecally (i.t.) did not. The inconsis-

tency may be partially attributed to different pain models adopted in these two studies.

Our results are largely consistent with the findings of Wang's team [10,11] on intraperitoneal injection of large dosage of IFN-α, but it is evident that a much higher dosage of IFN-α (> 2.5 × 106 μ/kg) is needed in the intraperitoneal injection.

In order to further explore the molecular mechanism of the analgesic effect of IFN-α, we analysed the mRNA expression levels of Gi and Gs proteins in the spinal cord and DRG. The results showed that IFN-α administration increased the expression level of Gi protein in both the spinal cord and DRG. There was no difference in Gi or Gs mRNA levels between the IFN-α group and morphine group. Compared with the groups in which IFN-α and morphine were administered individually, the combination of IFN-α and morphine achieved an even higher Gi mRNA level in the spinal cord. IFN-α administration also decreased the Gs mRNA level in the spinal cord and DRG, while there was no difference between the groups in which IFN-α and morphine was given individually. Combined administration of IFN-α and morphine brought an even lower level of Gs mRNA level at DRG, compared with both individual administration groups. In other words, intrathecal injection of 100 ng IFN-α and 0.03 mg/kg morphine had almost the same effect on G protein expression in the spinal cord and DRG of CCI rats. We found that Group CIM showed a higher Gi protein mRNA level and a lower Gs protein mRNA level, compared with Group CI and Group CM. Unexpectedly, this did not agree with the behavioural observations that there is no significant difference in PWT values between the IFN-α/morphine combination group (CIM) and the two groups of individual administration (CI and CM), respectively. We proposed three possible reasons: 1) The analgesic effects of both IFN-α and morphine are mediated by the μ-opioid receptor. If the combined dosage exceeds the capacity of all μ-opioid receptors, the "ceiling effect" will occur, which was confirmed by the fact that the PWT values remained stable 0.5 h and 2 h after intrathecal administration. 2) Group CIM had a higher PWT value than both Group CI and Group CM, but the difference is not statistically significant. 3) There may exist unknown G-protein-coupled receptors besides μ opioid receptors in the spinal cord and DRG involved in the analgesic effects of IFN-α and morphine.

Is there any other pathway to mediate the analgesic effect of intrathecal IFN-α on CCI rats? As a noxious neurotransmitter in the central nervous system, Glu can increase neuronal excitability and central sensitization. Some researchers have suggested that both the inflammatory pain caused by bee venom and neuropathic pain in spinal nerve ligation (SNI) model rats are related to the imbalance between excitatory amino acids (EAAs) and inhibitory amino acids (IAAs) in the cerebrospinal fluid, which is a possible mechanism of pain maintenance [15,16]. Our experimental results showed that the content of both Glu and GABA in the cerebrospinal fluid of rats in Group C increased 2 hours after intrathecal administration. Both IFN-α and morphine can reduce the Glu content in the cerebrospinal fluid, and the decrease of Glu in the combination group (CIM) was more evident than that in the IFN-α group. IFN-α also exhibited the same inhibitory effect on the release of GABA in the CSF of CCI rats. Given that IFN-α can inhibit the content of Glu in the cerebrospinal fluid of CCI rats as observed, IFN-α is supposed to have the capacity to adjust the imbalance between EAAs and IAAs, and thus reduce the excitability of the central nervous system and raise the pain threshold.

It has been found that chemokine (cc-motif) ligand 6 (CXCL-6) is common in patients with neuropathic pain, and there is an inflammatory reaction of the nervous system in patients with peripheral neuropathic pain [1]. In this experiment, we determined the content of CXCL-6 in the cerebrospinal fluid, and found that there was no statistically significant difference across all groups.

Liu *et al*. [9] found that there were type-I interferon receptors in the superficial dorsal horn (laminae I-II) in the spinal cord and dorsal root ganglion, which were co-expressed with the receptors of the neuropeptide calcitonin gene-related peptide (CGRP) and the neuropeptide substance P. They also found that perfusion of spinal cord slices with IFN-α reduced the frequency of excitatory postsynaptic current, which is related to glutamate concentration. More direct research evidence is needed to investigate the exact mechanism by which the intrathecal administration of IFN-α elicits analgesic effects.

This study has some limitations. The sample size is relatively small, which may limit the sensitivity of detecting differences. The expression level of μ opioid receptor protein was not detected, so the direct evidence of "upper limit effect" when the combined dosage of IFN-α/morphine exceeded the capacity of all μ opioid receptors could not be provided. This may require further research to obtain more direct evidence to study the exact mechanism of the analgesic effect caused by the intrathecal injection of IFN-α.

Conclusions

In conclusion, the intrathecal injection of IFN-α has an analgesic effect on CCI model rats, with an effect comparable to that of well-established morphine. The underlying mechanism may be that IFN-α can activate the G-protein-coupled μ receptors in the spinal cord and dorsal root ganglion, as well as inhibit the glu-

tamate release. In this study CXCL-6 was not supported to be involved in neuropathic pain maintenance in CCI rats.

Ethics approval and consent to participate

This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the First Hospital of Shanxi Medical University. Written informed consent was obtained from all participants.

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Disclosure

The authors report no conflict of interest.

References

1. Bäckryd E, Lind AL, Thulin M, Larsson A, Gerdle B, Gordh T. High levels of cerebrospinal fluid chemokines point to the presence of neuroinflammation in peripheral neuropathic pain: a cross-sectional study of 2 cohorts of patients compared with healthy controls. Pain 2017; 158: 2487-2495.
2. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33: 87-107.
3. Blalock JE, Smith EM. Human leukocyte interferon: structural and biological relatedness to adrenocorticotropic hormone and endorphins. Proc Natl Acad Sci U S A 1980; 77: 5972-5974.
4. Dafny N. Is interferon-alpha a neuromodulator? Brain Res Brain Res Rev 1998; 26: 1-15.
5. Guo YY, Xue ZX, Nan JJ, Guo SP, Yang BZ, Lv JP. Efficacy of paravertebral nerve block with interferon alpha-2b to prevent postherpetic neuralgia. Chinese J Pain Med 2015; 21: 830-833+839.
6. Jiang CL, Son LX, Lu CL, You ZD, Wang YX, Sun LY, Cui RY, Liu XY. Analgesic effect of interferon-alpha via mu opioid receptor in the rat. Neurochem Int 2000; 36: 193-196.
7. Lee JK, Park SH, Sim YB, Jung JS, Suh HW. Interaction of supraspinally administered interferon-α with opioid system in the production of antinociception. Arch Pharm Res 2010; 33: 1059-1063.
8. Li S, Xue CX. Efficacy observation on paravertebral nerve block using interferon α-2b in treatment of neuralgia dua to spinal metastatic of tumors. 2019; 41: 2727-2730.
9. Liu CC, Gao YJ, Luo H, Berta T, Xu ZZ, Ji RR, Tan PH. Interferon alpha inhibits spinal cord synaptic and nociceptive transmission via neuronal-glial interactions. Sci Rep 2016; 6: 34356.
10. Wang JY, Zeng XY, Fan GX, Tang JS, Yuan YK. Analgesic effect of interferon-alpha on neurogenic pain in mice. Chinese J Pain Med 2006; 1: 32-35.
11. Wang JY, Zeng XY, Fan GX, Yuan YK, Tang JS. mu- but not delta- and kappa-opioid receptor mediates the nucleus submedius interferon-alpha-evoked antinociception in the rat. Neurosci Lett 2006; 397: 254-258.
12. Wang YX, Chun LJ, Chang LL, Jiang CL, Lu CL, Song LX, You ZD, Shao XY, Cui RY, Liu XY. Distinct domains of IFNalpha mediate immune and analgesic effects respectively. J Neuroimmunol 2000; 108: 64-67.
13. Wang YX, Cui GY, Shen J, Huang AJ, Liu XY, Chen YZ, Jiang CL. Analgesic domains of interferon-α. Neuroreport 2001; 12: 857-859.
14. Wang YX, Song LH, Chen YZ, Jiang CL. The analgesic domain of interferon-alpha2b contains an essential proline(39) residue. Neuroimmunomodulation 2002; 10: 5-8.
15. Xue QF, Yang TD. The change and significance of four kinds of amino acid in cerebrospinal fluid of rats with neuropathic pain. Chongqing Med J 2011; 40: 3242-3243, 3265, 3331.
16. Yan LH, Hou JF, Liu MG, Li MM, Cui XY, Lu ZM, Zhang FK, An YY, Shi L, Chen J. Imbalance between excitatory and inhibitory amino acids at spinal level is associated with maintenance of persistent pain-related behaviors. Pharmacol Res 2009; 59: 290-299.