Natural ACTH Relieves Acute Inflammation of Gout by Changing the Function of Macrophages

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Research

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Abstract

Objective: Gout is a common arthritis caused by deposition of monosodium urate crystals. Macrophage is crucial in the process of monosodium urate (MSU) -induced inflammation. Although it has been reported that adrenocorticotrophic hormone (ACTH) in nature can be used to cure urarthritis, the mechanism concerning macrophage is still not clear. This study aims to explore how natural ACTH can alleviate urarthritis through functional changes in macrophage.

Methods: We analysed the variations in VAS pain scores of five patients, knowing the time of action, and detecting the level of cortisol and ACTH in patients 24 hours after the application of ACTH. The effect of natural ACTH on joint inflammation and the level of cortisol in blood in mouse model was evaluated by studies in vivo. In vitro studies we evaluated the effect of natural ACTH on macrophage and revealed different functions of ACTH and dexamethasone on macrophage in the transcriptional level.

Results: In patients with acute gout, natural ACTH can quickly alleviate pain and has no effect on the level of cortisol and ACTH. Natural ACTH is able to ease the swelling and inflammatory cell infiltration caused by arthritis, without changing the level of cortisol. Besides, natural ACTH in vitro can alleviate acute gouty inflammation by regulating phagocytosis and polarization of macrophage, which also exert different effects on the transcription of some related genes.

Conclusion: Natural ACTH is able to alleviate acute gouty inflammation by regulating macrophage, and this effect differs from that of dexamethasone in the transcriptional level.

Introduction

Gout is a kind of crystal related arthropathies, caused by deposition of monosodium urate (MSU) in joints. It is directly related to hyperuricemia caused by purine metabolic disorders and/or decreased excretion of uric acid, making it a kind of metabolic rheumatism (1). Apart from joint damage, patients with gout may also have kidney disease and other metabolic syndromes, such as hyperlipidemia, hypertension, diabetes and coronary heart disease, etc (2). It strongly recommends the colchicine, non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids (oral, intra-joint or muscle injection) being seen as the first-line drugs for the treatment of acute gout by 2020 American College of Rheumatology Guildlines for the Management of Gout (3). However, patients with gout may have other complications such as hypertension, diabetes, chronic kidney disease and hormone intolerance, etc. Therefore, some first-line drugs may not be effective and even cannot be used for treatment, which poses a great challenge to the treatment of gout.

Adrenocorticotrophic hormone (ACTH), is a kind of polypeptide hormone secreted by pituitary gland of vertebrates. It can promote the tissue hyperplasia of adrenal cortex and the generation and secretion of cortical hormone. Natural ACTH, whose generation and secretion are directly regulated by corticotropin-releasing factors (CRF) secreted from hypothalamus (4), is an important hormone in terms of maintaining the normal morphology and function of the adrenal gland. At present, natural ACTH is commonly used to treat collagenosis like rheumatoid arthritis (5, 6) and systemic lupus erythematosus (7, 8), while it does not serve as a first-line drug for the treatment of acute gout. In fact, ACTH was first used to treat acute gout more than half a century ago (9). Since then, some studies showed that ACTH had equal effects with NSAIDs and steroids, and had a safety profile (10–12). Besides, gout patients who were treated with ACTH had a stable level of blood pressure and serum potassium (13), without showing apparent adverse reactions. A study showed that ACTH was able to be used to treat gout patients who had various complications or who could not bear standard treatment, and the effective rate could be as high as 77.9–100% (14). Previous studies believed that inflammatory factors like IL-1 β , secreted when there is acute gouty arthritis, stimulate anterior pituitary gland to generate natural ACTH. The ACTH can bind to the receptor, MC-2R, on adrenal and therefore induce the secretion of corticosteroid, finally leading to the downregulation of inflammatory reaction (14, 15). Studies in recent years have extended our knowledge involving the mechanism of acute gout. It is said that ACTH can also bind to receptor MC-3R on macrophages to stimulate the transduction of melanocortin and reduce the release of chemokines which are responsible for the accumulation of neutrophil (14), thereby alleviating inflammation. It also has less adverse effect compared to corticosteroids. However, it is still unclear how natural ACTH affects the function of macrophage, and it is crucial to further study the mechanism.

The aim of this study is to explore the mechanism by which natural ACTH alleviates inflammation caused by acute gout through macrophage. We conducted studies both in vivo and in vitro, finding that natural ACTH can alleviate the inflammation existing in the

joints of mice with acute gout, and is able to affect the polarization and phagocytosis of macrophage to some extent, which functions in different ways in contrast to typical corticosteroids like dexamethasone.

Materials And Methods

Patients

In total, five patients with gout were included in the current study. The patients with gout fulfilled the 1977 American Rheumatism Association (now the ACR) preliminary criteria for acute arthritis of primary gout and the 2015 ACR/ European League Against Rheumatism (EULAR) gout classification criteria. The onset time of gout in all patients was more than 48 hours before we have had an adequate intercommunication and treated them with natural ACTH after reaching an agreement.

Mouse model

Six-week-old C57BL/6 mice were bought from Shangahi JieSiJie Laboratory Animal Limited Corporation and were fed under sterile conditions. Experiments involving animals were conducted according to protocols approved by the Animal Committee of Fudan University.

The gout mouse model was established by using MSU crystal. The three groups of mice were anesthetized by inhaled 3% isoflurane (Forane; Abbot, Chicago, IL, USA) and then intraarticularly injected with 50 μ l (1 mg) of MSU suspension in normal saline in the right foot pad. The left foot pad was injected with 50 μ l of normal saline as a control. Natural ACTH was injected with 800 μ l at a concentration of 0.25 U/ml and 2.5U/ml per mouse subcutaneously 30 minutes later. Blood was collected from each group of mice 8 hours later before the mice were executed. Collected blood was centrifuged at a speed of 3000 rpm, 15 minutes to collect the serum, with the temperature being kept at 4 °C during the whole process. Before the injection of MSU crystal, the joint thickness of hind joint was measured for 3 times by the caliper. Average value was calculated and recorded. The joint thickness was measured once again after 8 hours and was compared with the value measured before. The swelling of joint was signified by the ratio of average right joint thickness to that of left.

Histopathology

Mice were sacrificed after joint index evaluation and the joint tissue sections were prepared for haematoxylin and eosin (H&E) staining. The mice joint was cut into 5 μ m slices and embedded with paraffin.

ELISA

ELISA kit from eBioscience was used to detect the concentration of serum cortisol and factors like IL-1 β , IL-6, TNF- α , TGF- β in the supernatant of cells.

Natural ACTH

Natural ACTH (25U per ampoule) was purchased from Shanghai NO.1 Biochemical & Pharmaceutical Limited Corporation. Triple distillation H₂O was used to dissolve it into different concentrations according to the design of experiment.

Cell culture, pre-treatment and model

THP1 cells were bought from National Collection of Authenticated Cell Cultures and kept in the RPMI 1640 culture medium containing 10% FBS in humid conditions with 5% CO₂. The cells were subcultured every 48 to 72 hours. When there had enough cells, the cells were transferred to cell culture plates. PMA was added at a ratio of 1:10000 for 48 hours so as to induce cultured cells into macrophages. Culture medium was changed before experiment and was disposed by different methods according to specific experiments.

When the experiment started, 100 μ g/ml MSU crystal was added into THP1 cells for stimulation. 1.25×10^{-4} U/ml, 2.5×10^{-4} U/ml natural ACTH and 100 nM dexamethasone were applied 30 minutes later, respectively. Cells of their supernatant was collected after 48 hours for further detection.

Realtime quantitative PCR (qPCR) detection system

TRIzol was used to extract total RNA and ReverTraAce qPCR RT kit (Toyobo) was used for reverse transcription. qPCR was conducted on CFX96 Touch Real-Time PCR detection system (Bio-Rad) with the application of SYBR Green Kit (Roche). The RNA expression in the samples was normalized to the expression of control housekeeping genes (mouse GAPDH), and the relative mRNA levels of target genes were calculated by the $2^{-\Delta\Delta C_t}$ method.

Flow cytometry

Single cell suspension was stained by anti-Arg-1 (17-3697-82; eBioscience) and anti-iNOS (12-5920-82; eBioscience) antibody. The antibodies were used at a ratio of 1:100, incubating for 40 minutes without light, with the temperature kept at 4 °C. FITC-Latexbeads (500290; caymanchem) were added immediately when the experiment began. Canton C6 flow cytometer was used to conduct the experiment and collected data were analyzed by Flowjo.

Intracellular ROS determination

Reactive Oxygen Species Assay Kit (DCFH-DA, Beyotime, Jiangsu, China) was used to detect the level of intracellular ROS. After stimulation, the culture medium was removed, and DCFH-DA was added to serum-free medium at a ratio of 1:1000 for cell incubation, which lasted for 20 minutes with the temperature kept at 37 °C. Then after being washed by serum-free medium for 3 times, cells were observed and detected by fluorescence microscope and flow cytometer, respectively.

Determination of mitochondrial function

Variations involving the opening of mitochondrial permeability transition pore (mPTP) were detected by Mitochondrial Permeability Transition Pore Assay Kit (Beyotime, Jiangsu, China) in order to determine the functions of mitochondrion. After stimulation, culture medium was removed and PBS was used to wash the cells for 1 to 2 times. Proper amount of Calcein AM was added to incubate the cells for 30–45 minutes at 37 °C without light. Then fresh medium was applied for another 30-minute incubation at 37 °C without light. Finally, the medium was removed and cells were washed 2–3 times with PBS before observation under a fluorescence microscope and detection using a flow cytometer.

Statistical analysis

The data were analyzed with GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). All experiments were performed at least in triplicate, and the data are expressed as the mean±standard error of the mean (SEM). Repeated measures analysis of variance (ANOVA) followed by the Student–Newman–Keuls (S–N–K) test were used for post hoc analyses of differences among groups. P values <0.05 were considered to indicate statistically significant differences.

Results

Natural ACTH can quickly alleviate inflammation caused by acute gout, without affecting the level of cortisol and ACTH in patients.

VAS pain scale was used to evaluate the pain suffered by 5 male patients who were treated with natural ACTH. All patients suffered less to some extent 6–12 hours on average after the application of the drug. The level of cortisol and ACTH in blood lied in normal range 24 hours after the drug application. An additional file shows this in more detail (Additional file 1).

The swelling and inflammation shown in the joint of mice model can be alleviated by natural ACTH, without evident effect on the level of cortisol.

MSU crystal was injected through the mice joint to make models of acute gouty arthritis for detection. The right joint showed significant swelling after solely injecting MSU crystal when compared to the control group and the other joint of treated mice. Subcutaneous injection of ACTH at a concentration of 0.25 U/ml per mouse had no effect on the swelling of joint while 2.5 U/ml per mouse was able to alleviate it (Fig.1A). Severe inflammation was observed in the MSU injected group by HE staining, while mice treated with natural ACTH had less (Fig.1B). No significance was shown within groups when detecting the concentration of cortisol in blood by ELISA (Fig.1C).

Natural ACTH weakens the phagocytosis of macrophage in terms of MSU and Beads. We added natural ACTH with different concentrations into THP1 cells, using dexamethasone as the control drug, to evaluate the changes of phagocytosis of THP1 cells. Both natural ACTH and dexamethasone could inhibit the ability of phagocytosis when compared with the experimental group where MSU was added solely. When the concentration of natural ACTH was higher, the effect of inhibition was more severe (Fig. 2A). Both natural ACTH and dexamethasone could inhibit the ability of macrophage to engulf FITC-latexbeads in contrast with the control group while higher concentration of natural ACTH had stronger effect of inhibition (Fig. 2B).

Natural ACTH can promote the macrophage M2 polarization and downregulate the level of pro-inflammatory factors.

We detected the polarization of macrophage M2 and the level of related inflammatory factors. THP1 cells can be stimulated by MSU crystal to release inflammatory factors like IL-1 β (Fig. 3A), IL-6(Fig. 3B) and TNF- α (Fig. 3C). The application of natural ACTH and dexamethasone could significantly lower the level of these factors. When natural ACTH existing, the level of TGF- β did not change. Only when dexamethasone was applied, the level of TGF- β was decreased (Fig. 3D). When using flow cytometry to detect and analyze the polarization markers like iNOS/Arg-1, we found that THP1 cells could be stimulated by MSU crystal and display significant M1-type polarization. Low concentration of natural ACTH could inhibit the trend and promote macrophage to polarize to M2 type, while high concentration of natural ACTH led to no significant change (Fig. 3B).

Natural ACTH can downregulate the ROS level of gouty macrophages and protect the function of mitochondrion, which can be related to the inhibition of XDH generation.

We studied mitochondrion-related function of THP1 cells. As shown in Fig. 4A, MSU crystal could significantly increase the generation of ROS, while both natural ACTH and dexamethasone could inhibit the process. We further explored whether natural ACTH can protect the mitochondrion by detecting the opening of mPTP, which could be promoted by MSU crystal as shown in Fig. 4B. The function could be reversed by natural ACTH and dexamethasone. Besides, the protective effect of natural ACTH was stronger than that of dexamethasone, and had a positive correlation with its concentration. When detecting the genes that are related to the generation of ROS, the group treated with natural ACTH displayed lower mRNA expression of XDH, while the dexamethasone group displayed lower mRNA expression of NOX3 (Fig. 4C).

Natural ACTH has a different anti-inflammatory mechanism from dexamethasone on the transcription of Inflammatory-related genes.

PCR array was used to analyze transcriptional differences existing between natural ACTH and dexamethasone when concerning anti-inflammation (Fig. 5A). Fig. 5B to Fig. 5J showed differences between natural ACTH and dexamethasone in metabolism (Fig. 5B), inflammation (Fig. 5C), phagocytosis (Fig. 5D), melanocortin receptors (Fig. 5E) and some other related genes (Fig. 5F-Fig. 5J). The expression of most genes was not affected by the addition of natural ACTH, though there were some up-regulated genes like NR3C1, NR1H2, IL-37, Fc γ RIIIA and GAS6 together with some down-regulated genes like NLRP3, MC2R and MC3R. Dexamethasone showed significant differences in the expression of ANGPTL4, PDP1, PDHA1, VDR, IL1RN, IL-10, Mertk, PTX3, DDIT4 and SMAD3 compared with natural ACTH.

Discussion

It can be traced to half a century ago when natural ACTH was applied to treat acute gout. Lots of studies have shown, natural ACTH can improve the function of kidney and have lipid-lowering effect (15) when compared to traditional medicine like NSAIDs and corticosteroids. Besides, ACTH can be used to treat hypoadrenia, glucocorticoid resistance and even those who have contraindications. Currently, natural ACTH is seldom used to treat acute gout because the available formulation of ACTH in most countries bears a high cost. We conducted experiments concerning the treatment of acute gout using natural ACTH, exploring its application in the treatment of acute gouty arthritis in mice and its function of regulation in mediating THP1 cells, which is stimulated by MSU. In the animal experiment, we found high-concentration of natural ACTH, when subcutaneously injected into the mice, could effectively alleviate the joint swelling and inflammation caused by MSU crystal stimulation. Although low-concentration of natural ACTH may not ameliorate the swelling, it could still prevent the accumulation of inflammatory cells. In the meantime, the level of cortisol in mice blood did not show significant changes, indicating that the application of natural ACTH will not affect the level of cortisol in mice.

When acute gout attacks patients, macrophages within articular cavity and those transformed from monocyte existing in blood will engulf MSU, secreting pro-inflammatory factors to induce inflammation (16), and thus playing an important role in the progression and development of inflammation. When focusing on this aspect, we added natural ACTH and dexamethasone into THP1 cells which had been stimulated by MSU, finding that both of them were able to inhibit the THP1 cells when considering its phagocytic function on MSU crystal. High concentration of natural ACTH could strongly inhibit the cells, the effect of which could be similar to dexamethasone. In order to verify that the inhibitory function on THP1 cells does not specifically aimed at MSU, we used FITC-latexbeads to detect the phagocytic ability of THP1 cells. The latexbeads with FITC could be engulfed by macrophage, as detected by fluorescence microscope or flow cytometry. The results are similar to that with MSU stimulation, which confirms that natural ACTH is able to inhibit the phagocytosis of THP1 cells and further prevent the MSU-stimulated secretion of inflammatory factors.

Apart from phagocytosis, macrophage will develop into pro-inflammatory M1-type macrophage during the early period of inflammation (17). MSU crystal can stimulate the cells to generate IL-1 β , IL-6, TNF- α and induce inflammatory infiltration, driving the persistence of gouty inflammation (18). Our previous results showed that natural ACTH could alleviate the inflammation in the mice joint and weaken the phagocytosis of macrophage, which may be caused by the polarization of M2-type macrophage promoted by natural ACTH as we implied. Unsurprisingly, natural ACTH inhibited the MSU-stimulated THP1 cells to secrete M1-type inflammatory factors, the effect of which was equal to that of dexamethasone. In the meantime, when natural ACTH was added, Arg-1 in THP1 was expressed abundantly, suggesting that natural ACTH can inhibit inflammation by promoting M2-type polarization of THP1.

The function of immune cells depends on their metabolic activity to a large extent. Therefore, the cells need to develop metabolic adaptation so as to support their various immunological functions. Macrophage, as a crucial member participating in innate immunity, is strictly regulated by metabolic pathway and metabolites (19). We cannot ignore the role of mitochondrion, which is considered as the center for cell metabolism, in regulating immune cells. In recent years, more and more regulatory mechanisms have been gradually revealed. For example, mitochondrial reactive oxygen species (mtROS), generated alongside the process of electron transfer chain, may trigger innate immune signals and damage the cells, the extent of which depends on the volume of mtROS and when does it occur (20). Studies showed that inflammation may cause an increase of ROS in the macrophage mitochondrion and inhibit the function of oxidative phosphorylation (21, 22), indicating the impaired function of mitochondrion. We implied that natural ACTH can protect the mitochondrion of THP1 cells to some extent, and carried out studies in this aspect. THP1 cells could generate much more ROS in the mitochondrion when stimulated by MSU, while natural ACTH and dexamethasone could inhibit the process with similar inhibitory effect. mPTP is a group of protein complex existing between the inner and outer membrane of mitochondria. It is a type of non-specific channel, playing a significant role in the survival and apoptosis of cells, and involved in various fields, such as ischemia/reperfusion, cancer, aging and neural degeneration (23). mPTP allows ions whose relative molecular mass is relatively low to permeate freely under physiological conditions. It can maintain the mitochondrial membrane potential and the balance of ions within and outside the cells by driving ATP synthase through oxidative phosphorylation. However, apoptotic signals will stimulate the mPTP to open thoroughly, making soluble matter permeate nonselectively, which then leads to the imbalance of ions and membrane potential depolarization, causing apoptosis or necrosis (24, 25). mPTP can be used to detect the impairment of mitochondrial function, and thus we measured the protective function of natural ACTH on mPTP. Results showed that a great amount of mPTP existing in THP1 cells opened when being stimulated by MSU, indicating the function of mitochondrion was damaged. To the contrary, natural ACTH and dexamethasone reversed the phenomenon, and the function of natural ACTH was superior, suggesting natural ACTH is protective to mitochondrion in THP1 cells and maintains the functionality of mPTP. Meanwhile, we selected several genes, such as XDH, MPO, NOX1 and NOX3, all of which are associated with the generation of ROS to conduct qPCR test, and focused on XDH. Natural ACTH can inhibit the expression of XDH in MSU-stimulated THP1 cells, and XDH is able to turn products of purine metabolism into uric acid (26). Thus, we imply that natural ACTH can inhibit the generation of uric acid and protect the function of mitochondrion. However, more studies are needed to confirm the proposal.

When treating acute gout, patients who cannot react to colchicine or NSAIDs, or those who suffer from renal insufficiency, will always consider glucocorticoid as treatment. Although glucocorticoid are effective, it cannot be ignored that side-effects such as central obesity, infection, calcium loss, osteoporosis, diabetes, and stomach ulcers, etc. (27), will also bring inconvenience to patients. It is a big concern on how to largely avoid side-effect while receiving treatment. We doubt whether there is difference existing between them when concerning the effect of treatment, and whether natural ACTH is a better choice for treating acute gout,

which is also a question worth investigating. In addition to the results of the PCR array, we selectively chose some intriguing genes about metabolism, inflammation, phagocytosis and some others that may be involved during the process of exerting the anti-inflammatory effect. In metabolism-related genes, natural ACTH did not significantly affect the expression of them except NR3C1, a receptor of glucocorticoid within cells, we speculated that it has permissive effect on glucocorticoid. DXM upregulated the expression of ANGPTL4 and downregulated the expression of PDP1, PDHA1, NR3C1 and VDR, which reflected that DXM might have negative effect on the metabolism of sugars, lipids, proteins and osteocytes (28, 29) while playing the role of anti-inflammation. In inflammatory-related genes, natural ACTH did not significantly affect the expression of IL1RN and IL10, and upregulated the expression of NR1H2 and IL37 while down regulated the expression of NLRP3. As for DXM, it upregulated the expression of IL1RN and IL10 and did not significantly affect the expression of the others. This might suggest that ACTH and DXM had different mechanisms of anti-inflammatory in hepatic acute phase and immune responses (30, 31). In phagocytosis-related genes, natural ACTH did not show a significant impact on the expression of genes while DXM upregulated all of them, which might connect to the function of engulfing apoptotic cells like MERTK (32), innate resistance to pathogens and inflammatory reactions such as PTX3 (33) and FcγRIIIA (34). In addition, we also investigated some genes that may be involved in hormone and its receptor pathways like classic receptors of ACTH on macrophages MC2R and MC3R, a kind of proto-oncogene BCL6 (35), DNA damage induced transcription protein DDIT4 (36), a transcriptional coactivator for steroid receptors and nuclear receptors PGC-1α (37), an intracellular signal transducer and transcriptional modulator SMAD3 (38) and a ligand for tyrosine-protein kinase receptors GAS6 (39). After testing and analyzing the genes above, we found that natural ACTH had a similar therapeutic effect as dexamethasone while did not have a major impact on the most of the genes' expression at the same time. It seemed that natural ACTH was doing well in treating acute gout inflammation while avoiding the side effects of drugs.

To summarize, we explored the function of natural ACTH in treating acute gout, which includes alleviating the inflammation and regulating macrophage. At the same time, we also found that natural ACTH is different from corticosteroids on the level of gene transcription. Natural ACTH is an attractive therapeutic option for patients who may be problematic with NSAIDs, steroids or colchicine. Moreover, ACTH is nowadays widely available and inexpensive at least in China and most European countries (13). In our study, natural ACTH showed a lot difference from dexamethasone in terms of the transcription of inflammatory and metabolic related genes. It is still worth studying whether there is difference between natural ACTH and corticosteroids and whether natural ACTH can be used as a safe alternative to corticosteroids.

Conclusion

Through the collection of patient data and in vitro and in vivo experiments, we found that natural ACTH could alleviate the inflammatory response by inhibiting the aggregation of inflammatory cells and changing the function of macrophages, and verified that this effect is different from that of dexamethasone at the transcriptional level.

Abbreviations

ACTH: Adrenocorticotrophic hormone, ANGPTL4: Angiopoietin-related protein 4, Arg-1: Arginase-1, ATP: adenosine-triphosphate, AXL: Anexelekto, BCL6: B-cell lymphoma 6, CRF: corticotropin-releasing factors, DCFH-DA: 2, 7-Dichlorodi-hydrofluorescein diacetate, DDIT4: DNA Damage Inducible Transcript 4, ELISA: enzyme linked immunosorbent assay, FcγRIIIA: Low affinity immunoglobulin gamma Fc region receptor III-A, FITC: Fluorescein Isothiocyanate, GAS6: Growth arrest-specific protein 6, HE: hematoxylin-eosin staining, IL-1β: interleukin-1 β, IL1RN: Interleukin 1 Receptor Antagonist, IL-6: interleukin-6, IL-10: interleukin-10, IL-37: interleukin-37, iNOS: Nitric oxide synthase, inducible, MC-2R: melanocortin 2 receptor, MC-3R: melanocortin 3 receptor, MERTK: MER Proto-Oncogene, Tyrosine Kinase, MPO: Myeloperoxidase, mPTP: mitochondrial permeability transition pore, mTOR: mammalian target of rapamycin, MSU: monosodium urate, NLRP3: NLR Family Pyrin Domain Containing 3, NOX1: NADPH oxidase 1, NOX3: NADPH oxidase 1, NR1H2: Nuclear Receptor Subfamily 1 Group H Member 2, NR3C1: Nuclear Receptor Subfamily 3 Group C Member 1, Glucocorticoid receptor, NSAIDs: Nonsteroidal Antiinflammatory Drugs, PBS: phosphate buffered saline, PDHA1: Pyruvate Dehydrogenase E1 Subunit Alpha 1, PDP1: Pyruvate Dehydrogenase Phosphatase 1, PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PMA: Phorbol-12-myristate-13-acetate, PTX3: Pentraxin-related protein PTX3, qPCR: quantitative Real-time PCR, ROS: reactive oxygen species, RPMI: Roswell Park Memorial Institute, SMAD3: Mothers against decapentaplegic homolog 3, TGF-β: transforming growth factor-β, THP1: Tohoku Hospital Pediatrics-1, TNF-α: Tumor Necrosis

Factor- α , TYRO3: Tyrosine-protein kinase 3, VAS: visual analogue scale, VDR: Vitamin D3 receptor, XDH: Xanthine dehydrogenase/oxidase

Declarations

Ethics approval and consent to participate

Additional ethical approval was not required.

Consent for publication

Not applicable.

Availability of supporting data

All data generated or analysed during this study are included in this published article and are publicly available.

Competing Interest

The authors declare no relevant conflicts of interest.

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Authors' contributions

All the authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Tables

Table 1: Natural ACTH can quickly alleviate inflammation without affecting the level of cortisol and ACTH.

Patient	Sex	Age	VAS rating									blood cortisol level (normal range)**, ug/dL	blood ACTH level (normal range)**, ug/dL
			0 hours	6 hours	12 hours	24 hours	48 hours	72 hours	4 days	5 days	6 days		
1	Male	33	6	5	3	2	2	2	4*	2	1	4.88 (2.3~11.9)	19.45 (3~32)
2	Male	30	3	3	2	2	2	1	1	0	0	7.13 (2.3~11.9)	8.47 (3~32)
3	Male	27	10	8	5	7	5	5	5	3	3	5.54 (2.3~11.9)	15.17 (3~32)
4	Male	54	8	6	8	6	6	5	4	3	2	18.31 (6.2~19.4)	19 (6~48)
5	Male	40	7	6	6	4.5	4	4	4.5	4	2.5	12.45 (6.2~19.4)	23.6 (6~48)

The pain VAS (0H-6d) of five acute gout patients who were treated with natural ACTH and the level of cortisol and ACTH in their blood. For pain intensity, the scale is anchored by “no pain” (score of 0) and “worst imaginable pain” (score of 10). Patients can choose one score between 0 and 10 to describe the pain they are suffering. Score of 1 to 3 represents there is a slight pain and the patients can bear it; score of 4-6 indicates that the pain can also be tolerated although it may affect the quality of sleep; score of 7-10 means patients cannot tolerate the gradually strong sense of pain. *: patients once carried heavy object during this period. **: Blood tests were performed 24 hours after treatment of natural ACTH. Variations displayed in the level of cortisol and ACTH due to the circadian rhythm, and the results may differ slightly when detected in different periods.

Figures

Figure 1

Natural ACTH can alleviate the swelling and inflammation of mice joint.a:The swelling of joint was signified by the ratio of average right joint thickness to that of left joint.b: Histological changes of the rightjoint of mice with acute gouty arthritis. Bars, 2.5mm(left), 250μm(right). c: ELISA was used to detect the level of cortisol in mice serum (pg/ml). n=3. *, P < 0.05; **, P < 0.01***, P < 0.001n.s., no significance.

Figure 2

Natural ACTH can inhibit the phagocytosis of THP1 cells.a: Flow cytometry was used for detection after 48 hours. Macrophages showed different degrees of phagocytic inhibition after the addition of natural ACTH and DXM.b:the ability of macrophages to phagocytose LATEX-BEADS can also reduced by natural ACTH and DXM. Data are mean ± SEM and are representative of at least three independent experiments. *, P < 0.05; **, P < 0.01; ***, P < 0.001,****, P < 0.0001 n.s., no significance.

Figure 3

M1 type Macrophage can be turned into anti-inflammatory trend of M2 type by natural ACTH.a: ELISA was used to detect the concentration of IL-1 β , IL-6, TNF- α and TGF- β . b:The trend of polarization was determined by the ratio of iNOS fluorescence intensity to that of Arg-1. Data are mean $\pm$ SEM and are representative of three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., no significance.

Figure 4

Natural ACTH downregulates the ROS level of macrophage and inhibits the opening of mPTP. a: Fluorescence microscopy was used to evaluate the fluorescence intensity of ROS which was represented by MFI. b: The results analyzed by fluorescence microscope and flow cytometry, showed fluorescence intensity was negatively correlated with the damaged mitochondrial function. Bars, 100µm. c-f: qPCR was used to detect the expression of related genes. *, P < 0.05; **, P < 0.01; n.s., no significance.

Figure 5

Natural ACTH is different from dexamethasone in terms of the transcription of inflammatory-related genes. a: Gene heat map showing the effect of natural ACTH and dexamethasone on the gene transcription of THP1 cells. b-j: Expression of some related genes. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., no significance.