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Molecular Metabolism in Treatment of High Altitude Polycythemia with Duoxuekang Capsule

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JN and ZZ conducted the experiments in this study. DW and ML contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

Key words
Duoxuekang capsule, Molecular metabolism, High altitude polycythemia, Tibetan medicine

ABSTRACT

The objective of this study was to explore the molecular metabolic mechanism of Duoxuekang capsule curing high altitude polycythemia (HAPC) from two aspects of efficacy and mechanism. Sixty SPF grade male SD rats aged 1 month were purchased from the experimental animal institution of Sichuan Provincial People's Hospital and tested. According to the weight of rats, they were randomly divided into 6 groups, each containing 10 rats. Partial living environment of rats was simulated by high altitude hypobaric chamber, and group N and group M were established. Three groups of rats were given different doses of Duoxuekang capsules by gavage, and DXKH group, DXKM group and DXKL group were established. One group of rats were given Nordicam and group C was established. The activities of LDH and EPO in kidney tissue and SDH in the brain tissue of the rats in the group using Duoxuekang capsule were significantly higher than those in the group M ($P<0.05$). Their RBC, HGB, HCT and whole blood viscosity were under M's performance, and the effects of DXKH and DXKL groups were more significant. There were sixteen differential metabolites in the treatment of HAPC with Duoxuekang capsule, involving nine metabolic pathways. Four of them were related to the pathogenesis of HAPC, including glycerol, pyrimidine, arginine, proline and glutathione metabolism ($P<0.05$). Duoxuekang capsule has obvious anti-hypoxia effect and can improve the pathological changes of viscera in model rats. The pathogenesis of HAPC is complex, and Duoxuekang capsule can act on multiple targets of the body, such as oxidative stress, inflammatory reaction, etc., so as to improve the body's metabolites and treat HAPC.

INTRODUCTION

The main pathogenesis of high altitude polycythemia (HAPC) is that when the patient is at high altitude, the symptoms of high altitude hypoxia occur due to the increase of altitude and the decrease of oxygen content. High altitude hypoxia leads to the red blood cells proliferation in patients, increases the blood viscosity, and eventually leads to pathological changes in organ function, tissue function and system function of patients (Abdias *et al*., 2018; Vizcarra-Vizcarra *et al*., 2022). At present, HAPC is considered as a kind of polyemia, which leads to headache, dizziness, limb weakness, insomnia and other symptoms of patients, affecting their normal life and work (Luobu *et al*., 2019; Yuexin *et al*., 2020). Therefore, many scholars have carried out in-depth research and discussion on HAPC.

With respect to HAPC some studies give evidence for the pathogenesis and diagnostic methods of HAPC (Xiaotong and Xiangren, 2020; Yi *et al*., 2021). Xiaotong and Xiangren (2022) found that the plasma proteome in the patient's body can effectively reflect the condition of HAPC patients, which is an important part of the pathogenesis of HAPC, and therefore, changes in plasma proteome content in patients can be used to diagnose HAPC. Shuoning (2020) reported a HAPC model rats and analyzed the changes in the structure and function of their erythrocyte membrane, so as to explore the therapeutic effect and mechanism of salidroside on HAPC. Ying and Yangjin (2022) found that many patients with HAPC also suffered from peptic ulcer bleeding, so he designed experiments to explore and analyze the relationship between HAPC and

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peptic ulcer bleeding. Xiaoyan *et al*. (2021) included 95 patients with HAPC in the study, analyzed the clinical characteristics of HAPC patients through gastroscopy and clinical observation, and provided guidance for the diagnosis and prevention of HAPC.

In view of above, the current study on HAPC is mostly focused on the clinical diagnosis and the clinical characteristics of HAPC. There is little attention in studies involving Duoxuekang capsule as a commonly used therapeutic drug for HAPC. To this end, the study established a rat model of HAPC, explored the efficacy and mechanism of Duoxuekang capsule on HAPC, and provided theoretical guidance and data support for the application of Duoxuekang capsule in the treatment of HAPC.

MATERIALS AND METHODS

Experimental materials

Sixty SPF grade male SD rats aged about 1 month were selected for the experiment. The average weight of the rats was 200±20g. The rat license number was SCXK (Sichuan) 2017-16. All rats were fed adaptively in the experimental animal room for 1 week. The experiment began after the rats were fully adapted.

Establishing HAPC rats model

HAPC model rats were established, and whether the establishment of HAPC model rats was successful was evaluated according to Pharmacological Experimental Methodology. With weight as the standard, the rats were categorized into 6 groups stochastically, and each group included 10 rats. The living environment of rats was simulated by high altitude low pressure hypobaric chamber, and a plain control group (N) and a high altitude model group (M) were established. By gavage, three groups of the rats were given different doses of Duoxuekang capsules, namely high dose group (DXKH), middle dose group (DXKM) and low dose group (DXKL). One group of the rats were given Nuodikang capsules, namely Nuodikang drug group (C). Except for the group N, the rats in other groups were fed daily in a simulated environment with the altitude of 5000m. The rats in the drug groups were continuously administered for 40 days, while the rats in the groups N and M were given sterile distilled water by gavage according to the standard of 1ml/100g every day.

Evaluation of the modeling of HAPC rat model

The primary component analysis (PCA) and hematological test were used to evaluate whether the HAPC rat model was successfully replicated from the aspects of physiological and metabolic.

Blood samples were collected form N, M, DXKH and DXKL in a non-anticoagulant vacuum blood collection vessel and incubated in a 37°C water bath for centrifuged 3000 rpm, 5 min at 4°C. The supernatant was re-centrifuged at 13000 rpm for 10 min at 4°C and the supernatant stored at -80°C.

For LC-MS analysis to 100μL of thawed sample several 350μL of the extraction solution methanol, acetonitrile and water, 2:2:1, and 20μL of L-2-chlorophenylalanine was and mixed added well, pipe, ultrasonicated for 10 min, and then place it for 1h at -20°C. Subsequently, the sample was centrifuged 12000 rmp for 15 min at 4°C. Supernatant (0.4ml) was dried in a vacuum concentrator, and resuspended in 100 μL of the extraction solution (acetonitrile and water 12:1) put it into the sample bottle for liquid chromatography mass spectrometry (LC-MS) analysis through UHPLC system. The specific settings were as follows: Phase A: water mixed with 25nM ammonium acetate and ammonia; Phase B: acetonitrile. The flow rate to 500μL per minute and the injection volume to 2μL under 25°C were set. The equilibrium buffer is set as the initial mobile phase.

Observation indicators

The efficacy of Duoxuekang capsule in treating HAPC was analyzed according to the pathological morphology of rat lungs. The evaluation indicators included succinate dehydrogenase (SDH) activity in rat brain tissue, lactate dehydrogenase (LDH) activity in kidney tissue, kidney tissue activity (EPO), erythrocyte (RBC), hemoglobin content (HGB), hematocrit (HCT) and whole blood viscosity at different shear rates ($1S^{-1}$, $5S^{-1}$, $50S^{-1}$, $100S^{-1}$, $200S^{-1}$).

The differential metabolites of the drug groups and the group M were obtained according to PCA, t-test and variable importance in projection (VIP), and the conditions were: $P<0.01$, $VIP>1$. MetPA database analysis was used for metabolic network analysis. Through the above indicators, the mechanism of Duoxuekang capsule in treating HAPC was analyzed.

Data processing

The data generated during the experiment were processed by software SPSS 22.0. Since the data required in the experiment were all metrological data, all data are recorded by mean ± standard deviation. If the square difference is even, LSD test is used; If the variance is not even, Tamhane's T2 test is used. $P<0.05$ indicates significant difference.

RESULTS

The pathological examination results of lung sections of the rats in each group are shown in Figure 1. There

were obvious inflammatory cell infiltration in the lung tissue of the group M, and most of them were neutrophils. The lung interstitium was significantly thickened, and the number of cells was less than that of the group N, and the alveolar structure was compressed, and the number was largely reduced compared with N. In the group C, the tissue structure was clear, there were a small amount of inflammatory cells infiltration, and most of them were neutrophils, and the lung interstitium was thickened. In the group DXKH, the tissue structure at all levels was clear, and the number and shape of alveolar were normal. In the group DXKM, the tissue structure at all levels was clear, there were inflammatory cells infiltrating in the pulmonary interstitium, and most of them were lymphocytes. The number of pulmonary foam cells increased. In the group DXKL, the tissue structure at all levels was clear, the lung interstitium was thickened, a small amount of inflammatory cells were infiltrated, and fibrous tissue hyperplasia occurred in a few parts.

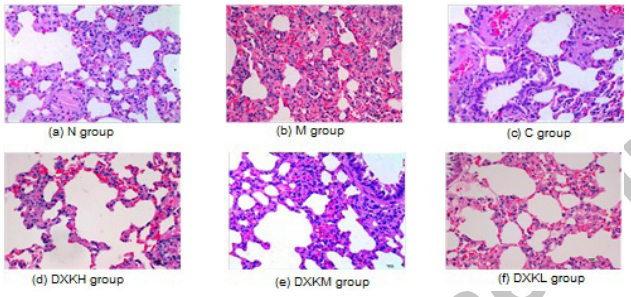

Fig. 1. Effect of different concentration of duoxuekang on pathological characteristics of lung of rats suffering from high altitude polycythemia (×400). A, N: Plain control; B, M: high altitude model group; C, C: Nusdikand drug group; D, DXKH: high dose duoxuekang group; E, DXkM: middle dose duoxuekang group; F, low dose duoxuekang group.

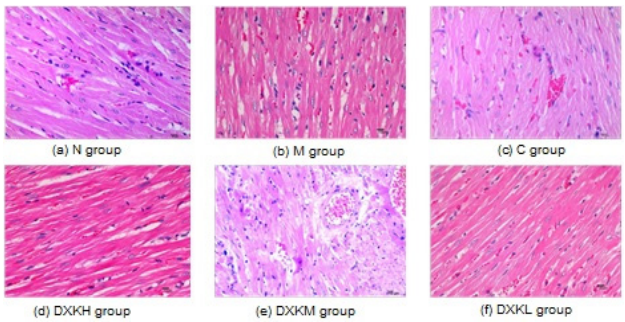

Fig. 2. Pathological examination of cardiac slices of rats in each group (×400). A, N: Plain control; B, M: high altitude model group; C, C: Nusdikand drug group; D, DXKH: high dose duoxuekang group; E, DXkM: middle dose duoxuekang group; F, low dose duoxuekang group.

The pathological examination results of cardiac sections of the rats in each group are shown in Figure 2. The tissue outline of the group N is clear, and the arrangement of fibers is normal and relatively neat. In the group M, there was edema in tissue fibers, but the degree was mild, accompanied by granular degeneration. In the group C, the tissue contour was clear, the arrangement of fibers was normal and relatively neat, with a small amount of tissue expansion and hyperemia. In the group DXKH, the tissue contour was clear, the arrangement of fibers was normal and relatively neat, and a small amount of tissue fibers appeared edema, but the degree was mild. In the group DXKM, the tissue contour was clear, the chromatin depth of different parts was different, and a few cells were arranged in disorder. In the group DXKL, the tissue contour was clear, the arrangement of fibers was normal and relatively neat, and a small amount of tissue fibers appeared edema, but the degree was mild.

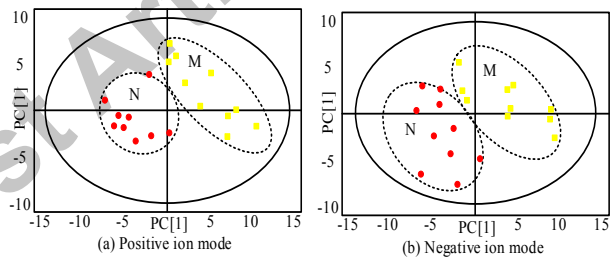

Fig. 3. PCA analysis of HAPC model.

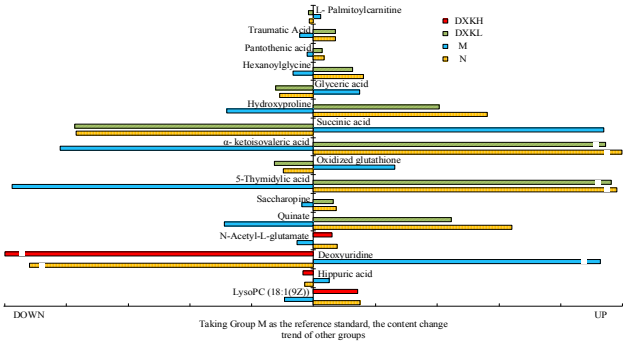

Fig. 4. Potential serum differential metabolites of Duoxuekang capsule in the treatment of HAPC.

The PCA analysis results of HAPC model are shown in Figure 3. The sample points of the groups N and M are obviously different, and they gather in a certain area. This can indicate that during the feeding process, the normal physiological metabolism of rats has deviated, which is quite different from the metabolic distribution track of the group N, so it can be judged from the physiological metabolism level that the HAPC model has been successfully copied.

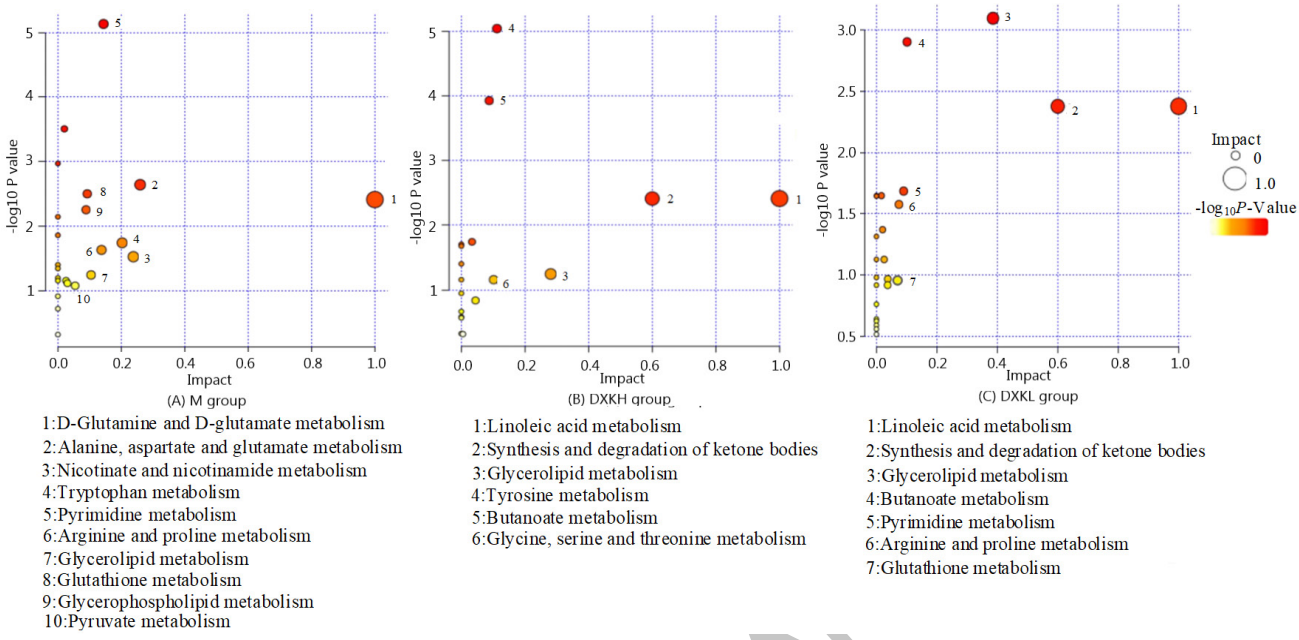

Fig. 5. Pathway analysis of M, DXKH and DXKL groups rats.

The potential differential metabolites of HAPC with high-dose and low-dose Duoxuekang capsule was screened, and on this basis, the differential metabolites that coincided with the metabolites of rats in the group M was screened. The screening results are shown in Figure 4. The 16 differential metabolites can improve the metabolic changes caused by HAPC.

The pathway analysis of the groups DXKH, DXKL and M is shown in Figure 5. The MetPA database was used to screen the main metabolic pathways corresponding to each Duoxuekang capsule in the treatment of HAPC Differential Metabolites, and then analyze its metabolic network. There are 6 pathways involved in the group DXKH, of which 3 metabolic pathways overlap with the group M. The group DXKL mainly involves 7 pathways. Among them, 4 metabolic pathways overlap with the group DXKH and 4 metabolic pathways overlap with the group M. There are 4 pathways in the groups DXKH and DXKL that overlap with the group M, including glycerol metabolism, pyrimidine metabolism, arginine and proline metabolism and glutathione metabolism.

DISCUSSION

During the PCA analysis, no matter in the positive ion mode or negative ion mode, the sample points of the groups N and M are obviously different, and they gather in a certain area. The experimental results proved the literature (Ying and Yangjin, 2022; Zhen *et al*., 2021), which can show that during the feeding process, the normal physiological metabolism of rats has deviated, which is significantly different from the metabolic distribution track of the group N, so it can be judged from the physiological metabolism level that the HAPC model has been successfully copied.

In the experiment, the RBC, HGB and HCT of the groups DXKH and DXKL were under M's data. Both high-dose and low-dose Duoxuekang capsules can effectively and significantly improve RBC, HGB and HCT of patients with HAPC. The whole blood viscosity of the groups DXKH, DXKM and DXKL was significantly lower than those of the group M at different shear rates ($P<0.05$), indicating that the high and low dose of Duoxuekang capsule significantly improved the blood viscosity of HAPC. In the examination of pathological structure of heart and lung sections, the groups DXKH and DXKL had clear tissue structure, less inflammatory factor infiltration, and the damage of tissue microstructure was significantly improved compared with the group M, which showed that Duoxuekang capsule could effectively protect the tissue damage caused by hypoxia. The results of the study are consistent with previous studies content (Shouning *et al*., 2020; Prem *et al*., 2018; Peng *et al*., 2021; Ashour, 2020; Jun *et al*., 2022), and confirm the effectiveness of the study.

After 40 days of the experiment, the SDH activity in the brain tissue of each drug group was above M's data. The SDH activity of brain tissue in the group DXKM was higher. This can show that the high, medium and low dose of Duoxuekang capsule can significantly improve the

SDH activity of rat brain tissue. The activities of LDH and EPO in renal tissue of the rats in each drug group were significantly lower than those in the group M. The activities of LDH and EPO in renal tissue in the groups DXKH and DXKL decreased. It means that the high, medium and low dose of Duoxuekang capsule could effectively reduce the LDH and EPO activities of rat kidney tissue, while the high and low dose of Duoxuekang capsule had better effect on reducing the LDH and EPO activities of kidney tissue. The experimental data are consistent with the existing research results (Yue *et al*., 2019; Rolando *et al*., 2000).

The results showed that Duoxuekang capsule could cause the relative content change of 16 metabolites in the body of HAPC rats. These 16 metabolites were the differential metabolites of Duoxuekang capsule in the treatment of HAPC. A total of 4 pathways overlap with those of the group M. Consistent with past studies (Feng *et al*., 2019; Heinrich *et al*., 2019), Duoxuekang capsule has excellent targeting effect in the treatment of HAPC, and the above four pathways may be the mechanism of Duoxuekang capsule in the treatment of HAPC.

CONCLUSION

The study verified the therapeutic effect of Duoxuekang capsule on HAPC rats during the experiment, and obtained the relevant targets and metabolic pathways of Duoxuekang capsule treating HAPC, so as to analyze the specific mechanism of Duoxuekang capsule. The research provided ideas and theoretical support for the treatment and research of HAPC. The study did not combine molecular biological technology to conduct in-depth and comprehensive discussion on the metabolic regulation mechanism of Duoxuekang capsule in the treatment of HAPC, so it needs to be further improved in the follow-up study.

DECLARATIONS

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IRB approval

This study was approved by the Advanced Studies Research Board of Chengdu University of Traditional Chinese Medicine, Chengdu, China.

Ethical approval

The study was carried out in compliance with guidelines issued by ethical review board committee of Chengdu University of Traditional Chinese Medicine, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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