Experimental evaluation of the anti-inflammatory effect of calcite treated with Tibetan technology

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Experimental evaluation of anti-inflammatory effect of calcite processed by Tibetan technology

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SUMMARY

Calcite (lime spar, $CaCO_3$) is widely used in Tibetan medicine to treat diseases based on metabolic disorders. This paper describes the results of a study of the original technology for the preparation of calcite, including calcination and treatment in different media (water, sour milk, beer). Using X-ray phase analysis, scanning electron microscopy, and IR spectroscopy, it was shown that, in the samples under study, films with varying degrees of crystallinity, containing Ca (OH), are formed on the calcite surface.$_2$... According to the data of pharmacological studies, the most pronounced inhibitory effect on the alterative and exudative phases of the inflammatory process is characteristic of calcite treated with beer ($CaCO_{3(beer)}$). The activity of the studied samples and the reference drug (indomethacin) is arranged in decreasing order: $CaCO_{3(beer)}$ - indomethacin - $CaCO_{3(water)}$ - $CaCO_3$ calcined - $CaCO_3$ (sour milk).

Key words: calcite, X-ray phase analysis, scanning electron microscopy, IR spectroscopy, anti-inflammatory activity.

RESUME

Calcite ($CaCO_3$) is a widely used in Tibetan medicine mineral for treatment of diseases caused by disturbances of metabolic disorders. Present paper aimed at a study of the original technology of preparation of calcite including calcination and slaking in the different media (water, sour milk, beer). Using X-ray phase analysis, scanning electron microscopy and IR-spectroscopy the formation of films with different crystallinity containing of $Ca(OH)_2$ at the surface of calcite samples was shown. These changes were formed as a result of epitaxy-caused deformation. According to pharmacological data the most pronounced inhibitory effect on alterative and exudative phases of the inflammatory process was observed for calcite sample treated by beer ($CaCO_{3(beer)}$). Anti-inflammatory activity of test samples and indometacin (reference compound) was following, $CaCO_{3(beer)}$ - indomethacin - $CaCO_{3(water)}$ - $CaCO_3$ calcined - $CaCO_{3(sour\ milk)}$ (in decreasing order).

Keywords: calcite, X-ray phase analysis, scanning electron microscopy, IR-spectroscopy, antiinflammatory activity.

The difficulty of developing effective and safe agents for the treatment of inflammatory diseases lies in the fact that inflammation is a polyvalent and dynamic process with many alternative and intersecting pathways in the metabolic process that exist both at the level of intracellular interactions of signaling cascades and at the level of regulation of the production of inflammatory mediators [10]. In accordance with this, the effect on only one target of pathogenesis does not always provide a sufficient pharmacological effect [16].

Currently, in the arsenal of modern medicine there is a large number of anti-inflammatory drugs, the use of which is limited due to the numerous side effects that negatively affect the implementation of physiological processes in the body. In this regard, the study of drugs of Tibetan medicine seems to be relevant, since for some compositions in the experiment, the polyvalence of their action and high efficiency have already been proven [1, 6], but further developments are hindered by the lack of research on the study of Tibetan technology for the manufacture of drugs. A few publications in this area indicate the need to study Tibetan technologies, since it is assumed that the processing of raw materials before obtaining medicines affects the yield of active substances,

Compositions containing calcite and used in the arsenal of traditional Tibetan medicine as sedatives and balancing agents are very promising in this regard [8]. Calcite ($CaCO_3$) Is a natural mineral from the group of carbonates, widely used in Tibetan medicine for the treatment of mucus diseases [11], ie diseases based on disorders of the immune system, metabolism and neurohumoral regulation. A detailed description of the processes of preparation of individual components of complex medicinal preparations containing both organic (plants, various organs and tissues from animals) and inorganic components (metals, minerals, etc.) is given in the Tibetan essay on the preparation of medicinal elixirs "Kunsal-Nanzod" [3]. In particular, according to this essay, the scheme of calcite processing consists in firing it with subsequent quenching in different media (Table 1).

Table 1

Obrabcalcite failure (based on materials from Kunsal-Nanzod, Part 1, P.15)

Processing method	Properties after processing
Roasting + chang (beer or barley wine strength 9-14 °)	Acquires hot properties, used in warming compositions
Roasting + gift (sour milk)	Becomes heat-neutral and cold, used in balancing formulations
Firing + water	Acquires cold properties, used in cool formulations
Firing without subsequent extinguishing	Has a strong heat, corrodes, rejects rot

Purpose of the study: to reproduce the Tibetan technology of calcite processing and to determine the pharmacological activity of the obtained samples at various stages of the inflammatory process.

MATERIALS AND METHODS

In the laboratory of biomedical research of the Institute of General and Experimental Biology of the Siberian Branch of the Russian Academy of Sciences, three samples of calcite were prepared, which, according to the texts of the Tibetan source [3], were calcined at t = 800 ° C, and then extinguished in water, sour milk and beer, respectively. X-ray phase analysis (XPA), IR spectroscopy and scanning electron microscopy (SEM) of the samples were carried out under the guidance of Academician V.V. Boldyrev. Scanning electron microscopy was performed on a Hitachi TM-3000 device. XRD was performed on a Bruker D8-GADDS multifunctional diffractometer with a Hi-Star two-coordinate detector (Cu-Kα radiation, graphite monochromator, 0.5 mm collimator, focusing on the sample, reflection shooting). Diffraction patterns were obtained by two

in different ways: in a standard way from the entire mass of the sample and under a small incidence of the primary beam from a thin surface layer. Data processing was carried out using software packages supplied with the diffractometer (GAD-DS, EVA, WIN-METRIC) and a PDF-2 powder database. The IR spectra of incomplete internal reflection (ATR) were recorded on a DigiLab Ex-calibur 3100, Varian FT-IR spectrometer. The spectra were recorded in the range 4000–600 cm – 1 with a resolution of 2 cm – 1 on a MIRacle ATR, Pike attachment.

The anti-inflammatory activity of calcite samples was studied in the laboratory for the safety of biological activity of substances of the Institute of Oceanology and Biology, Siberian Branch of the Russian Academy of Sciences. Pharmacological studies were carried out in the winter-spring period on 144 white male Wistar rats with an initial weight of 180-200 g. laboratory practice rules ". Before the start of the experiments, animals meeting the criteria for inclusion in the experiment were divided into groups taking into account age, weight and the principle of randomization. The experimental work was carried out in accordance with the "Rules for carrying out work with the use of experimental animals" (Appendix to the order of the Ministry of Health of the USSR No. 755 dated 12.08.77), "Rules,

In the study of anti-inflammatory activity, the classical models of aseptic inflammation (AV) have been reproduced, which make it possible to assess the effect of the investigated agents at different stages of this process, as well as allow one to determine some of the mechanisms of their anti-inflammatory action.

All animals were divided into 6 groups, 8 animals in each group: control (AB + H_2O); experimental 1 (AB + $CaCO_3$ unquenched); experimental 2 (AB + $CaCO_{3(water)}$); experimental 3 (AB + $CaCO_{3(spoiled\ milk)}$); experimental 4 (AB + $CaCO_{3(beer)}$); experimental 5 (AB + indomethacin). Samples of calcite were injected intragastrically to animals at an experimental therapeutic dose of 130 mg / kg (pre-established), in the form of an aqueous suspension. The reference drug, indomethacin (Sopharma, Bulgaria), was used at an isoeffective dose of 10 mg / kg of animal weight. The control group of animals received distilled water in an equivalent volume at all stages of the study.

The effect of agents on the processes of alteration and tissue regeneration was assessed by the dynamics of healing of a musculocutaneous defect caused by subcutaneous administration of 0.5 ml of 9% acetic acid with simultaneous intraperitoneal administration of dextran at a dose of 300 mg / kg [5]. Samples under study $CaCO_3$ and the reference drug was administered 1 hour before the injection of the acetic acid solution, and then daily 1 time per day for 28 days. On the 7th, 14th and 28th days of the experiment, the area of necrotic tissue was estimated using a planimetric method.

The study of antiexudative activity was carried out on the model of acute aseptic inflammation caused by subplantar injection of 0.1 ml of a 3% formalin solution into the hind right limb of a rat [7]. 3 hours before the subplantar injection of formalin and 5 and 18 hours after the initiation of inflammation, the animals were injected intragastrically with the test agents and the reference drug. Animals of the control group received distilled water in an equal volume and in a similar mode. Evaluation of the antiexudative effect of the funds was carried out by the oncometric method 24 hours after the introduction of formalin, calculating the percentage of suppression of paw edema in relation to the control.

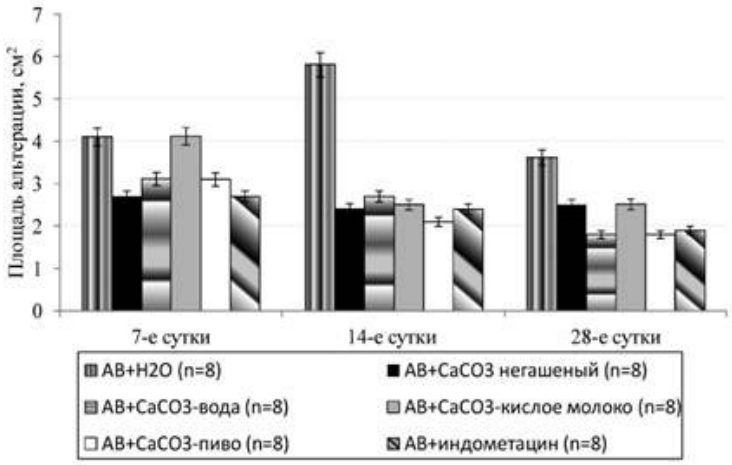
The proliferative stage of the inflammatory process was studied by the method of F.P. Trinus et al. [9]. Rats under ether anesthesia were implanted under the skin in the back area under aseptic conditions with sterile cotton balls weighing 15 mg, after which the wound was sutured in layers. CaCO Sample Solutions$_3$ and indomethacin was injected intragastrically once a day for 7 days, after which the ball with the formed granulation fibrous tissue (GFT) was removed and dried to constant weight at 70 ° C for 24 hours. The proliferative response was assessed by the difference between the weight of the dried granuloma and the initial weight of a cotton swab.

results pharmacological research processed Withusing nonparametric Mann-Whitney U-test using Statistica 6.0. Differences between groups were considered significant at p ≤ 0.05 [4].

RESULTS AND DISCUSSION

On the model of the alterative stage of the inflammatory process, it was found that the course administration of the investigated agents to one degree or another has an anti-alterative effect, reducing the degree of tissue destruction caused by the injection of a phlogogenic agent. So, calcite treated with beer, at an experimental-therapeutic dose of 130 mg / kg of animal weight, reduces the area of alteration on the 7th, 14th and 28th days of the experiment by 24, 64 and 50%, respectively, compared with these animals control group (Fig. 1).

The introduction of quicklime calcite and calcite treated with water has a moderate anti-alterative effect, reducing the degree of tissue destruction at all periods of observation. At the same time, on the 7th day of the experiment, against the background of the introduction of quicklime calcite and calcite treated with water, the area of necrosis decreases by 34 and 24%, respectively, compared with the control. On the 14th and 28th days of the study, in the group of animals receiving quicklime calcite, the alteration process decreases by 59 and 31%, respectively, and in the group receiving calcite treated with water - by 54 and 50%, respectively, compared with indicators in animals of the control group.

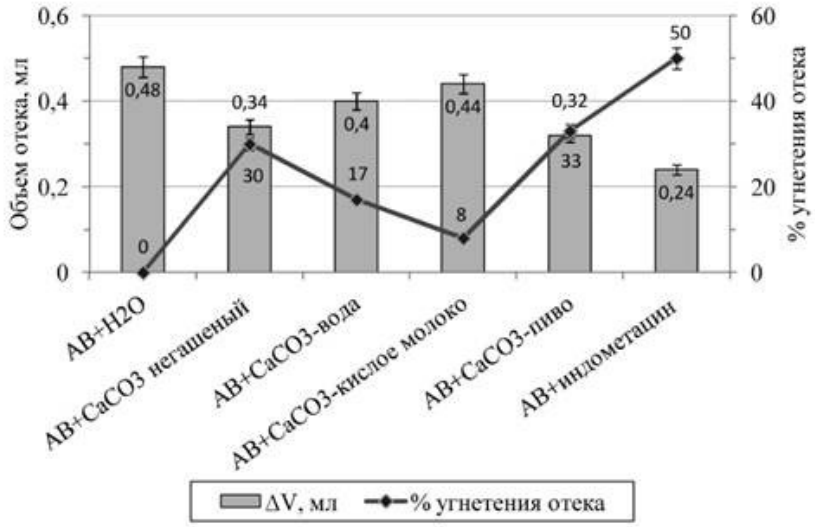


Rice. 1. Influence of calcite samples on alteration and tissue regeneration in acute aseptic inflammation in white rats (p ≤ 0.05).

The anti-alterative activity of the samples under study decreases in the following order: CaCO (beer) > indomethacin> CaCO (water) > CaCO quicklime> CaCO (spoiled milk) ...

Thus, the results obtained indicate that during the period of the alterative stage of the inflammatory process, accompanied by damage to the cellular elements of the tissue, metabolic disorders (the predominance of catabolic reactions), the separation of oxidation and phosphorylation processes, it is necessary to use warming agents that gently correct metabolic processes in the focus of inflammation. This is exactly what CaCO has. treated with beer, which, due to its "warm" properties, apparently improves the redox processes in the damaged zone, limits the accumulation of intermediate products of glycolysis, inhibits the development of metabolic acidosis. Anti-alterative action of CaCO(beer) comparable to that of the reference drug. Calcite treated with water, sour milk, as well as quicklime calcite have a moderate anti-alterative effect, which is due to their "cold" or excessively hot properties.

When evaluating the antiexudative effect, it was found that quicklime calcite and beer-treated calcite exhibit pronounced anti-inflammatory activity at the exudation stage, reducing the degree of limb edema by an average of 31% compared with the control (Fig. 2). Probably, the antiexudative action of the $CaCO_{3(beer)}$ lies its warming property, which helps to improve blood circulation and microcirculation, to reduce vascular permeability, due, apparently, to its membrane-stabilizing effect, as well as to minimize energy deficiency and optimize energy metabolism in the focus of inflammation. $CaCO_3$ quicklime, which has a strong heat, is slightly inferior in its effect to $CaCO_{3(beer)}$... Calcite, treated with water or milk, has a moderate anti-exudative effect, reducing the severity of exudative-infiltrative phenomena by 17 and 8%, respectively, compared with the control.



Rice. 2. Influence of calcite samples on exudation in acute aseptic inflammation limbs in white rats ($p \leq 0.05$).

The anti-inflammatory activity of the investigated agents at the exudation stage decreases in a number of:

indomethacin - $CaCO_{3(beer)}$ - $CaCO_3$ quicklime - $CaCO_{3(water)}$ - $CaCO_{3(spoiled\ milk)}$...

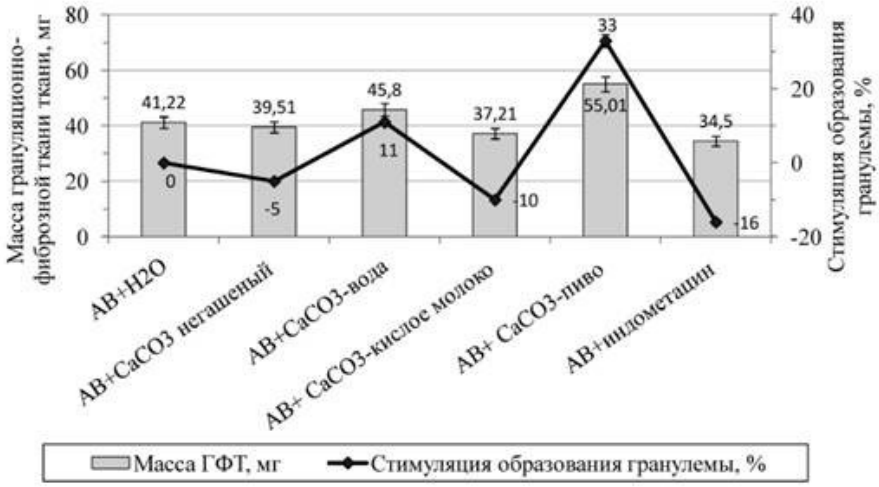
When simulating the proliferative stage of the inflammatory process, it was found that with the introduction of quicklime calcite and calcite treated with milk, a decrease in proliferation intensity by 5 and 10%, respectively, is observed, which indicates a tendency towards the manifestation of their antiproductive activity (Fig. 3). At the same time, inhibition of the proliferative process in the group of animals treated with $CaCO_{3(spoiled\ milk)}$, close to that of indomethacin. Sample "$CaCO_3$- sour milk "characterized by a property that is neutral in heat and cold, apparently has an inhibitory effect on kinin formation reactions, prevents excessive proliferation of connective tissue, thereby contributing to a better restoration of the normal structure and function of the damaged area.

Calcite, treated with water, slightly increases proliferative activity, stimulating the formation of granulomas by 11%. The introduction of calcite, treated with beer, has a pronounced productive activity, stimulating the development of granulation-fibrous tissue by 33% compared with the data in the control group of animals.

The antiproliferative activity of the investigated agents decreases in the following order:

indomethacin> $CaCO_{3(spoiled\ milk)}$> $CaCO_3$ quicklime> $CaCO_{3(water)}$ > $CaCO_{3(beer)}$

The experimental data on the anti-inflammatory activity of calcite quenched in different media are consistent with the data of X-ray phase analysis and scanning electron microscopy. According to SEM data on the surface of calcite samples ($CaCO_{3(beer)}$) there are islands of other phases. According to XRD data, calcite was found in all samples without changing the unit cell parameters within the experimental error (calcite cards 05-0586 and 86-0174 from PDF). The presence of other crystalline phases is concentrated mainly on the surface. Calcium oxide CaO identified by card 82-1690, calcium hydroxide $Ca(OH)_2$ - by card 84-1263. The differences in the samples quenched in the indicated media are mainly in the different state of the thin surface layer (Table 2). No crystalline phases were found on the surface of the sample quenched in sour milk, according to XRD data. Apparently, it is covered with a rather thick layer of a non-crystalline film. Sample $CaCO_{3(beer)}$ differs in that the $Ca(OH)_2$covering the surface of calcite is in a finely dispersed state and, possibly, there is some additional noncrystalline film that attenuates the intensity of diffraction peaks. The state of the surface can influence the dissolution processes and thus alter the pharmacological activity.



Rice. 3. Influence of calcite samples on proliferation processes in aseptic inflammation in white rats ($p \leq 0.05$).

table 2

Changes in the anti-inflammatory activity of $CaCO_3$ after processing in Tibetan technologies

$CaCO_3$ и вид гашения	Противовоспалительная активность			Изменения в образцах по данным РФА	
	Антиальтерат.	Антиэксудат.	Пролиферат.	С поверхности	В целом весь образец
$CaCO_3$ негашеный	+	++	«-П»	Хорошо окристаллизованные фазы (узкие пики) гидроксида кальция, кальцита, следы оксида кальция	Кальцит, следы гидроксида кальция
$CaCO_{3(вода)}$	++	+	«+П»	Хорошо окристаллизованная фаза (узкие пики) гидроксида кальция, следы кальцита	Кальцит, следы гидроксида кальция
$CaCO_{3(пиво)}$	++ (= индометацину)	++	«+П»	Мелкодисперсная фаза (уширенные пики) гидроксида кальция, слабая интенсивности пиков	Кальцит
$CaCO_{3(кислое молоко)}$	–	–	«-П»	Кристаллических фаз не обнаружено	Кальцит

Примечание. «++» – выраженная, «+» – умеренная, «-» – не выраженная противовоспалительная активность; «+П» – пролиферативная активность, «-П» – антипролиферативная активность.

We believe that the observed changes in the powder diffraction patterns and the pharmacological activity of the samples are associated with disordering due to the complete or partial amorphization of calcium hydroxides on the surface. Perhaps the reason

disordering is the structural misfit of the lattices of calcite and the reaction products on its surface [12, 13, 14]. A more detailed study of this effect is planned for the future.

When comparing the IR spectra of samples of raw calcite and calcined calcite after quenching in sour milk ($CaCO_3$ (spoiled milk)), the appearance of new low-intensity bands was found: doublets at 2921 and 2854 cm^{-1}, as well as at 1794 and 1745 cm^{-1}... In addition, a broad band is noted with several maxima in the region of 1036–1166 cm^{-1}... These bands, not related to calcite, were recorded exclusively in samples quenched in sour milk. Since milk contains proteins and various fatty acids, it can be assumed that at the moment of boiling, molecular compounds containing hydroxyl groups are absorbed on the surface of calcite. In this case, two stripes in the range from 2930 to 2850 cm^{-1} can be attributed to the linked stretching vibrations of the OH-group. Bands in the range of 1740-1800 and 1036-1166 cm^{-1} belong to the skeletal vibrations of absorbed molecules and their exact assignment is very problematic (the regions of stretching and deformation vibrations of C-H, CC, CN and C-O bonds).

The results of pharmacological experiments indicate that the course administration of the studied agents to animals, to one degree or another, has an anti-inflammatory effect. So, quicklime calcite at an experimental therapeutic dose of 130 mg / kg has moderate anti-alterative and pronounced anti-exudative activity, and also tends to decrease the intensity of proliferation. The experiment has shown that calcite, quenched with water, has a pronounced anti-alterative and moderate proliferative effect. Calcite treated with sour milk exhibits moderate anti-alterative and anti-exudative activity, and also tends to have an anti-productive effect. For calcite treated with beer, pronounced anti-alterative and anti-exudative effects were revealed, and there is also an intensification of the proliferative process through the formation of granulation-fibrous tissue in the focus of inflammation. Thus, the pharmacological activity of the studied samples and the reference drug is in decreasing order: $CaCO_3$(beer) - indomethacin - $CaCO_3$ (water) - $CaCO_3$ quicklime - $CaCO_3$ (spoiled milk)... The data obtained confirm the information of Tibetan sources about the change in the properties of calcite after processing.

CONCLUSION

1. Reproduced the technology of calcite processing according to the texts of the Tibetan source "Kunsal-Nanzod": treatment with beer (to obtain a warming medicine), treatment with sour milk (to obtain a neutral one) and treatment with water (to obtain a medicine with cool properties).

2. The fact of the influence of the method of calcite processing on the degree of severity has been established. antiphlogistic activity. The most pronounced anti-inflammatory effect, at the stage of alteration and exudation, is characteristic of a sample of calcite, which was extinguished in beer after firing. This effect is apparently due to its warming property, which corrects redox processes in the focus of inflammation.

3. The study of ancient knowledge on the technology of preparation of medicines is necessary for objective assessment of pharmacotherapeutic efficacy and establishment of the mechanism of action of multicomponent compositions, which include calcite.

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