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THERAPEUTIC EFFECT OF ELECTROCHEMICALLY ACTIVATED AQUEOUS SOLUTIONS AT CERTAIN INFECTIONS IN PETS

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ABSTRACT

Studies have been carried out to determine the therapeutic effect of electrochemically activated aqueous solutions anolyte and catholyte in some species of pets (dogs, cats, gerbils, hedgehogs) with various infections. Studies have been conducted in patients with bite wounds, dermatitis, conjunctivitis and viral diseases. It has been found that anolyte and catholyte have a pronounced healing effect not only when used as adjunct therapy to the conventional but also in the cases of self-administration.

Key words: anolyte, catholyte, infections, therapeutic effect.

Introduction

Escalating microbial resistance is a major threat to public health as it reduces the efficacy of antimicrobial therapy. The choice of agents to treat the respective infections is constantly decreasing. This in turn leads to increased morbidity and mortality, as well as to rising health care costs (Singh et al., 2006). New strategies need to be advanced and new antimicrobial agents to be identified in order the next generation of medications to be develop for the control of the microbial infections.

In recent years, it has been reported that electrochemically activated aqueous solutions (EAWS) that are produced by water in the electrolysis process have properties that make them proven to be effective in the treatment of a number of diseases. Anolyte destroys bacteria and many viruses, fungal flora, has antiseptic, anti-inflammatory, antiallergic, anti-itching and anti-edematous action. Catholyte or "living water" is a solution with alkaline properties. It has immunostimulating, antioxidant and regenerative properties (accelerates tissue healing), when adding certain minerals helps with diabetes, hypertension, osteoporosis and other diseases. It is characteristic for EAWS that their properties change depending on their redox potential, which is the most important parameter of the activated solutions. It characterizes the restoring - oxidating activity of the substances in a solution and their ability to import or receive electrons. The anolyte has a high ORP (up to 1200 mV) due to the presence of strong oxidants in its composition and its ability to remove electrons from other compounds and biological objects, thus causing oxidation and impairment of their viability (Ashbakh, 2008, Atanasov et al., 2014; Karadzhov et al., 2014). As a result of the electrolysis of the aqueous salt solution, strong oxidants are accumulated in the anode: chlorine radicals – chlorine dioxide, hypochlorous acid (HClO), hypochlorite ions (ClO^-), hydrochloric acid (HCl); chlorine (Cl_2) and oxygen radicals like atomic oxygen, ozone and hydrogen peroxide.

This composition and the high redox potential, determine the properties of the anolyte, which is a bright, clear solution with chlorine odor. Owing to their low content of oxidants, anolytes have very little chemical buffering, there by they are environmental safety, both in their production and use, and also after their final intended use. The anolyte has a local (topical) healing effect. This means that it acts (on the bacteria or the inflammatory infectious outbreak) only upon direct contact. When it is inflammation of the lungs or other diseases where direct contact is not possible, the

anolyte does not have this effect. The properties of EAWS vary depending on their redox potential. The characteristic of the anolyte and the catholyte is that the first one has reduced electron activity and has strong expressed oxidant properties (oxidant), and the second one has increased electron activity and reducing properties (reducer). The substances in the anolyte and the catholyte resulting after the electrochemical activating process are in a metastable chemical condition for a time interval different for the anolyte and the catholyte, after which they restore the state of an inactivated aqueous electrolyte (Aschbakh, 2008; Benyaeva et al., 2009; Gluhchev et al., 2018).

While the anolyte differs from the water with a slight odor of chlorine and a slightly sour, astringent taste, the catholyte about the taste, smell and color practically does not differ from the water. It differs from water by several parameters, of which the most important for explanation of its healing properties are ORP, acidity / alkalinity (pH) and the presence of active micro- and macro-elements. It has now been found that the catholyte has antioxidant and immunostimulating properties and accelerates the regeneration of tissues. It is an affordable and simple means of maintaining the balance between acid-forming and alkali-forming products, as it has a pH of 7 to 12 depending on the activation. In summary, catholyte possesses antioxidant and immunostimulating properties, accelerates tissue regeneration, stimulates energy production processes (ATP), regulates hydrocarbon and lipid exchange, increases the amount of erythrocytes in anemia and irradiation, and other healing properties which are mainly related to its antioxidant and alkalizing action (Ashbakh, 2008, Gluhchev et al., 2015, Ignatov et al., 2015).

The qualities of EAWS have provided grounds for broad application in both the human and the veterinary field.

The aim of the present study is to test the therapeutic effect of electrochemically activated water solutions - anolyte and catholyte in animals with various infections and in different ways of administration.

Materials and methods

Electrochemically activated water solutions. They were obtained with an apparatus Asbach-1 included in the plumbing installation in a third stage of activation and activation time of about 2 minutes: the anolyte with pH 5.8; the catholyte with pH 8.5.

Animals

Patient 1. Male cat John, 3 months of age with purulent conjunctivitis.

Patients 2, 3, 4 and *5* are yard (street) cats living together and showing clinical signs of panleukopenia, that is confirmed by laboratory test with Anigen – FPV – antigen positive test (Fig. 1).

Patient 2. Male cat Yancho – 8 months of age with severe gastroenteritis, weight loss and poor overall condition.

Figure 1: Chromatographic ELISA test for cat panleucopenia showing a positive result after fecal sample dropping from patient 2 (left) and patient 7 (right).

Patient 3. Male cat Sincho – 2 years of age with prolapse of the third eyelid, gastroenteritis, lack of appetite and weight loss.

Patient 4. Male cat Vesco – 18 months of age with purulent haemorrhagic rhinitis, severe gastroenteritis, lack of appetite, weight loss and poor general condition.

Patient 5. Female cat Elie – 10 months of age with gastroenteritis, vomiting and depression.

Patients 6 and 7. Male gerbils – 7 months of age with signs of dermatomycosis. There are scarred spots on the head in the area of the muzzle. The one of these animals has bigger spots and larger area of damage.

Patient 8. Female cat – 5 weeks of age, European shorthaired breed, with a characteristic color of the hair cover, which had refused to eat for 2-3 days, has vomiting and diarrhea with a malicious smell and yellow-brown colour, and from the next day already with blood in the stools. Degraded general condition. Accelerated breathing, ruffled fur, coarse hair. The hair cover around the anal area is stained with watery bad smelling stools. Internal body temperature is 40.3 °C. The Anigen - FPV - antigen test was positive (Figure 1). Panleucopenia was diagnosed.

Patients 2, 3, 4, and 7 were diagnosed with panleukopenia and treated with catholite by indipenden vets, on independent and different times, at different places.

Patient 9. Female dog Ariana, breed Golden Retriever, 5 years old with serous conjunctivitis. The right eye secretes serous exudate; the lining of the eye was very red and inflamed with no other signs and with no changes in the normal general condition of the animal. Left eye was normal. After ophthalmological observation, no foreign body was found, nor was there evidence of entropy or other morphological disorders of the eye which directed us to bacterial infection. We have not done additional microbiological tests to identify the causative agent.

Patient 10. Female dog Sue, Dakel breed, 9 years of age with a lot of bites on the back area of the body that were caused during a walk by another dog, breed Weimaraner. The animal had deep bites in the back area of the body and torn *musculi obliquus esterna et interna sinistri*. The animal showed signs of stress and shock. The muscles were surgically treated and sutured.

Patient 11. Female hedgehog found in very severe health condition in the nature and was taken for healing. The hedgehog had signs of severe inflammation in the oral cavity, with rotten pieces of jaw and teeth, as well as nerve signs (circling). After antibiotic therapy and surgery procedureof the mouth and palate by our colleagues, it gradually begins to accumulate mass and become vital. During this treatment period, anolyte was included as an additional therapeutic agent.

Microbiological studies

For the isolation of microorganisms materials from affected areas of the patients' skin 6 and 7 were used. Cultures were prepared on the following nutrient media produced by Antisel (Sharlau Chemie SA, Spain): ● agars – Mueller – Hinton, Endo, Cetrimide, Chapman, Chapek and Saburo, as well as Colorex Chromogenic Orientation Agar (Rida-Com Bulgaria, HiMeida Laboratories Pvt Ltd. Mumbai India); ● broths – dextrose and Tarotzi. Cultures were cultuvated at 37 °C for 24 – 72 hours and those for fungi – for 7 – 14 days at 22 °C.

Microscopic observation of native materials and after staining using the classical methods of Gram and Romanovski-Giemza were done under an impersion microscopy at an increase of 1200 x.

Identification of isolated microorganisms was performed by microscopic examination of Gram stained preparations, taking into account the peculiarities in the solid and liquid media and the biochemical properties with the help of Polymicrotest (Rida-Com Bulgaria, HiMeida Laboratories Pvt

Ltd. Mumbai, India) and also probes for oxidase and catalase activity were done. The isolation and identification of the bacteria was carried out in accordance with the Bergey International Identifier (Holt et al., 1994), and the inditification of the fungi were carried out according to Murray et al. (2003).

Determination of the sensitivity of the isolated bacteria to antimicrobial agents was performed using the classic agar-gel diffusion method of Bauer et al. (1966). Standard disks for antibiocograms (NCZPD – Sofia) and prepared by us after inoculation of bacterial suspensions in an exponential growth phase at a concentration of 2.10^6 cells / ml on Mueller-Hinton agar were used. Cultivation was carried out at 37 °C for 24 hours under aerobic conditions. The results are interpreted by the three-step system of Bauer et al. (1966) after measuring the diameters of the inhibition zones in millimeters.

Chromatographic ELISA (Anigen – FPV-ag) was used to detect feline panleucopenia virus antigens in faeces.

Results

Patient 1. Therapy was conducted only with **catholyte**, dropped into the eyes in the morning and evening daily for 5 days. On the second day there was an improvement in the condition, on the third day the effect was very good, and on the fifth day - there were no signs of disease.

Patient 2. Treatment in a clinic was conducted for 5 days with hyperimmune serum against panleukopenia, antibiotic amoxicillin with clavulonic acid and general consolidation therapy. After this time, the patient was taken for home-tretment without being able to eat. Only **catholyte** with 5% honey dissolved in it was administered orally 4 times a day for 4 days. On the second day the improvement of the condition was visible, and on the third day cat ate by its-self and no signs of gastroenteritis were seen. In the following days, his general condition and appetite were fully recoverd.

Patient 3. Therapy was conducted only with **catholyte** given instead of drinking water for 2 weeks. On the third day there was an improvement in the condition. The third eyelid was still noticeable, but the cat started to eat. In the following days, the signs of gastroenteritis disappeared, the appetite rised up, and on the 10th day the general condition of the animal was very good. The third eyelid was not visible any more, gastroenteritis was missing, appetite fully recovered.

Patient 4 and 5. Similar with the previous patient, therapy was conducted with only **catholyte** given instead of drinking water for 2 weeks. They also had an improvement in the general condition on the third day. Signs of rhinitis are considerably weaker and the cat Vesko starts to eat some foods. In the following days, the signs of gastroenteritis in the both animals Vesko and Eli disappeared, the appetite rised up, and on the 10th day the general condition of the animals was very good. There were no rhinitis and gastroenteritis, and the appetite was completely restored.

Patients 6 and 7. *Staphylococcus epidermidis*, *Pseudomonas fluorescens* and *Microsporium nanum* were isolated from the skin samples of the both patients. Local tretment with **anolyte** (once a day) and **catholyte** (once a day) in the first few days did not provide a visible result, but after a week of treatment, the hairless spots began to diminish, the skin regenerated and hair growth appeared.

The results of the antibiogramme presented in Tabl. 1 and Fig. 2, show the resistance of the strains to most penicillin antibiotics and sensibility to those of the other test groups, including broad spectrum antibiotics such as amphenicols, tetracyclines, aminoglycoside-aminocyclitols. Strains also exhibit sensitivness to chemotherapeutics such as sulfonamides and quinolones. Because of the

good effect of the therapy with EAWS, which can be seen in Fig. 3, no antibiotic therapy was required.

Table 1: Sensitivity of the isolated bacteria *Staphylococcus epidermidis* to antimicrobial means *in vitro*

Antimicrobial mean	Contents of the disc (µg)	Inhibitory zones in mm and sensitivity of the strains	
		P 6	P 7
Chloramphenicol	30	19 – S	23 – S
Tetracycline	30	31 – S	25 – S
Clindamycin	2	26 – S	22 – S
Oxacillin	1	10 – R	12 – R
Amoxycillin+Clavulanic acid	10	11 – R	21 – S
Penicillin G	10	8 – R	16 – R
Cefuroxime	30	17 – I	22 – S
Cefotaxime	30	18 – I	19 – I
Kanamycin	5	22 – S	23 – S
Novobiocin	30	21 – S	11 – R
Gentamicin	10	20 – S	19 – S
Ciprofloxacin	5	35 – S	30 – S
Sulfamethoxazole+Trimethoprim	23,75/1,25	31 – S	28 – S

P 6 – patient 6; P 7 – patient 7; S – sensitive; I – intermediate; R – resistant.

Figure 2: Sensitivity to antimicrobial agents *in vitro* of *Staphylococcus epidermidis* isolated from patient 6 (above) and 7 (below).

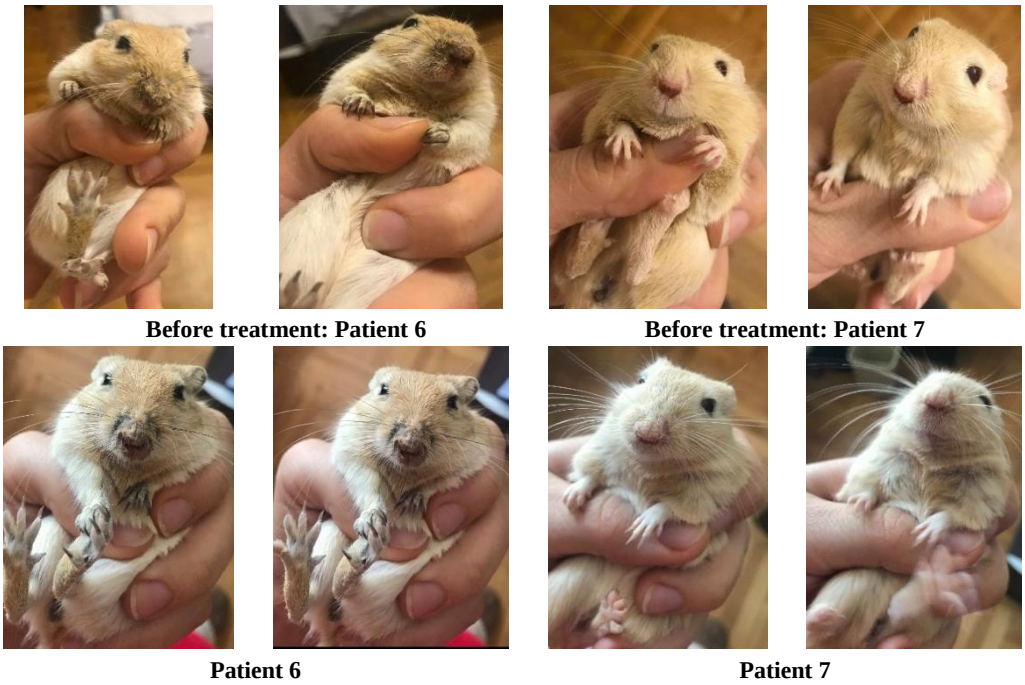

Figure 3: Patients 6 and 7 before treatment (above) and after one-week administration of anolyte and catholyte (below).

Patient 8. The treatment was performed with hyperimmune serum against panleukopenia, antibiotic amoxicillin with clavulonic acid, general supportive therapy and immunostimulants. For home tretment of the hosts was given a **catholyte** for oral administration 3 times a day (morning, afternoon, evening). After treatment, in the first 36–48 hours, the faeces began to normalize, with no blood in them, vomiting stopped, and the animal improved its general condition. On the 3rd day of therapy there was a visible result and improvement of the condition of the kitten. After the course of treatment, the animal was completely cured.

Patient 9. Therapy is conducted with an **anolyte** alone, in the form of a lint compress for a few minutes – at least 5 and the same prescription to the owner's during the home therapy, morning and evening daily for 7 days. On the second day there was an improvement in the condition, on the third day the effect was very good and the on the fifth day there were not signs of conjunctivitis (Figure 4).

Patient 10. Post-operative treatment with general supportive therapy, antibiotics treatment, anti-inflammatory and analgesic agents. To the owner was given **catholyte** for administration in the form of soaked lint compresses and washing of the opened wounds and surgical ones, along with conventional therapy. After about 10 days of treatment, there was a full regenerative effect and complete wound closure as shown in Fig. 5.

Patient 11. Hedgehog found in very severe health. At the middle of the treatment, after antibiotic therapy and mouth and palate surgery, vitamins and **anolyte** per os were used and the animal began to recover at a very good rate. At the beginning the condition of the hedgehog was very poor and weighs only 300 g, which is underweight for an adult hedgehog. At the end of the treatment, before putting it back into nature, weighs around 600g, which is already a normal weight for an adult female hedgehog.

As can be seen from Fig. 6, after treatment with antibiotics and with anolyte the hedgehog is in very good condition. When an anolyte is included in the treatment, the hedgehog shows greater vitality and activity. According to the owner, anolyte therapy has visibly accelerated the recovery of the animal.

Figure 4: Patient 9 before treatment (left) and after once week therapy with anolyte (right).

Figure 5: Patient 10 before treatment (left) and after 10 days tretment with catholyte (right).

Figure 6: Patient 11 before the therapy with anolyte after the surgecal procedure (left) and 10-day anolyte administration (right).

Discussion

The results of our research definitely confirm the significant therapeutic effect of anolyte and catholyte obtained by electrochemical activation of pure water without addition of salts. The local administration of catholyte in conjunctivitis tretment proved that it is an effective and innocuous means of rapid therapy. The per os administration of catholyte with 5% of honey in it leads to rapid recovery of appetite and improving of the general condition of patients with panleukopenia. Our results show that cat panleucopenia can only be cured with catholyte given instead of drinking water

for 2 weeks. This complementary therapy proves to be an effective one for rapid and complete recovery from severe disease. It is necessary to start the treatment timely and not to be interrupted. According to our results, its minimum duration is 10–14 days, but for a faster recovery and a secure and lasting result it can last for another 1–2 weeks. This therapeutic approach is very well suited to yard cats where it is difficult to apply daily specific and maintenance therapies.

These results of the study are consistent with the studies of Ashbach et al. (Ashbakh, 2008) who conducted experiments in 1995 on the study of the influence of activated solutions on various parts of the immune system and the results obtained show that the immunostimulating action of the activated solutions, and first of all the catholyte, directly depends on the parameters of the redox - potentials varying from +150 to +350 mV and reaching up to – 840 mV. Drinking a catolyte, according to these reports, improves general body and mibd condition, helps patients recover faster, improves blood and immune system performance.

The high sensitivity of the isolated from gerbils staphylococcai to antibiotics from different groups is probably due to the fact that the tested animals were not treated with antibiotics at all. Therefore, the representatives of their skin microflora, which are obviously the isolated staphylococci, have not developed resistance. As a result of their isolated cultivation, they have not obtained resistant microorganisms from the outside, nor plasmids with genes for resistance. This fact reaffirms the great role of excessive use of antibiotics for the construction and spread of resistant mutants and in the opposite side - resistant strains do not appear when abstaining from antibiotics.

At present, the development of many diseases, including inflammation and infections, is associated with the destructive action of oxidants – free radicals. These are molecules having electrons in their outer electron shell that are not in pairs, making them chemically particularly active and "aggressive". They seek to bring back their electron in lack, removing it from the surrounding molecules. Changes in cell membrane molecules caused by free radicals' attack have a devastating effect on the cardiovascular system: the blood components become "sticky," the walls of the vessels are drenched with lipids and cholesterol, resulting in thrombosis, atherosclerosis, and other illnesses (Ashbakh, 2008)

Many antioxidants neutralize free radicals by giving them electrons. As a result of the reaction, they themselves become free radicals but weaker and incapable of harm, and then, through complex biochemical transformations, they are removed from the body. This also explains the mechanism of the antioxidant action of the catholyte, taking into account its negative ORP. The living organism is a complex aqueous solution. As in all aqueous solutions, there are oxidizers and restorers constantly interacting with each other. In this way ORP plays a huge role in the normal course of life. Any fluid that the body receives possesses and has specific by its own ORP. The redox potential of the catholyte with values from -50 to -70 mV is as close as possible to the physiological significance of the ORP of the blood and tissues of the organism (Khachatryan, 2003). Catholyte is an accessible and simple means of maintaining the balance between acid-forming and alkali-forming products, as it has a pH of 7 to 12 depending on the activation.

The results we obtained are consistent with Bakhir's data (2009 a, b), by Tasheva et al. (2010) Gurgulova et al. (2010; 2011) and our previous studies (Popova et al., 2016 a, b, c), according to which the anolyte destroys bacteria, viruses, patogenic fungi and spores. It is harmless to humans and to the environment. Ozone, atomic oxygen, hydrogen peroxide, chlorine dioxide, etc. are contained in it. Ashbakh (2008) firstly established the selective antibiotic properties of the anolyte by

conducting chronic tonsillitis (rinsing of the tonsillas with anolyte). By conventional microbiological methods, they find that the anolyte selectively destroys the pathogenic microflora (in the case of *S. haemolyticus* of groups A and B, *S. aureus* and other bacteria) but does not affect microorganisms that are not involved in the throat inflammation process(such as micrococcai, non-hemolytic streptococci), or anolyte exhibits selective antimicrobial activity. They confirm these results in a number of experimental and clinical studies, clarifying that this selective antimicrobial activity of the anolyte directly depends on its ORP and occurs only at certain values. This property of the anolyte gives it an immense advantage over the antibiotics which, by destroying the pathogenic flora, destroy the normal bacterial flora that is necessary for the normal existence of a certain organism, which leads to some secondary diseases – candidosis and other fungal diseases, dysbacteriosis, and fermentative functions, etc. The use of super-oxidized solutions as wound care products is a modern concept. The moisturizing effect and minimal toxicity found with the use of such solutions make them a good choice for wound healing. Preliminary results suggest that this non-antibiotic technology offers a broad new paradigm for the prevention and treatment of acute and chronic wounds (Dalla Paola et al., 2006).

The bactericidal and fungicidal action of the anolyte is due to the combined effect of the hydrogen concentration, oxidation-reduction potential and dissolved chlorine. Anolyte is a strong acid but is different from hydrochloric acid or sulfuric acid. These acids have a high level of ionization, and when oxidation occurs, H^+ is used and a new H^+ is generated. Under the action of anolyte, no new H^+ is generated since it is produced only by electrolysis of the saline solution. Thus, the solution in its full effect is not corrosive to the skin and the organic materials (Hussein et al., 2013). The large amount of $HOCl^-$ due to low pH (3) is considered to be a major factor in the disinfection efficacy of the anolyte.

It is known that nitrogen compounds such as proteins are chlorine-consuming, which means that organic nitrogen (N^+) will react immediately with the free available chlorine residue ($HOCl^-$) to form non-germicidal N-chloro-compounds. Thus, proteins reduce the effectiveness of disinfection of the total chlorine residue. Therefore, in order to ensure the efficiency of disinfection with the help of electrochemically activated aqueous solutions, pre-treatment is necessary to minimize the available organic contamination, which is a basic principle for the application of other types of chemical disinfectants. Lower efficacy of the EAWS in high content of biological leavings in environment has been established in our previous studies of bovine fertilizers and depleted sludge from a municipal sewage treatment plant (Popova et al., 2018) and other authors such as Peev (2017) studies of water from lagoons for the storage of bovine manure and Dimitrova et al. (2013) in the study of the bactericidal effect of EAWS on biosylams.

Conclusion

Catolyte, administered locally, twice daily for 5 days, has been shown to be an effective and harmless agent for rapid therapy of purulent conjunctivitis in cats.

Anolyte, administered as a lint compress for several minutes twice a day for 7 days, also proved to be an effective self-administered agent for treatment of serous conjunctivitis.

Electroactivated water solutions show a good therapeutic and regenerative effect not only applied to the mucouses but also to deep skin wounds – after surgical intervention as well as on biting wounds that are particularly dangerous for infectios injures.

The internal administration of catholyte instead of drinking water for 10–14 days has a therapeutic effect in a severe, and particularly dangerous cats' viral disease such as panleucopenia. It can be given alone or with the addition of 5% honey as a complementary therapy to the conventional such. Its effect is indicative in cats with proven panleucopenia - both clinically and after an antigen test, as patients are diagnosed and treated with catholyte at different times and places and have no connection with each other.

Electroactivated water solutions (catholyte and anolyte) show a very good therapeutic effect, applied to rodents such as gerbils and hedgehog, as well as like a general treatment of skin infections or per os administration as a supportative therapeutic and generally reinforcing agent along with the convencional therapy.

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