

PROTECTIVE EFFECT OF VIT.E WITH CYPROHEPTADINE AGAINST LETHAL EFFECTS OF EXUDATES FROM BURNT RAT SKIN

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ABSTRACT: To evaluate the protective effect of vit.E with cyproheptadine against lethal effects of exudates from burnt rat skin. the steps of the procedure were: **Step 1:** A pouch was made with air on the dorsal side of the albino rats and dipped in hot water. Tyrode solution was injected into the pouch and withdrawn after two minutes. The procedure is repeated for not more than 5 times to collect maximum exudates. **Step 2:** Albino rats were divided into three groups receiving three different doses of exudates and monitored for 24 hours for the mortality. The lethal dose is determined and served as control group. **Step 3:** Albino rats are divided in five groups. Group 1 received cyproheptadine and Group 2,3,4&5 received same dose of cyproheptadine with increasing doses of Vit.E. (0.9 to 7.2mg/100g) and monitored for 24 hours for the mortality. Group 1 and 2 showed same reduction in mortality rate. Group 3 and 4 showed increase in mortality rate while group 5 has the same effect as that of group 4. Vit.E. with cyproheptadine reduced the mortality rate confirming the presence of free radicals in the exudates along with antihistaminic and anti serotonin properties of cyproheptadine .

INTRODUCTION

Literally speaking, burns induced injury and death are ridiculous, Compared to adults, children are the major victims, caused by accidents with kettles, pans, hot drinks and bath water¹⁻³. Burns cause damage in a number of different ways, but by far the most common organ affected is the skin. However, burns can also damage the airway and lungs, with life threatening consequences. Airway injuries occur when the face and neck are burnt, Respiratory system injuries usually occur if a person is trapped in a burning place and is forced to inhale the hot and poisonous gases. Fortunately, marked decreases have seen in both mortality rates and length of hospitalizations since 1970⁵. This improved prognosis due to better understanding of the systemic effects of massive burns and discoveries of better ways to prevent wound infection and facilitate healing of skin surface¹.

Rocha E Silva and Rosenthal⁶ have reported that the exudates from burnt rat skin contain histamine, bradykinin, adenosine derivatives and possibly serotonin and other as yet unidentified pharmacologically active and toxic substances^{1,4}. It was confirmed by Rao:5 that two newer antihistaminic and anti serotonin compounds Cyproheptadine and BC 105, possess significant protective effect against the lethal effects of exudates from burnt rat skins. It is also stated that burns release free radicals and prostaglandins, which also account for the mortality³.

MATERIAL AND METHODS

The method of Rocha E sliva and Rosenthal⁶ was utilized to obtain the exudates. Animals weighing between 120 and 150g were anesthetized with thiopental sodium (40mg/kg, i.p.) and then 30ml of air was injected beneath the dorsal skin to raise a pouch of 7x4x3cm. This dome was submerged in water at 95° C for 15 to 20 sec. After scalding, the animal was suspended by its paws and 5 ml of tyrode solution was injected into the pouch. The animal was then shaken for 2 min after which the fluid was withdrawn by syringe and needle. The washing was repeated at regular intervals

after burning and a maximum of 5 washings were done to each animal Determination of control dose Animals were divided into three groups often each. The exudates were injected intraperitoneally at three different doses. Group I received (2ml/100g), Group 2 received (4ml/100g) and Group 3 received (6ml/100g). Animals were monitored during the next 24 hrs for the mortality. The dose, which is prior to the 100% mortality dose, is selected as the control dose, to prevent overloading in the peritoneal cavity and for better efficacy

Drug Treatment Animals were divided, into five groups of 10 each. Group I received cyproheptadine (0.05mg/100g, s.c.). Group 2,3,4 and 5 received same dose of cyproheptadine (0.05mg/100 g, s.c.) and 0.9,1.8,3.6 and 7.2mg/100g, s.c., of vit E respectively. The animals were monitored for the next 24 hrs for the mortality.

Animals: Albino rats of either sex (120-150 g) were used. The animals were bred and housed under standard environmental condition and fed with standard diet and water ad libitum. The institutional animal ethics committee has approved the animal studies.

RESULTS

Table1: Percentage mortality of rats injected with exudates from burnt rat skin

Groups	Control dose of exudate (ml/100g,i.p.)	% Mortality in 24hrs.
1	2	50
2	4	70
3	6	100

Table 1 indicates the percentage mortality of animals when exudates were administered intraperitoneally. A dose of 6ml/100g) showed 100% mortality.

Table2 indicates the influence of drugs on toxic effect of exudates. Groups showed almost partial reduction in mortality rate (40%). In case of Vit-E added groups, at lowest dose (Group 2) of vit E) it has no effect in reduction of mortality rate.

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Table 2: Percentage mortality of rats injected with 4ml/100g, 1hr after administration of test drugs.

Groups	Drug	Dose mg/100g,s.c.	Mortality in 24hrs.
1	Cyproheptadine	0.05	40
2	Cyproheptadine + Vit-E	0.05+0.9	40
3	Cyproheptadine + Vit-E	0.05+1.8	30
4	Cyproheptadine + Vit-E	0.05+3.6	40
5	Cyproheptadine + Vit-E	0.05+7.2	40

At the dose of 1.8 and 3.6 mg/100g (Group 3 and 4), the mortality rate was reduced to 30% and 20% respectively. Group 5 received the highest dose and showed no reduction in the mortality rate when compared to the prior dose (Group 4).

The maximum and minimum reduction of mortality rate were at 3.6 and 1.8 mg/100g respectively.

DISCUSSION

The present investigation carried out on the effect of Vit-E in enhancing the protective effect of cyproheptadine against lethal effect of exudates from burnt rat skin reveals that moderate decrease in mortality was there with increasing dose of Vit-E, suggestive of destructive role of free radicals in burns exudates.

Rocha E SIL V A. et al⁴ have already reported that exudates contain histamine and serotonin. Our present study reveals the presence of free radicals in the exudates from burnt rat skin. Though many research projects suggested the beneficial role of various anti-oxidant in burns⁷⁻¹³, the protective action against exudates is not yet reported. It may therefore be

anticipated that the histamine, serotonin and free radical contents of the exudates are the major cause of death in animals and the drugs protect the animals due to their antihistamine, anti serotonin and anti-oxidant properties only.

In clinical cases of extensive burns, antihistaminic, antiserotonin and antioxidants are rarely used. They are therefore possibly therapeutically important in the management of cases of extensive burn. Fat soluble vitamins rarely cause hypervitaminosis in burnt patients and so the safety of Vit.E should be extensively studied.

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Drug Profile

Diacerein

Mechanism of Action : *Rhein*, the active metabolite of diacerein inhibits the production of interleukin-1 beta by human monocytes which in turn reduces the collagenase production and collagenolytic activity in articular cartilage. Diacerein decreases the number of urokinase receptors on chondrocytes to normal levels and reduces fibrinolytic activity of synovial fibroblasts. It, dose dependently inhibits superoxide anion production, chemotaxis and phagocytic activity of neutrophils and macrophage migration and phagocytosis. In Patients with active osteoarthritis, diacerein increases the lymphocyte number and synovial membrane fluidity and reduces the ratio of chondroitin 6-sulphate to chondroitin 4 and thereby protects the proteoglycan aggregation and helps the articular cartilage to resist compression under load. It does not alter renal or platelet cyclooxygenase activity and may therefore be tolerated by patients with prostaglandin dependent renal function.

Pharmacokinetics : Oral diacerein undergoes first pass metabolism and is deacetylated to its active metabolite *rhein*, which is metabolized to glucurono and sulpho-conjugates. In healthy volunteers, the maximum plasma concentration (C_{max}) of *rhein* was 3.2 mg/L at 2.2 hours after administration of a single oral dose of diacerein 50 mg. The plasma protein binding is approximately 99%. Area under the plasma *rhein* concentration time curve (AUC) from time zero to infinity was 21.2 mg/L.h, apparent volume of distribution was 13.2L, terminal elimination half life ($t_{1/2}$) was 4.3 hours, apparent total plasma clearance was 1.6L/h and renal clearance (CL_R) was 0.13 L/h. The total quantity excreted in the urine is approximately 30%. The elimination half life of *rhein* is approximately 4.5 hours. *Rhein* is eliminated in urine 80% as sulfo and glucurono conjugated forms and 20% in unchanged form.

For doses ranging between 50 and 200 mg of diacerein capsules in a single intake, all the pharmacokinetic parameters are independent of the dose. The concomitant administration of diacerein capsules with food delays the absorption but increases the bioavailability presents a low accumulation. Among patients with severe renal impairment (creatinine

clearance less than 30 ml/min), the area under plasma concentration-time curve and elimination half life are doubled and urinary elimination is reduced by half. Among elderly subjects, taking into account the good tolerance of diacerein capsules, it is not necessary to modify the dose, despite slower elimination.

Indications : Diacerein is indicated for the symptomatic treatment of osteoarthritis of the knee or hip.

Warnings and Precautions : (i) Diacerein should not be administered to children (less than 15 years), (ii) Caution is advised in patients with inflammatory organic disease of colon (ulcerative colitis, Crohn's disease, etc.) or abdominal painful syndrome of unspecified cause. (iii) With prolonged treatment with any medication, a complete blood test, including liver enzymes and urinalysis should be conducted every 6 months. (iv) Diacerein capsule should not be used during pregnancy, and in a woman during the breast feeding period. **Drug Interactions :** The concomitant administration of hydroxides of aluminium, calcium or magnesium may cause reduction in the absorption of diacerein from gastrointestinal tract.

Side effects : (A) Diarrhea, soft stools and abdominal pain. (B) A yellow-brown colouring of the urine and pigmentation of the colonic mucosa (colonic melanosis) can be observed occasionally. (C) Other side effects like pruritis, eruptions and eczema may occur.

Dosage and Administration : As diacerein may cause acceleration in intestinal transit time during the first 2 weeks of treatment, it is recommended that therapy be started with one capsule per day administered with the evening meal for 4 weeks. The capsules should be swallowed whole with water, preferably in the middle of the meals.

The duration of treatment should not be less than 6 months. In clinical trials diacerein has been administered for up to 2 years with no safety problems. NSAID or analgesics for the first 2-4 weeks of treatment, may be needed. Modification of the dosage of diacerein capsules in patients with hepatic impairment is not required.

Renal impairment : In mild to moderate renal insufficiency, it is not necessary to modify the dosage of diacerein capsules while in patients with severe renal insufficiency (creatinine clearance less than 30 ml/min), dose should be reduced by half.