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INHIBITION OF CROTON OIL-INDUCED OEDEMA IN RAT EAR SKIN BY TOPICAL NICOTINAMIDE GEL

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Abstract

Nicotinamide, a physiologically active form of niacin (nicotinic acid), in combination with zinc is being assessed in clinical studies for the treatment of inflammatory skin diseases such as acne vulgaris and bullous pemphigoid. Consequently the aim of the present study was to investigate the anti-inflammatory effect of Nicotinamide gel 2%, 3% and 4% (Apex Laboratories Private Limited, Chennai, India) against croton oil-induced ear oedema skin inflammatory model in Wistar rats. Ear oedema was induced by topical application of irritant solution of croton oil to the right ear only. A total of 24 Wistar rats were randomly grouped into four groups each containing six animals. Croton oil-induced ear oedema control rats (Group I) and Nicotinamide gel 2% (Group II), 3% (Group III) and 4% (Group IV) dissolved in the irritant solution of croton oil was applied for 21 days. The topical application of Nicotinamide gel significantly reduced the oedema ($p<0.001$) in comparison with the croton oil applied toxic control group (untreated) at all the three concentrations 2%, 3% and 4%. The present study revealed that the Nicotinamide gel 2%, 3% and 4% (Apex Laboratories Private Limited, Chennai, India) is the potential agent that could offer a novel therapeutic option against croton oil-induced ear edema in Wistar rats.

Key words: Nicotinamide, Croton oil, Inflammation

Introduction

Skin is responsible for the communication between an organism and the environment and is constantly subjected to exogenous stimuli. The skin is able to activate a defense mechanism aimed at pathogen elimination and tissue repair. Initiation of the defense response is characterized by the infiltration of neutrophils and the release of several pro-inflammatory mediators, which starts the inflammatory process. Currently available therapeutics to treat chronic inflammatory skin diseases are mostly ineffective and produce a plethora of side effects, the search for more effective and safer treatment alternatives is necessary.

Croton oil-induced inflammation is due to the activation of phopholipase A2, which releases arachidonic acid from the cell membrane. Arachidonic acid, in turn, is metabolized to prostaglandins (PG's) and leukotrienes. Substances able to inhibit edema could be inhibitors of cyclooxygenase (COX) and/or 5-lipoxygenase [1]. Nicotinamide, also known as niacinamide or nicotinic acid amide, is the water-soluble, active form of vitamin B3. One of the studies suggest the role of Nicotinamide as an anti-inflammatory agent in a mouse model of septic shock [2]. It has been increasingly studied for many different indications in the field of dermatology but more research is needed to clarify its value. Nicotinamide in combination with zinc is being assessed in clinical studies for the treatment of inflammatory skin diseases such as acne vulgaris and bullous [3]. Delivery of drugs to the skin is an effective and targeted therapy for local dermatological disorders.

Hence a study was planned to investigate the effect of topical formulation of Nicotinamide 2%, 3% and 4% gel on croton oil-induced ear oedema in Wistar rats.

Methods

Animals

24 adult male Wistar rats weighing 150–200 g were obtained from Central Animal Research Facility, Manipal University, Manipal (India). Animals were housed in polypropylene cages, maintained under standard conditions with temperature (22–24^0 C), 12-h light/12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to norm caloric standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and to tap water. The animals were acclimatized to the laboratory conditions for one week before the start of the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/03/2014) and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment (Government of India), Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Drugs

Nicotinamide 2%, 3%, 4% gel was obtained from Apex Laboratories Private Limited, Chennai (India).

Induction of ear oedema by irritant solution of croton oil

The irritant croton oil were prepared by dissolving 4 parts of croton oil, 10 parts of ethanol, 20 parts of pyridine, and 66 parts of ethyl ether. The test compounds were dissolved (5 mg/ml strength) in the croton oil.

Experimental procedure

A total of 24 Wistar rats (male, pathogen free, 6-8 weeks old) were used in the experiment. All the rats were grouped into 4 groups each consisting 6 animals. The treatment was carried once for a period of 21 days with one time per day application of the drugs. The left ear of each rat was kept as control (untreated) with respect to the right ear. The toxic control and the test animals received the drugs in following manner:

- Group I: Toxic control rats- 0.02 ml of croton oil solution, applied on either side of the right ear.
- Group II: Test drug treated rats- 0.02 ml of croton oil solution containing dissolved Nicotinamide gel (2%; Apex Laboratories) 0.5 mg/ml were applied on either side of the right ear.
- Group III: Test drug treated rats- 0.02 ml of croton oil solution containing dissolved Nicotinamide gel (3%; Apex Laboratories) 0.5 mg/ml were applied on either side of the right ear.
- Group IV: Test drug treated rats- 0.02 ml of croton oil solution containing dissolved Nicotinamide gel (4%; Apex Laboratories) 0.5 mg/ml were applied on either side of the right ear.

After one hour of the last day application of drugs, animals were sacrificed by cervical dislocation. Both ears were removed and discs of 8 mm diameter were punched. The discs were weighed immediately and the difference in weight between the treated and the untreated ear indicated the degree of inflammatory

oedema.

The percentage increase in the oedema of the treated ear was calculated by the following formula:

$$\% \text{ ear oedema} = \frac{\text{Wt. of test ear} - \text{Wt. of control ear} \times 100}{\text{Wt. of control ear}}$$

Data analysis

Using SPSS 20.0, data were expressed as mean ± standard error of mean (SEM) and analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test. P value less than 0.05 was considered as statistically significant.

Results

The topical application of Nicotinamide gel significantly reduced the oedema (p<0.001) in comparison with the croton oil applied toxic control group (untreated) at all the three concentrations 2%, 3% and 4%. The anti-inflammatory effect was more pronounced in the Nicotinamide 4% gel treated group when compared to rest other groups.

Discussion

The present study revealed that the Nicotinamide gel 2%, 3% and 4% (Apex Laboratories Private Limited, Chennai, India) is the potential agent that could offer a novel therapeutic option against croton oil-induced ear edema in Wistar rats. Nicotinamide has been reported to exert its anti-inflammatory action partly through suppression of neutrophil chemotaxis [4], as also seen in a mouse arthritis model [5]. One of the studies suggests that nicotinamide is able to scavenge oxygen radicals, it does not scavenge nitric oxide [4].

However, nicotinamide inhibits nitric oxide synthase mRNA induction in activated macrophages [6]. Nicotinamide increases the biosynthesis of ceramides by keratinocytes [7].

It is known that the sphingosine derived from degradation of surface ceramide inhibits protein kinase C (PKC) and decreases basal cell proliferation dependent on PKC [8].

Some studies have shown that transient increases in ceramide levels are associated with the induction of keratinocyte apoptosis [9, 10]. This effect could offset the anti-apoptotic effect of nicotinamide exerted through Poly (ADP-ribose) polymerase (PARP) inhibition [4]. Further, clinical evaluation has to be performed to precisely define the role of Nicotinamide gel 2%, 3% and 4% in skin inflammatory conditions of human subjects.

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Table 1: Percentage oedema (On day 21)-

Groups and Doses	Mean ±SEM
Group-I 0.02 ml of croton oil solution (Toxic control)	39.93±6.03
Group-II 0.02 ml of croton oil solution + Nicotinamide 2% gel (0.5 mg/ml)	9.88±2.39***
Group-III 0.02 ml of croton oil solution + Nicotinamide 3% gel (0.5 mg/ml)	15.26±1.63***
Group-IV 0.02 ml of croton oil solution + Nicotinamide 4% gel (0.5 mg/ml)	5.66±1.20***

*** $p<0.001$, when compared to Group-I (Toxic control)