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The effects of oral vitamin A (retinol) on chemically–induced corneal ulcer in guinea pigs

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ABSTRACT

The effects of the healing propensity of vitamin A (retinol), a vital micro-nutrient were studied on chemically–induced corneal ulcers in guinea pigs. Corneal regeneration following alkaline injury was studied by fluorescent dye staining of the cornea under cobalt blue light, while digital photographs were taken every 6 hours. Results showed that administration of vitamin A syrup reversed the epithelial damage, produced accelerated wound healing, restored the eyelid status, light-reflex and corneal lustre, abolished corneal infiltrates and lacrimation in the treated group while the positive control (untreated) group had slow healing process as the ulceration continued unhindered. It is, therefore, concluded that vitamin A administered systemically facilitates the rate of epithelial regeneration in the cornea following traumatic injury or damage.

Key words: Alkali injury, corneal ulcer, retinol, wound healing.

Introduction

The eye is a complex and highly developed photosensitive organ that permits a fairly accurate analysis of the form, light intensity and colour reflected from objects. The organ is delicate that any 'harm' done to the transparent tissue distorts vision to a specific degree. The cornea is a clear transparent anterior portion of the fibrous coat of the eye comprising about one-sixth of its surface. Its curvature being greater than that of the remainder of the bulb, enables it to function as important refractive medium. It is continuous at its periphery into the sclera. It is composed of five layers: epithelium, Bowman's membrane, substantia proprio (stroma), Descemet's membrane and a layer of endothelium.The cornea is richly supplied with sensory nerve endings via the first division of the trigeminal nerve which innervates the whole of the eye and its appendages, giving warning of injuries and pain spots. The corneal transparency is based on its avascularity [1], though there are small loops of ciliary vessels which permeate the periphery for the nourishment of the tissues. The connective tissue cells in the cornea are flat and fewer than those present in the sclera. The corneal epithelium is thin, compact with smooth optical surface compared with the conjunctival epithelium which is thicker and overlies loose episcleral tissue. The corneoscleral junction is the area of transition from transparent collagenous bundles of the cornea to the white opaque fibres of the sclera, highly vascularised with blood vessels and being involved in corneal inflammatory processes. Corneal ulcer is a pitting of the cornea caused by an infection with bacteria, fungi, viruses, or protozoa, *Acanthamoeba specie* and sometimes from traumatic injury, which can either be chemical or mechanical. Bacteria (staphylococci, pseudomonas, or pneumococci), can infect and ulcerate the cornea after the eye is injured, a foreign body or object lodges in the eye or the eye is irritated by a contact lens. Other bacteria such as gonococci and viruses (herpes) can

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also cause corneal ulcer while fungi may cause slowly growing ulcers [2].Corneal ulcers cause pain, sensitivity to light and increased tear production all of which may be mild depending on the intensity of ulceration. A whitish yellow spot of pus may appear in the cornea, while ulcers, may in some instances develop over the entire cornea, penetrating deeply. The deeper the ulcer the more severe the symptoms and complications such as perforation of the cornea, displacement of the iris and destruction of the eye [3].
Vitamin A is generic term for a large number of related compounds. Retinol (an alcohol), and retinal (an aldehyde) are often referred to as preformed vitamin A, stored in the liver in esterified form. Vitamin A increases the lustre nature of the conjunctiva. Its deficiency does not lead to corneal ulceration but causes epidermoid changes in the epithelium, causing inability to secrete mucus and consequent dryness. It is commonly called anti-infective vitamin because of its requirement for the normal functioning of the immune system [4]. Vitamin A is used in the prevention and treatment of some ocular and somatic conditions of the epidermal tissues [2] and plays an important role in the biochemistry of vision. The main objective of this study was aimed at evaluating the effects of the healing propensity of vitamin A (retinol), a vital micro-nutrient, on chemically–induced corneal ulcers in guinea pigs.

Materials and methods

Twelve guinea pigs of the same colony, and either sexes, were procured from DEGOZ farms, Onitsha, Anambra State, Nigeria and quarantined for acclimatization. External eye examination was conducted by the optometrist using a pen torch and a +10.00DS lens as a magnifier. The sclera, limbal and corneal regions of each guinea pig were screened for any abnormality, and only those with normal sclera, cornea and limbal structure were used for the study. Additionally, each animal showed positive test of corneal sensitivity, that is, presence of corneal reflex before commencement of protocol. All the experimental procedures followed the recommendations of the committee for research and ethical issues of the International Association on Ethical Standard for Investigations on Experimental Animals[5].The guinea pigs whose weights were between 420 and 440g (mean 430.6 ± 5.5g) were randomly divided into three groups of A, B and C, of four animals per group. Animals in group A served as positive control while animals in group B served as the test group, and group C animals were used as negative control. The guinea pigs in each group were housed separately to allow free movement with access to food and water *ad libitum*. A solution of sodium hydroxide was prepared by dissolving 4.0gm NaOH pellets in 100ml double distilled water, and allowed to cool. This gave 1M NaOH (one molar solution) which was used in the induction of corneal ulcer in guinea pigs in groups A and B. Animals in groups A and B were anaesthesized using 0.4% lignocaine (Hans-E, Lembeke, Hamburg, Germany) solution to abolish corneal reflex. Thereafter, one drop of 1M NaOH was applied to the corneal region using a droptainer (drop dispenser) on right eye (OD) of the guinea pigs in groups A and B. The overflow of the sodium hydroxide was rinsed using wet cotton wool. Observations were made after 24 hours for induction of corneal ulcer using sterile fluorescein ophthalmic strip, a pentorch and a +10.00DS magnifier. Treatment with Vitamin A (retinol) syrup (Campharm Ltd, Orlu, Nigeria) commenced on the second day and continued till the 7th day of corneal ulceration only on group B animals while animals in group A were left untreated and group C animals were fed freely. The vitamin A syrup was administered twice daily by gavage. The vitamin syrup is composed of vitamin A palmitate 10,000IU (5.5mg equivalent to 5 ml).The weights of the animals and general behavior were monitored and evaluated on daily basis and the following parameters were observed in vitamin A treated group and compared with the guinea pigs in groups A and C; eyelid status, conjunctival status, lacrimation, epithelial morphology, eyelid oedema, light reflex, corneal fluorescein staining, discharges, corneal lustre/infiltrates and interior ocular structures. The composite results were presented in tables and plates were used to show the healing processes.

Results

Corneal ulceration set in 24hours after induction with caustic soda in groups A and B. There was slight variations in the weights of the animals. Group A animals which had corneal ulcers but not treated lost 0.5% weight while group B guinea pigs that received vitamin A syrup (retinol) gained 0.23% in weight, animals in group C which served as negative control showed no change in weight. During the 2nd day of retinol therapy, the white stringy mucous discharges in group B animals were gradually disappearing and by the 3rd day the discharges have completely disappeared while corneal infiltrates were still present. By the 4th, 5th, 6th and 7th day of therapy, the alkali injury has been healed while in group A animals, marked epithelial degeneration, corneal infiltrates and oedema persisted. These results are presented in tables 1 and 2. Plate 1

shows normal cornea of the guinea pigs while Plate 2 shows 24hr after induction of corneal ulcer using 1M Na0H. Plate 3 shows the extent of ulceration 48 hr after the induction, before the commencement of therapy while Plates 4 and 5 show the degree of corneal ulceration and medication at the 3rd day of ulceration and medication respectively. Plate 6 shows the corneal status at the end of the 7th day of medication with vitamin A.

Discussion

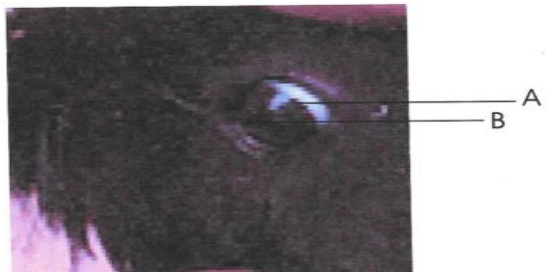
Ulcer is an open sore or lesion of the skin or mucous membrane of the body, with loss of substances sometimes accompanied by formation of pus. Simple corneal ulcers result from trauma, caustics, intense heat or cold, and in the present study, sodium hydroxide was used to induce the corneal ulcer in the guinea pigs. The course of corneal ulcer depends on the severity of the infection, the nutritional state of the patient, and in the absence of therapeutic management several courses can develop such as, the ulcer may heal without scarring, or may penetrate deeply to expose the Descemet membrane, or penetrate the corneal stroma substantia propria or the ulcer may perforate leading to escalation and deterioration.Vitamins are crucial micronutrients that are needed by the body in small quantities for homeostasis, growth, cell generation and general well being [6]. In the presence of retinoic acid, the mucous cells divide at a high rate and the progeny rapidly matures for fully differentiated mucous and ciliated cells. When such cells grow in the absence of retinoic acid or collagen substrates, they fail to mature into normal columnar mucous cells thereby aggravating the corneal ulceration. This scenario is presented in group A guinea pigs whose ulcers were not treated with vitamin A or retinol. On the other hand, vitamin A enhanced healing of corneal epithelial regeneration in group B animals. The corneal epithelial cells are more firmly attached to one another and are capable of sliding action which is demonstrated in the early stages of healing of a corneal abrasion[7]. Ideally, healing commences by a process of cell migration or sliding of cells to fill the defect followed by healing stage which involves proliferation of new epithelial cells[8]. This cellular mitosis which occurs within 72 hours of the injury enhances the covering and thickness of the injured area [9] and this is shown in Plate 3. However, on the 3rd day of retinol therapy, healing had commenced but can only be noticed with fluorescein stain examination as depicted in Plate 4 and more prominent on day 4 as shown in Plate 5. According to a study[10], vitamin A deficiency caused a marked drop in the amount of cross reacting antigen in a variety of epithelial tissues and in the control (group A) no healing was observed by the fourth day of ulceration due to sluggish response to cellular mitosis, while at the same period group B animals had achieved substantial healing. The eyelid status had returned to normal, the oedema receded, no discharges or infiltrates, light reflex was present but not complete due to incomplete healing, the conjunctiva had returned to normal as shown in Tables 1 and 2. By the 6th day of drug administration, guinea pigs in group B had developed a healthy cornea, but the interior structures were not completely accessible because deeper layers of the cornea were affected as depicted in Plate 6, involving the Bowman's membrane and stromal layer which provide resistance to injury and infection but not capable of regeneration when destroyed[8]. Since caustic soda was used to induce the corneal ulcer, saponification of the lipids occurs in the corneal epithelium, binding to the mucoproteins and collagen in the stroma, thereby making the injury more penetrating on the cornea, unlike acids which will precipitate tissue protein, coagulating and forming barriers which would prevent deep penetration. The healing in group A animals was observed to be complete by the third week of ulceration and the healing was sluggish in the absence of exogenous retinol because cellular mitosis was slow; though the animals in this group had only dietary source of vitamin A (retinol) which was not enough to effect quick healing. In group C animals, no reasonable loss or gain in weight was observed as well as in other study groups showing that the alkali injury did not cause any significant weight loss. The functional and structural integrity of the epithelial cells are dependent on vitamin A content of the body and should be incorporated in therapeutic regimen when wound healing is being contemplated particularly after ocular surgery. This is because vitamin A is vital for normal development and differentiation of non-squamous epithelium in which deficiency of vitamin A will result to keratinization. We conclude that oral vitamin A has demonstrated immense help in controlling and accelerating wound healing in corneas that have suffered epithelial damage, and might be of beneficial effect post injury following refractive ocular surgery.

Table 1: Ocular Status of the Guinea pigs after 24 Hours of Alkali Injury

S/N	Parameter	Group A	Group B	Group C
1	Eyelid status	Moderately inflamed	Moderately inflamed	Normal
2	Conjunctival status	Moderate hyperemia	Moderate hyperemia	Normal
3	Lacrimation	Severe	Severe	Absent
4	Epithelial Morphology	Cellular eruption	Cellular eruption	Normal
5	Edema	Present	Present	Absent
6	Light reflex	Absent	Absent	Present
7	Corneal fluorescein staining	Staining present and marked	Staining present and marked	Absent
8	Discharge	White stringy mucous discharge	White stringy mucous discharge	Absent
9	Corneal lustre	Absent	Absent	Present
10	Corneal infiltrates	Present	Present	Absent
11	Interior ocular structures	Not accessible	Not accessible	Accessible

Table 2: Health Status of the Cornea at the Seventh Day of Therapy

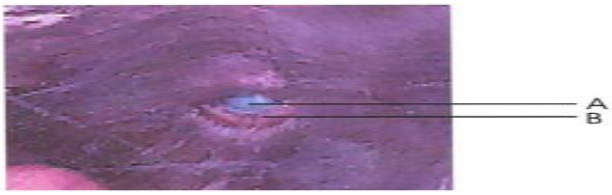
S/N	Parameter	Group A	Group B	Group C
1	Eyelid status	Almost normal	Normal	Normal
2	Conjunctival status	Almost normal	Normal	Normal
3	Lacrimation	Slightly present	Nil	Nil
4	Epithelial Morphology	Marked degeneration	Totally regenerated	Normal
5	Edema	Mild	None	Nil
6	Light reflex	Absent	Present and bright	Present and bright
7	Corneal fluorescein staining	Present but reduced slightly	Absent	Absent
8	Discharge	Present	Absent	Absent
9	Corneal lustre	Absent	Present	Present
10	Corneal infiltrates	Minimal	Absent	Present
11	Interior ocular structures	Not accessible	Not completely accessible	Accessible



KEY
A- Sclera. B- Cornea

PLATE 1. Normal Cornea

Fig 1: Plate 1, showing normal cornea(A. Sclera ,B. Cornea)

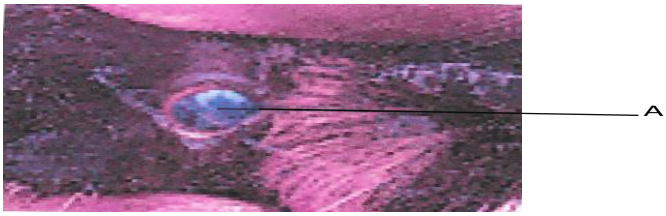


KEY
A - Corneal ulceration. B - Eyelid
PLATE 2. 24 hours after corneal ulcer induction

Fig 2: Plate 2, showing 24 hours after corneal ulcer induction(A. Corneal ulceration,B. eyelid)

Plate 3. 48 hours after corneal ulcer induction in GP B.

Fig 3: Plate 3, showing extent of ulceration 48 hours after induction

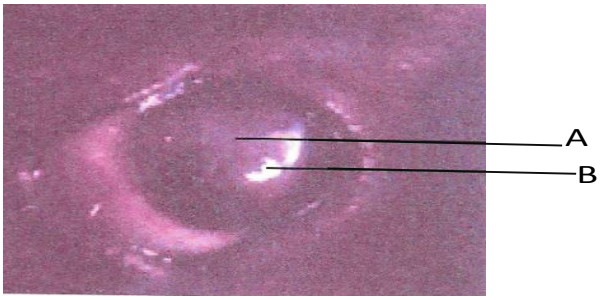


NO FLOURESCEIN STAIN
Key
A- Corneal Ulceration
PLATE 4. Corneal ulceration at the third day of drug administration

Fig 4: Plate 4, showing corneal ulceration at the third day of drug administration(A-Corneal Ulceration)

UNDER FLOURESCEIN STAIN
KEY
A- Corneal Ulceration
PLATE 5. Corneal ulceration at the third day of drug administration

Fig 5: Plate 5, showing corneal ulceration



KEY
A- Nebula B- Corneal Sheen

PLATE 6. Healed corneal ulcer

Fig 6: Plate 6, showing healed corneal ulcer (A-nebula,B-corneal sheen)

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