Potassium for baldness treatment | experiment

The hair follicle is a highly dynamic organ found only in mammals. It is an important characteristic for mammals. (1) Hair plays a key role in providing protection against heat loss. The correctly functioning follicles are crucial to many mammals' survival because loss of fur or faulty colouration can lead to death from the cold or predation. (2) Hair also protects the epidermis from minor abrasions or ultra violet light by forming a barrier such as eyebrows and eyelashes. (3, 4)

Hair loss is not life threatening for humans. However hair plays a significant role within society. (2, 5) A full head of hair connotes feelings of youthfulness and health. Balding men are often regarded by society as older, weaker and less desirable. As a bald man, women relate to this as a statement of illness. After all, we know when we see cancer patients; this is often the terrible results of chemotherapy causing hair loss. (6) Hair has not only been a symbolic indicator of gender, but of social, religious and professional status as well. e. g. Early Christian monks and priests shaved the hair on the crowns of their heads. This clearly visible mark symbolized their vow of chastity, and their lack of concern with vanity and the biblical tale of Samson- a man who has the strength to destroy the Philistines as long as his hair remains long and uncut. As soon as Delilah cuts his hair, he loses all of his strength. (7) In addition human hair provides indications of sexual development through onset of secondary sexual characteristics such as beard and upper pubic hair development. (8-10)

Hair disorders

Androgenetic alopecia is caused by miniaturizing of hair follicles located in the frontal or crown part of scalp and are hereditarily more sensitive to

androgen. In their hair cycles, the androgen shortens the anagen phase of follicles and shifts them to the catagen phase earlier than usual.

Structure of hair follicle

At the base of the hair follicle is the dermal papilla. It is feed by the bloodstream which carries nourishment to produce new hair. The dermal papilla is a very important structure to hair growth because it contains receptors for male hormones and androgens. Androgens regulate hair growth and in scalp hair and may cause the hair follicle to get progressively smaller so the hairs to become finer in individuals who are genetically predisposed to this type of hair loss. Hairs that are outside of the body are thin flexible tubes of dead fully keratinised epithelial cells. These vary in size, colour, length, diameter and cross-sectional shape.

Growth cycle of hair

Research findings have recognized that they are three main phases of the hair growth cycle – a growth phase (anagen phase I-VI), a regression phase (catagen), and resting phase (telogen). (montagna W, the structure and function of skin 1974)

Hair is produced in the growth phase where cell division occurs in the matrix of the hair bulb outside the dermal papilla, then the keratinocytes move up the follicle, differentiating into the different layers of the hair and the surrounding sheaths. In addition, melanocytes in the bulb transfer pigment into the hair-forming keratinocytes giving the hair colour. At the end of the anagen phase the hair follicle enters into a catagen phase. During the catagen phase the cell division and pigmentation stops. Hair follicle shrinks to about 1/6 of the normal anagen length. The lower part is destroyed and

the dermal papilla breaks away to rest below. The resting phase follows the catagen phase. During this time the hair does not grow but stays attached to the follicle while the dermal papilla stays in a resting phase below. At the end of the telogen phase the hair follicle re-enters the anagen phase. The dermal papilla and the base of the follicle join together again and a new hair begins to form. If the old hair have not already been shed the new hair pushes the old one out and the growth cycle starts all over again.

The duration of anagen length determines hair length. (kligman AM: The human hair cycle 1959)

Minoxidil

Minoxidil was introduced in 1970s as a treatment for hypertension. Hypertrichosis was a common side-effect in those taking minoxidil tablets and included the regrowth of hair in male balding. This led to the development of a topical formulation of minoxidil for the treatment of androgenetic alopecia in men and women. Despite much research over 20 years we still have only a limited understanding of how minoxidil stimulates hair growth. Nevertheless, understanding minoxidil’s mechanism of action is important, both from the point of view of developing more effective treatments for hair loss disorders and for the insights it may give into the biology of hair growth. 7 Minoxidil is a member of a group of drugs known as potassium (K+) channel openers.

KATP channel

Potassium channels are usually located in the plasma membrane. They allow the flow of K+ across membranes. They are regulated by intracellular levels of ATP and ADP.

KATP channel is an octameric protein complex which consists an inner ring of four inwardly recitifying K+ channel (Kir6. X) subunits forms the pore through which potassium ions pass, whereas the outer ring comprises of four regulatory sufonylurea receptor (SUR) subunits that can alter channel acticiry in response to intracellular nucleotides or extracellular drugs. Both subunits are very important to form a functional channel. SUR exists in three forms: SUR1, SUR2A, SUR2B and kir6. X in two: kir6. 1 and kir6. 2. 6, 8 Minoxidil does not open SUR1 channels. Recent studies showed that K+ openers act on K+ channels within the hair follicles themselves which can be stimulated to increase hair growth.

Rat whisker hair follicles

Previous work on KATP channel subunits of hair follicle has been performed mainly in red deer or human samples. However due to the extreme winter weather, red deer skin samples were not available. Therefore rat whiskers were used because of the large size of their follicles.

The whisker appears to resemble a guard hair of the pelage very closely except for its size and the large perifollicular blood sinus (Melaragno and Montagna, 1953; Vincent, 1913). An additional advantage accruing from the use of whiskers is that it is easier to collect these from the laboratory.

Vibrissae have the same essential structure as pelage hairs (Text-fig. 1) and also produce hairs in different cycles (Cohen, 1961).

The arrangement of the major vibrissal follicles on the upper lip of hooded rats is shown in Text-fig. 2. This arrangement is constant in rats of both sexes and at any age (Oliver, 1966).

Aims

Minoxidil applied to the surface of scalp is the most common treatment for balding. 4, 5Recently, there is evidence shown that minoxidil and other drugs stimulate hair growth in vitro via potassium channels. 3, 6Potassium channels are the channels on of hair follicles the cell membranes which open or close depending on the cell’s energy levels.

The aims are to confirm whether the presences of potassium channels are in hair follicle. I will able to make vertical cryosections of hair follicles and carry out histochemical staining of the skin using sacpic stain to investigate hair follicle structure. In addition, to learn the immunihistological technique by locating keratin and KATP channel using antibodies

Materials and Methods

Rat whisker and skin samples

White-haired red-eyed Hooded rats were used. These skin samples were obtained from a laboratory which the rats were bred for experimental use and were used for several projects.

These samples were dissected into 1cm2 pieces and stored in O. C. TTM (Sakura Finetek. Zoeterwoude, NL) at a temperature of -20oC until they were required.

Preparation of slides

Glass slides (76 – 26 – 1 mm, Fisherbrand, FB58622, UK) were cleaned and coated in poly-l-lysine (Sigma- Aldrich Co. St Louis, USA) to help sections adhere to the slide. The slides were then placed into a plastic slide carrier, and then rinsed thoroughly in distilled water to remove detergent and

soaked in absolute ethanol for 5 minutes. Afterwards, the slides were allowed to dry before being immersed in 10% (v/v) poly-l-lysine in distilled water for 5 minutes. Once dry, the slides were stored in the original box at room temperature until required.

Preparation of frozen tissues sections

Sections of the skin samples were made by using the Leica CM 1850 Cryostat (Leica Microsystem Nussloch GmbH. Heidelberger, Germany). O. C. TTM was applied to the chuck to form an even disc, and placed in the cryostat set at -27â„ ƒ to freeze solid. The frozen O. C. TTM disc was then placed into the sample holder of the cryostat and sliced until level. The skin sample was dissected and placed onto the O. C. TTM disc orientated so that longitudinal follicle could be sectioned. The sample was placed on its side to allow the blade to cut through dermis and fat simultaneously and therefore cut smoothly. O. C. TTM was applied around the sample and allowed to freeze solid, so that the sample was no longer visible. Sections of 5 µm thickness were cut and transferred to poly-l-lysine coated slides at room temperature. The slides were labeled, replaced into slide carriers and stored at -20 â„ ƒ until needed.

Investigation of hair follicle structure using Sacpic staining

Rat whisker skin was stained with Sacpic staining technique which involves several dyes; so different parts of the skin and hair follicles can be distinguished. Frozen sections were fixed in ice cold acetone for 15 minutes then rinsed by distilled water twice. They were immersed in Celestine blue staining solution for 5 minutes and rinsed in tap water. Afterward, the

sections were placed into Gill’s haematoxylin (VWR International Ltd. Poole, England) for 5 minutes and washed with tap water. The sections then placed into Scott’s tap water for 2 minutes and then washed by tap water and stained in 2% safranin for 5 minutes. The sections were rehydrated for 1 minute in each of the following – tap water, 70% ethanol and 95% ethanol. The slides then treated by absolute picric acid/ethanol for 3 minutes followed by rehydrated in tap water, 70% ethanol and 95% ethanol for 1 minute each. They were stained in picro-indigo carmine for 1 minute and rinsed by tap water, then rehydrated in tap water, 70% ethanol and 95% ethanol for 5 minute each. Finally, the sections were cleared in histoclear : ethanol (1: 1) and absolute histoclear(National Diagnosis. Hull, UK) for 4 minutes each. Coverslips (18-18mm, 22-50mm, Menzel-Glaser) were then mounted using histomount (National Diagnosis. Hull, UK). Sections were examined and photographed using a Nikon DS-Fi1-L2 camera head with a stand-alone control unit with a biological upright microscope.

Localisation of specific protein using Immunohistochemistry

Immunohistochemistry was performed to identify the locations of protein specific bind to the antibody. All incubations were carried out in a prepared histology wet box to prevent evaporation. The incubation periods for primary antibody were either 90 minutes at room temperature 25â„ ƒ or overnight at 4â„ ƒ.

The frozen sections were air dried in room temperature for 50 minutes then fixed in ice cold (4â„ ƒ) acetone for 10 minutes and followed by 2 washes in sterile PBS. The sections were circled with a Vector Immedge Pen to produce

a hydrophobic ring to reduce the volume of antibody applied. Endogenous peroxide activity was blocked by incubated the sections with 3% hydrogen peroxide in methanol for 20 minutes followed by 2 washes of in PBS. Non specific protein binding was blocked by 5% Bovine serum albumin (BSA) in PBS for 30 minutes. Then sections were incubated with the appropriate primary antibody (Santa Cruz Biotechnology, Inc. CA. U. S. A.). The primary antibodies were diluted with 1. 5% BSA in PBS: Cytokeratin 6/75 (D-13) 1: 20 (sc-22480), SUR-1 (C-16) 1: 7. 5 (sc-5789).

Slides were rinsed twice in PBS for 10 minutes while shaking before incubation with monoclonal anti-goat biotin conjugated secondary antibody (B6523, Sigma-Aldrich, St Louis. USA) diluted 1: 20 with 5% BSA in PBS for 30 minutes followed by 2 washes of PBS for 10 minutes. Then sections were incubated with ExtrAvidin®-peroxidase (E8386, Sigma-Aldrich, St Louis. USA) diluted 1: 20 with PBS for 30 minutes followed by 2 washes of PBS. Afterward incubated sections with AEC substrate solution(SK4200, Vector Laboratories Inc. Burlingame, CA) that made up immediately before use (2 drops of Buffer, 2 drops of hydrogen peroxide and 3 drops of AEC in 5ml of distilled water). The colour development was observed under the light microscope that usually took 5-30 minutes. The reaction was stopped by immersing the slides into distilled water. The sections were counterstained with Mayer’s haematoxylin for 5 second then blued in Scott’s tap water. The slides were rinsed in tap water and mounted with warm glycerol then leave to dry overnight. Sections were examined and photographed using a Nikon DS-Fi1-L2 camera head with stand-alone control unit with biological upright microscope.