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A NEW METHOD FOR BURNS TREATMENT - IMPROVING THE WOUND HEALING USING TOPICAL ADMINISTRATION OF ANABOLIC HORMONES AND BIOMATERIALS

O noua metoda de tratament a arsurilor - Imbunatatirea vindecarii plagilor prin aplicarea locala de hormoni anabolizanti si biomateriale

Abstract. Burns represent a pathology with major social impact and with severe repercussions upon the quality of life of the patients. This is mainly due to the complications like scar contractures which lead to functional impairment of the affected areas. The main objective of the study is to speed the wound healing of the burn areas with minimal scarring. Therefore we will investigate the influence of oxandrolone and of the recombinant growth hormone (rGH) on burn wound healing in rabbits. This will be a prospective double blind study on animals with symmetric burns on the anterior limbs and on the trunk. The two hormones will be delivered topically using two carrier biomaterials: the hyaluronic acid and collagen type I hydrogels on four groups.. On the opposite burn wound we will apply only the control represented by the biomaterial. The healing of the burn wounds and scars will be monitored clinically. The degree of contraction, the physical properties of the scars will be quantified using a goniometer and a tensiometer. Through microscopy, immunohistochemistry and biochemistry studies we will investigate the collagen type, the presence of myofibroblasts and of the neovascularization. The statistical analysis of the data and the interpretation of the results will be done side by side in each group and also between groups. In parallel we will aim the specialization of the early stage researchers, the dissemination of the results through articles publication, congress presentation.

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FIRST STAGE OF THE STUDY

The objectives of the stage:

I. Manufacturing burning devices and adapting them to the experimental animals used;
II. Adapting experimental model to the study;
III. Preparing gel from hyaluronic acid and RGH, oxandrolone and hyaluronic acid;
IV. Topical administration of recombinant growth hormone using the hyaluronic acid as carrier

I. Manufacturing burning devices and adapting them to the experimental animals used

• It is necessary to obtain a burned area of constant depth in order to check the effects of local treatment. For this purpose a combustion metallic device was designed, device that can be maintained at constant temperature. Two resin cast were made- one for the posterior limbs and another one for the flank, in order to comply as closely as anatomic topography

• According to the mold, the final device was made of copper, material with high thermal conductivity, and presents: a head (contact surface) and a thermally insulated handle;

II. Adapting experimental model to the study

• We have tested several doses and anesthetic mixtures to obtain sedation and effective analgesia for 40 minutes required maneuvers, while maintaining an efficient ventilation. Experimental animals, New Zealand male rabbits, with an average weight of 2.5 kg were pre-anesthetized using a mixture of Ketamine and Xylazin (1 ml: 0,7 ml)

• For experimental needs is required a clean surface that allows burns, applying the local treatment and fixing the dressing. This is obtained by shaving and further, by waxing. Procedure: After 15min of onset of anesthesia, rabbits were shaved on both flanks and posterior limbs.

• Failed to fully clean the skin of hair, so a thick layer of about 0.3 mm depilatory cream was applied and was left in place to act for 20 minutes.

• Depilatory cream was removed by washing with warm water. The skin was dried.

• As a mild local erythema appeared, for the next 24 hours, no other procedure was performed.

• To perform burns, rabbits were again anesthetized with a mixture of ketamine-xylazin

• The firing device was heated by immersion in boiling water, its temperature was measured with an infrared thermometer.

• Multiple protocols with varying temperature and contact time were used to obtain a 2^{nd} degree burn. We used 2,3,5,7 seconds contact time and temperatures of 42°C, 43°C, 45°C, 47°C.

• Before applying the heated device on the skin, Lidocain spray 1% was sprayed. The device was applied perpendicular to the skin and the contacte time was controlled with a metronome and ranged between 3-6 seconds.

• Pressure on the skin was the device's only weight, no additional pressure was applied manually

• Depth of burn was checked both clinically and by histopathological examination of the biopsies taken from the burned areas

• Optimal depth was obtained at a temperature of 43 degrees Celsius, with a contact time of 3 seconds for the flanks.

III. Preparing gel from hyaluronic acid and rGH, oxandrolone and hyaluronic acid

• The gel formulations were obtained by dispersing oxandrolone (Balkan Pharma), respectively, rGH (Sigma Aldrich) in the matrix of hyaluronic acid gel, prepared in advance.

• The concentrations of active principles were set so that after applying the dose for oxandrolone was of 1.2 mg/m2/day, and for rGH of 0.36 mg/m2/day.

• Preparation was performed in aseptic way.

• The formulas were tested in terms of consistency, viscosity (Brookfield DVIII Ultra spiral adapter) and adhesion in vitro, using the method of separation from the mucosa.

• The formulas have the optimal characteristics of consistency, viscosity, adhesion, microbiological purity to be used in the experimental study.

V. Topical administration of recombinant growth hormone using the hyaluronic acid as carrier

• 20 New Zealand male rabbits with an average weight of 2.5 kg were used. Blood samples were taken from each rabbit and the following parameters were investigated: alanine aminotransferase, aspartate aminotransferase, total and direct bilirubin, total proteinemia, albumin, glucose, IGF-1. After an initial preparation of the animal according to the protocol described at the second paragraph, four 2^{nd} degree burns were inflicted on each animal, under anesthesia with ketamine-Xylazin in the mentioned doses- one on each posterior limb and one on each flank. Each has witnessed its contralateral wound, thereby eliminating the differences between animals and ensuring high relevance of the results. Each rabbit has a control wound and a study wound.

• On the control wounds was applied only the biomaterial (hyaluronic acid) gel, while, on the study wounds was applied the rGH and hyaluronic acid gel. A semi-occlusive dressing was used to cover the treated area, to avoid gel's extravasation and possible contamination from the outside environment. The dressing was changed every two days for 2 weeks.

• The first week, Enroxil (enrofloxacin) 5mg/kg/day was given prophylactic and for 3 days the rabbits got ketoprophen 4mg/kg/day.

• After 2 weeks new blood samples were taken from each rabbit to evaluate the same parameters.

• After 10 weeks wound biopsies were taken for histological evaluation.