

## Effects of melatonin and alpha-lipoic acid on collagen and VEGF expression in palatal wound healing

Onur Kutlu<sup>1</sup>, Gülten Kavak<sup>1</sup>, **Aslı Erdoğan Öner**<sup>2</sup>, Selen Akyol Bahçeci<sup>2</sup>

<sup>1</sup>Department of Oral and Maxillofacial Surgery, İzmir Katip Çelebi University, İzmir, Türkiye,

<sup>2</sup>Department of Histology and Embryology, İzmir Katip Çelebi University, İzmir, Türkiye

### Background incl. aims

Reactive oxygen species (ROS) play a crucial role in the normal physiology of wound healing. The balance between high and low levels of ROS is critical during the healing process. As regulators of oxidative stress, antioxidants are proposed as targets for new therapies to accelerate wound healing. Alpha-lipoic acid and melatonin are antioxidants that have been shown to be involved in wound healing in many different organs. In addition to protective effects against oxidative stress, they also exert anti-inflammatory effects. In this study, we aimed to evaluate the effects of alpha-lipoic acid and melatonin on rat palate wound healing and whether they have a synergistic effect when used together.

### Methods

A total of 64 male and female adult Wistar rats were randomly divided into 4 main groups. All rats were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg) injections before creating a 5 mm diameter wound using a punch biopsy in the central area of the hard palate. Experimental groups were treated with daily intraperitoneal injections of alpha-lipoic acid (60 mg/kg/day) or melatonin (30 mg/kg/day) or both of them until sacrifice. Control group did not get any injection. 2 sub-groups were created for each group, according to sacrifice days; 5 or 10 days after wound creation. Palatal biopsies were fixed with 10% neutral buffered formalin. After decalcification for 8 days in decalcification solution, samples were dehydrated with increasing grades of ethanol, cleared with toluene, and embedded in paraffin. 5 µm thick sections were taken with microtome. Masson trichrome stain was performed to evaluate the amount of collagen fibers in sections. VEGF expression was determined using the immunohistochemistry method. After taking photographs of the sections, Image J software was used to evaluate the results of the stainings. Statistical analyses were performed using IBM SPSS version 24.

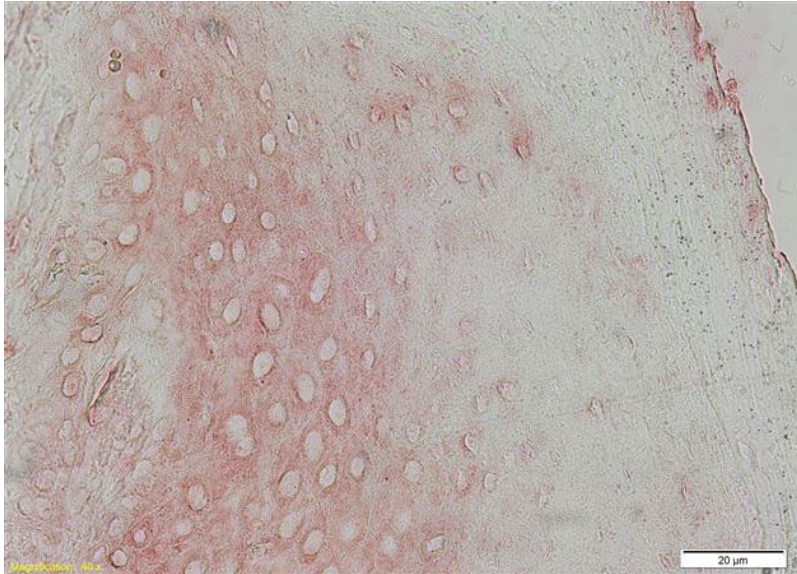
### Results

Collagen fiber density in the granulation tissue in the 5th day samples of the melatonin-treated group was significantly higher than that in the control group ( $p < 0.008$ ). On the 10th day, the collagen fiber density in the granulation tissue of the alpha-lipoic acid + melatonin group was significantly higher than that of the control group ( $p < 0.008$ ). In immunohistochemical staining using VEGF, positive immunoreactivity was detected in the epithelial areas of all group samples. Cytoplasmic and widespread VEGF expression was observed in all epithelial layers (except the stratum corneum-keratin layer), especially more prominently in the stratum granulosum. No specific and significant immunoreactivity was found in the granulation tissue or subepithelial connective tissue in the wound area. VEGF expression on the 5th day was significantly higher in the alpha-lipoic acid + melatonin group compared to the control group ( $p < 0.008$ ).

### Conclusion

Growth factors and collagen production are one of the key factors that determine the wound healing. According to our results, the combinatory application of alpha-lipoic acid and melatonin might be effective in palatal wound healing via collagen synthesis in the early period and via VEGF expression in the late period.

**Graphic:**



**Keywords:**

alpha-lipoic acid, melatonin, wound healing

**Reference:**

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