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The Hema Effects on Autophagy Mechanism in the Human Dental Pulp Stem Cells

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Autophagy is an intricate mechanism that allows the degradation of cellular constituents to improve cell homeostasis, recycling the injured, dysfunctional, or not necessary constituents.

The aim the work was to analyze the biological outcomes of HEMA on proliferation and autophagy in human dental pulp stem cells (hDPSCs). Human DPSCs were treated with several concentrations of HEMA. Autophagic markers such as microtubule-associated protein 1 light chain 3 (LC3-I/II) and ubiquitin-binding protein (p62) were evaluated through immunofluorescence. Beclin1, LC3-I/II, and p62 were determined using Western blotting. On considering the part occupied by the extracellular signal–regulated kinase (ERK) and its phosphorylated form (pERK) arbitrates numerous cellular events, such as autophagy, apoptosis and senescence, the involvement of ERK/pERK signaling was also measured. Then, the up-regulation of pERK and ERK levels, connected with nuclear translocation, shown that ERK pathway signaling could act as a promoter of autophagy in dental pulp stem cells treated with HEMA.

Methacrylate-based restorative materials are used in clinical practice to repair tooth injury, function, and at the same time guarantee the desired aesthetic component. These materials are commercialized in a viscous form and then they are exposed to a polymerization event to be transformed into their solid form. An incomplete polymerization can stimulate the release of monomers into the oral cavity, which move towards dentin micro-channels to enter the vascular system, promoting an inflammatory reaction, causing an alteration to the odontoblastic function and subsequent pulp tissue injury.

HEMA has been described to alter the homeostasis of various cell lines in vitro, inducing DNA damage, apoptosis, and necrosis and autophagy. Autophagy machinery plays a direct or indirect role in health and disease. Mitophagy could be considered a selective autophagy mechanism that induces the degradation of mitochondria in response to damage or stress. Damaged mitochondria produce high levels of reactive oxygen species (ROS) that trigger the mitophagy event. Autophagy has a dual role in tumor cells, acting as a tumor suppressor and, at the same time, enhancing tumor cell advance. Autophagy plays also a fundamental role in the suppression or not of inflammatory events. In the meantime, inflammatory processes can promote or block the autophagy pathway. The primary aim of the present study was to investigate the participation of autophagy in response to 3 and 5 mmol/L of HEMA treatment in hDPSCs as an adaptive machinery to guarantee cell homeostasis. For this reason, the capacity of LC3, p62, and ERK signaling to counteract on the control of autophagy enhanced by HEMA treatment in stem cells from dental pulp was assessed. Our outcomes provided evidence that hDPSCs express stemness markers, and are competent to differentiate into osteoblast and adipoblast lineages.

The decrease of cell proliferation and the change in classical cell morphology can thus be directly related with the HEMA treatment. The current work is focused on the metabolic changes that occur in the first 24 h of treatment. Especially, the expression at the confocal microscopy level and Western blotting analyses of autophagic markers such as LC3 and p62 and the molecular signaling pathway ERK, were assessed. These types of intracellular signal transduction cascades are activated through phosphorylation that promotes nuclear translocation. MEK/ERK signaling mediates autophagy, involving several mechanisms, including amino acid deprivation, aurintricarboxylic acid, B-group soyasaponins, and curcumin. Beclin-1 has been revealed to be regulated by MEK/ERK signaling to induce the autophagy event. In this work, we have provided evidence that after 24 h of 3 and 5 mmol L−1 HEMA treatments, an up-regulation of LC3 in parallel with a p62 down-regulation was identified. Furthermore, an important increase of ERK protein level was established, together with its nuclear translocation in treated samples, indicating that HEMA treatment stimulate an autophagic process through the positive modulation of ERK signaling. Our outcomes confirmed a reduced level of p62 in HEMA treated cells and in co-treated with HEMA and rapamycin samples and at the same time the data obtained underlined an overexpression of Beclin1 in HEMA treated cells; furthermore a significant increase in samples co-treated with HEMA and rapamycin in hDPSCs showed a decrease of p62 and an overexpression of Beclin1 when compared to untreated hDPSCs. ERK, pERK, and LC3-II evidenced an upregulation in 3 and 5 mmol/L HEMA when compared to control cells, while LC3-I displayed an opposite regulation. Rapamycin is a special prophylactic for the mammalian target of rapamycin (mTOR), which binds fk506-binding protein 12 kDa (FKBP12) to form a molecular complex that inhibits mTOR activity, and is considered an autophagy promoter. It was also studied the influence of rapamycin on the expression of classical autophagic markers in treated and untreated hDPSCs. In the current work, Western blot analysis indicated that rapamycin increased Beclin1 levels, but decreased p62 levels.

In response to HEMA injury, dental pulp stem cells activate autophagy as a pro-survival cytoprotective mechanism. Further studies are necessary to consider the strategic and therapeutic applications of this research in tissue repair and regeneration.