Resveratrol in Bone Regeneration

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The natural polyphenol Resveratrol (RSV) claims numerous positive effects on health due to the well documented biological effects demonstrating its potential as a disease-preventing agent and as adjuvant for treatment of a wide variety of chronic diseases. Since several studies, both in vitro and in vivo, have highlighted the protective bone aptitude of RSV both as promoter of osteoblasts' proliferation and antagonist of osteoclasts' differentiation, they could be interesting in view of applications in the field of dentistry and maxillofacial surgery.

Keywords: Resveratrol ; bone-regeneration ; craniofacial tissue ; alveolar bone loss ; bone defect ; resveratrol scaffold

1. Introduction

Resveratrol (trans-3,4′,5-trihydroxystilbene, RSV) is a naturally occurring polyphenol and stilbene derivative, obtained from food sources as such red wine and numerous fruits including grapes, peanuts, nuts, pistachios, cocoa, berries and some Asian medicinal herbs [1][2]. Resveratrol exists as cis- and trans-isomer. The preferred steric form is the trans-RSV that is relatively stable if protected from high pH values and light. Its synthesis in plants can be induced by microbial infections, ultraviolet (UV) radiations and exposure to ozone [3].

This compound has been studied for its several biologic effects and potential as a disease-preventing agent in the prevention and treatment of a wide variety of chronic diseases [4].

Resveratrol is protective against oxidative cardiovascular disorders [5], being in part responsible for the "French paradox", the phenomenon that explains a low incidence of cardiovascular diseases in a high-fat diet with contextually a moderate consumption of red wine [3]. This effect seems related to its antioxidant property [6], since it acts as a scavenger of a number of free radicals already at low doses (10 μM), modulates lipid metabolism, protects low density lipoproteins (LDL) against oxidative and free radical damage and inhibits platelet activation and aggregation [7].

However, this action in vivo may be more attributable to its gene regulator effect than a direct scavenging action. Resveratrol downregulates the expression and activity of the oxidase inhibiting NADPH oxidase-mediated production of reactive oxygen species (ROS), stimulates mitochondria biogenesis reducing mitochondrial superoxide generation and upregulates the tetrahydrobiopterin-synthesizing enzyme guanosine triphosphate (GTP) cyclohydrolase I preventing superoxide production from uncoupled endothelial nitric oxide synthase. In addition, RSV increases the expression of various antioxidant enzymes [8].

Due to its action on gene regulation, RSV also possesses anti-ageing properties. It shows a strong capability of Sirtuin 1 (Sirt1) activation, an enzyme belonging to the families of sirtuins, known also for their antiaging action. Resveratrol is able to modulate the activity of numerous proteins through Sirt1 stimulation, as the peroxisome proliferator-activated receptor coactivator-1α (PGC-1 alpha), the forkhead family of transcription factors (FOXO) and protein kinase B (Akt). It can also modulate the nuclear factor kappa B (NFκβ), which is an important sensor of toxic xenobiotics and oxidants, whose activation is a crucial cellular defence mechanism [9][10]. Moreover, its structural similarity to the synthetic oestrogen diethylstilbestrol lets it play a role as a phytoestrogen, making it able to bind to estrogenic receptors both in an agonist and antagonist manner, regulating the transcription of oestrogen-responsive genes, thus preventing age-related diseases [7]. Resveratrol modulates enzymes, pathways of inflammation mediators and induction of programmed cell death in activated immune cells, expressing also anti-inflammatory properties [3][11].

The cancer chemopreventive and anticancer activity of RSV is also known [1][12]. At 100 μM concentration, RSV seems to block the carcinogenesis principle stages of initiation [7], mainly due to induction of apoptosis via several pathways, such as mitogen-activated protein kinase (MAPK)- and p53-dependent mechanism [13], and to alteration of gene expression, bringing to cancer initiation, promotion and progression [11].

Dental caries and periodontitis are very common diseases worldwide and are the main causes of tooth loss. As a consequence of tooth loss, resorption of the alveolar bone incurred as well as a reduction of the amount of available bone for the insertion of dental implants [14].

In maxillofacial reconstructive surgery, traditional bone regeneration techniques involve autologous, homologous, heterologous, or allogeneic grafts. Autologous bone grafts are considered the best option due to a low risk of immunogenicity or disease transmission but are limited because of inadequacy for repairing larger bone defects, donor-site morbidity, and potential wound-based infections, as well as the prolonged operative times.

The use of stem cells, as MSCs (mesenchymal stem cells), DPSCs (dental pulp stem cells) or adipose-derived stem cells, is one of the most interesting approaches, but it is still limited by their low accessibility, difficult collection, and poor long-term stability. Stem cells also need the combination with scaffolds or biomaterials to improve their efficacy and stability.

Some researchers have focused on investigating biomaterials embedding osteogenic growth factors (e.g., bone morphogenic proteins, vascular endothelial growth factor, fibroblast growth factor, insulin-like growth factors) as a strategy to enhance bone regeneration and accelerate tissue healing. Despite their effectiveness in vitro, comparable in vivo results have been difficult to achieve, and it has been even more difficult to transfer their use into clinical protocols. Moreover, the lability and the easy denaturation under extreme treatments of growth factors must be considered.

In terms of cost and efficacy, small molecules (<1000 Da), synthetically manufactured or isolated from natural sources, easy to manufacture, stable, processable, affordable and able to activate particular signalling pathways that lead to osteoblastic growth and differentiation without risk of host immune reaction could represent a valuable approach to overcoming limits of other strategies [15]. Considering that RSV closely matches the aforementioned characteristics and possesses interesting biochemical activities, the aim of this review was to collect all information about RSV effects on bone regeneration, in view of its future application in dentistry and maxillofacial surgery.

2. Innovative Scaffolds Loaded with Resveratrol

Resveratrol is well known for its poor aqueous solubility and rapid metabolism and excretion as sulfate and monoglucuronide derivatives. The poor bioavailability is a major drawback of the drug. Different approaches have been used to improve solubility, stability and bioavailability of RSV by formulation of drug delivery systems (DDSs) in the form of scaffolds able to release RSV at the site of bone loss to improve and optimize its efficacy.

A scaffold loaded with RSV was prepared grafting RSV to polyacrylic acid (PAA, 1000 Da), PAA-RSV, and then incorporating this macromolecular drug into atelocollagen (Coll) hydrogels (Coll/PAA-RSV) [16]. The scaffold was tested in vitro both on chondrocytes and BMSCs and in vivo on rabbits with osteochondral defects by an implant. In vitro results showed that the scaffold could support the growth and maintain the morphology and phenotype of chondrocytes and BMSCs and it is able to protect them against reactive oxygen species, demonstrating an excellent cytocompatibility. After implantation of Coll/PAA-RSV scaffold for two, four and six weeks on rabbits, the inflammatory-related genes IL-1β, matrix metalloproteinases-13, cyclooxygenase (COX)-2 were downregulated while bone and cartilage related genes SOX-9, aggrecan, Coll II and Coll I were upregulated resulting in an anti-inflammatory functionality. Moreover, after 12 weeks, the osteochondral defects completely disappeared and the neo-cartilage was well integrated with surrounding tissue and subchondral bone. The distribution of Coll II and glycosaminoglycans in the regenerated cartilage was confirmed by immunohistochemical and glycosaminoglycan staining [16].

Resveratrol was grafted to the surface of porous poly-ε-caprolactone (PCL) by a covalent linkage with the carboxylic groups of acrylic acid (AA) to produce a scaffold with osteogenic effect, tested on BMSCs and in the rat calvarial defect model [17]. The ALP activity in stromal cells and the mineralization of the cell–scaffold composites resulted in being increased by the presence of RSV. In vivo osteoinductive effects were evaluated by implanting a scaffold in rat calvarial defects. After eight weeks, the increased bone regenerating capacity of the RSV-PLC scaffold was highlighted by X-ray and histological analyses [17].

A 3-D porous PLC scaffold was formulated with RSV-loaded albumin nanoparticles (RNP) to form a PCL-RNP-RSV composite scaffold with improved osteoconductive, osteoinductive, and osteogenic capacities [18]. Resveratrol was released from PCL-RNP in a sustained-manner for 12 days, until a total release of 64%. In vitro experiments on HBMSCs showed a significant increase in proliferation, ALP increase and mineralization induced by PCL-RNP-RSV compared with the PCL scaffold. PCL-RNP-RSV scaffold was also cytocompatible [18].

A chitosan-poly-ε-caprolactone composite nanofibrous scaffold for wound dressing able to simultaneously deliver ferulic acid and RSV was designed by Poornima and Korrapati [19]. In vitro cytocompatibility and hemocompatibility was evaluated, whereas, in vivo on Wistar rats, the full thickness skin wound healing was studied. The nanofibers were able to maintain a sustained release of actives, showed compatibility with keratinocytes and enhanced healing of skin wounds in vivo [19].

Since RSV could promote osteogenesis and inhibit adipogenesis in mesenchymal cell lines, electrospun drug-eluting fibers loaded with RSV were designed to be used in regenerative dentistry for the post-extraction preservation of the alveolar socket [20]. Uniform defect-free membranes based on fibres of PCL or poly(lactic) acid (PLA) containing RSV were produced and the kinetics of RSV release as well as their osteoinductive capacity on DPSCs were evaluated. An initial burst followed by a slow release was shown by a PCL-RSV membrane, while PLA-RSV presented a much slower and continuous release over time. In vitro experiments on DPSCs highlighted that the RSV concentration range released from the two nanofibers influenced osteoblast and osteoclast differentiation differently. In particular, both materials were able to promote DPSCs differentiation into osteoblast-like phenotypes, increasing gene expression of the osteogenic markers and inducing calcium deposition after 28 days of incubation. However, the RANKL-induced osteoclast differentiation was inhibited by PLA-RSV and only this reduced TRAP activity and cathepsin K gene expression. The PLA-RSV could represent a useful scaffold for dentistry applications able to limit the physiological remodelling process that could affect correct implant placement [20].

The combination of collagen scaffold containing RSV with human adipose stem cells (hASCs) for craniofacial tissue-engineering applications both in vitro and in vivo was evaluated by Wang et al. The collagen/RSV biocomposite scaffold used in hASCs differentiations demonstrated the complete biocompatibility of RSV and its role in the high differentiation rates of stem cells and in calcium deposition. The in vivo results on surgically induced oral mucosal defects indicated that the great effectiveness of scaffolds in promoting epidermal wound healing. Furthermore, on rats with critical-sized calvarial defects, the micro-CT analysis showed that hASCs-cultured collagen/RSV scaffold implanted had better bone mineralization and defect regeneration than hASCs-collagen scaffold without RSV. The overall results suggested that collagen scaffolds loading RSV were more effective than empty ones at enhancing epithelial and osteogenic differentiation of hASCs [21].

3. Resveratrol with Platelet-Rich Plasma and Other Hemocomponents

In the last few years, a new guided bone regeneration procedure was introduced in oral surgery, based on the use of non-transfusional hemocomponents, like platelet-rich plasma (PRP) [22], even if, in literature, there has still been little produced about the application of RSV together with them.

Surely, one of the most popular beneficial effects of RSV is the prevention of atherosclerosis and coronary heart disease. Wang et al. investigated RSV influence on the aggregation of platelets obtained from healthy, normotensive male volunteers and in hypercholesterolemic rabbits, using Born's method to measure the platelet aggregation rate [23]. Both in vitro and in vivo results suggest that RSV can inhibit platelet aggregation and this could be correlated with its cardioprotective effect. In vitro effects were evaluated using platelet-rich plasma (PRP) from healthy subjects. It was observed that the aggregation of platelets was significantly inhibited by 10–1000 µM RSV, in a concentration-dependent manner. In in vivo experiments, 4 mg/kg/day RSV administration showed in hypercholesterolemic rabbits, inhibition of ADP-induced platelet aggregation but had no effect on serum lipid levels [23].

Medication-related osteonecrosis of the jaw (MRONJ) is an adverse drug reaction that consists of progressive bone destruction in the maxillofacial area of patients exposed to the treatment with drugs associated with the risk of ONJ, in the absence of previous radiation treatment [24].

Usually, MRONJ is related to long-term treatment with bisphosphonates, the first-line treatment for metastatic bone cancer and osteoporosis.

The application of autologous platelet concentrates for the prevention and treatment of MRONJ has been recently investigated [25][26]. Bisphosphonates are commonly used in the therapy of osteoclast-mediated bone loss, even though this long-term treatment could be associated with pathological conditions, including osteonecrosis of the jaw, specifically named Bisphosphonates-related osteonecrosis of the jaw (BRONJ) [27].

In vitro effects of concentrated growth factors (CGF) and/or RSV on proliferation and differentiation of human osteoblasts, treated or not with bisphosphonates, were investigated by Borsari et al. [27]. Platelet concentration (also named CGF) was prepared centrifuging blood samples with a special machine that, at the end of the process, formed three fractions: the

platelet poor plasma (PPP) in the upper layer, free red blood cells (RBC) in the lower layer and CGF in the middle layer, used for the study. Resveratrol at 10 μM concentration was used. The results, obtained by both MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay and the evaluation of some osteogenic markers using ELISA (enzyme-linked immunosorbent assay) and immunohistochemical analysis, showed that in vitro osteoblast proliferation and differentiation is promoted by both CGF and RSV, which had a protective role on osteoblasts treated with bisphosphonates, especially zoledronate. This activity is also improved by co-treatment, making these findings promising for the clinical management of BRONJ [27].

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