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Article

Impact of Soft Tissue Pathophysiology in the Development and Maintenance of Bisphosphonate Related Osteonecrosis of the Jaw (BRONJ)

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Abstract: Since the first description of bisphosphonate-related osteonecrosis of the jaw (BRONJ) numerous research groups have focused on possible pathological mechanisms including the suppression of the bone turnover of the jaw, antiangiogenic effects and soft tissue toxicity. In our review we focused on summarizing the role of the soft tissues in the development and progression of BRONJ. The biological behavior of fibroblasts can be significantly influenced by bisphosphonates (BP) such as a concentration dependent reduction of cell viability. High concentrations of BP can induce apoptosis and necrosis of the cells. Comparable effects could be detected for keratinocytes. Compared to non-nitrogen containing bisphosphonates nitrogen-containing BP have worse effects on cell biology by blocking the mevalonate pathway. Next to this the cell architecture and the expression levels of several genes and proteins are significantly disturbed by BP. These inhibitory effects of BP are in accordance with BP related reduced angiogenesis and neovascularization and could underline the hypothesis that inhibition of fibroblasts and keratinocytes results in delayed wound healing and can induce and trigger BRONJ.

Keywords: gingiva; bisphosphonate; soft tissue; fibroblasts; keratinocytes; bisphosphonate associated osteonecrosis of the jaws

1. Background of BRONJ

Bisphosphonates are widely used in different benign and malignant diseases like Paget's disease, osteoporosis, multiple myeloma and bone metastases of breast- or prostate cancer. Beginning in 2003, Marx et al. and other international research groups reported a medication associated osteonecrosis of the jaw and called it bisphosphonate related osteonecrosis of the jaw (BRONJ) (1). Previous studies report that the incidence of BRONJ ranges from 0.94 to 18.6% (2, 3).

Over the last decades, different international research groups tried to analyze the pathophysiology of BRONJ [(4-7)]. Besides mechanism of the hard tissue disturbance, immune system disorder and anti-angiogenic effects, research focused more strongly on the effect of the soft tissue (5, 8, 9).

Besides the inhibitory effect of bisphosphonates (BPs) on osteoclasts and osteoblasts, especially nitrogen-containing BP interact with cell soft tissue cells like fibroblasts and keratinocytes (10, 11). After local accumulation of BPs and especially in combination with other cancer medication like chemotherapeutics und angiogenesis blocker, BPs might exert directly the oral mucosa via tissue toxicity. This could lead to gingiva injury followed by bone exposition, the main clinical sign of BRONJ (12, 13). Disturbed biological activity of the soft tissue could also results in delayed mucosal healing after tooth extraction or dentoalveolar surgeries in patients treated with BPs (14). Therefore

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soft tissue management plays an important role in oral surgery intervention: After resection of osteonecrosis, a watertight coverage by good vascularized local tissue is mandatory (15). In advanced stage of disease jaw can be rebuild by microvascular flaps e.g. the osseocutaneous fibular flap (16).

2. Characteristics of the oral mucosa

In comparison to other parts in the human body, the oral gingiva is unique showing special features. Unlike other epitheliums, the oral gingiva is in direct contact to the underlying bone. Under BP treatment, there is a direct cytotoxic effect by blood support as well as the BPs enriched underlying bone. No soft tissue layer such as fat, fascia, or muscle buffers the negative effect of BPs released from the underlying bone (17).

Under normal conditions the mucosal immune system suppresses the pathogenic organism like bacteria and fungi.

These physiological and anatomical factors are unique to the oral environment and may represent an important factor for course of disease of BRONJ. Furthermore this explains the fact that oral mucosa is strongly inhibited by BPs. Different research groups showed additionally *in vivo* and *in vitro* that BP can directly counteract and inhibit cells of the immune system like neutrophils and lymphocytes (18, 19). The missing link is seen in measurements of BP-concentration in oral soft tissue, which would support the theory.

3. Impact of bisphosphonate on fibroblasts

Collaboration of osteoblasts and osteoclasts are strongly required for normal bone turnover. This physiological link is disturbed in patients treated with BP (20). Under normal conditions, a bunch of molecules including RANK-L, osteoprotegerin (OPG) and interleukin 6 (IL-6) are produced by osteoblasts (21, 22). BP-treatment disturbs the RANK-L-OPG-system and IL-6 expression in osteoblasts by decreasing the production of RANK-L and IL-6 (23). In addition to cells of the immune system, e.g. T-cells, fibroblasts can produce RANK-L and OPG, too. Bacterial infection leads to inflammatory conditions by lipopolysaccharide (LPS). LPS has a direct effect on fibroblast, which increase the production of Il-6 and RANK-L (24). Fibroblast growth factor (FGF) is another important cytokine for bone metabolism. FGF induces BMP and RANK-L expression from osteoblasts (25). Taken together, BPs counteract not only with osteoblasts and osteoclasts. They also influence bone turnover via inhibition of fibroblasts.

4. Impact of bisphosphonates on keratinocytes

For a sufficient oral wound healing, viability of keratinocytes is mandatory. Reduction in cell viability may result in exposed bone, which could serve as ignition spark for BRONJ. Pabst et al could demonstrate that nitrogen-containing BPs have a strong influence on keratinocytes. BPs decrease cell viability, migration ability, and increase apoptosis rate (26). Going in details, keratinocytes interact with osteoblasts and osteoclasts by different cytokines. Via production of epithelial growth factor (EGF), keratinocytes induce differentiation of osteoclasts and RANK-L expression by osteoblasts (27, 28).

Taken together these studies support but can not terminal confirm the theory, that a drug holiday during oral surgical procedures could be beneficial for normal keratinocytes function which support wound healing and tissue regeneration (28, 29). Since during the administration an increased concentration of BP in these tissues is likely.

5. Bisphosphonates influence oral wound healing

Next to the direct cytotoxic effect on fibroblast and keratinocytes, different research groups could also detect a direct inhibition of wound healing and impaired mucosa function. The development of gastric erosion and ulcers is a well described side effect of BPs. Several studies could

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demonstrate that especially nitrogen containing BPs show a negative effect on different gastric cell types. Wallace et al showed in an ex vivo gastric chamber model, that the gastric mucosa is inhibited by nitrogen-containing BPs (30). Landesberg et al showed that bisphosphonate pre-treatment of oral mucosal cells inhibits proliferation and wound healing at clinically relevant doses and that this inhibition is not due to cellular apoptosis (31).

6. Summery

The development and maintenance of BRONJ is a multifactorial event. The adverse impact of BPs results in inhibition of cellular function of the hard tissue as well as inhibitory effects of the mucosal layer. Inhibition of fibroblast and keratinocytes lead to disturbed integrity of the mucosal layer and has a negative influence on bone metabolism via RANK-L-OPG-system. Beside this mucosal immune system is compromised and vulnerable for infection. Mucosal architecture is influenced by BPs and results in mucosal thinning.

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