



found in the roots of licorice plants (*Glycyrrhiza glabra*). GA is the most important active ingredient in the licorice root, and possesses a wide range of pharmacological and biological activities (anti-inflammatory, antiviral, antiallergic and anti-cancer properties). GA exhibits remarkable cytotoxic and anti-tumor properties; in particular this molecule inhibits cell proliferation in a series of cancer cells, including human cervix cancer cells, melanoma cells, hepatocarcinoma cells, human epithelial ovarian cancer cells, gastric cancer cells, human breast cancer, lung cancer cells etc. GA exhibits dual activity in cancer: anti-cancer and cancer chemopreventive activities. A large number of studies have investigated the molecular mechanisms underlying both activities. GA enhances antioxidant enzymes, inhibits oxidative enzymes and GA is an 11 β -HSD2 inhibitor. Moreover, GA exerts its cytotoxic activities via different mechanisms, including either the inhibition of NF- κ B, PKC, Ras and other anti-apoptotic proteins or the activation of Bid, kinase inhibitors, caspases and other pro-apoptotic proteins. View of the important GA action on many tumor lines, the aims of this study were:

- 1) Verify the cytotoxic effects of this molecule on PE/CA PJ15 (a squamous cell carcinoma of oral cavity)
- 2) Verify the cytotoxic effects of this molecule on normal gingival fibroblasts (HGFs).

Methods: Isolation and culture of HGFs: Cells were obtained (with informed consent) from patients subjected to gingivectomy of the molar region. The specimens were plated in tissue culture flasks with complete DMEM, at 37°C, 5% CO₂ atmosphere. The HGFs were used before the fifth passage. Culture of PE/CA PJ15 cell line: Cells were plated in tissue culture flasks with complete ISCOVE, at 37°C, 5% CO₂ atmosphere. Cytotoxic Assay: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to determine the GA concentration value able to provoke cytotoxicity. PE/CA PJ15 and HGFs were seeded into 96-wells culture plates and exposed to different concentrations of GA (range from 10 μ mol/L to 500 μ mol/L). Cells were incubated with 0.5 mg/mL MTT for 4 h at 37 °C. Purple formazan crystals were solubilized by adding 100 μ L of DMSO and the absorbance was measured using a microplate reader at a wavelength of 540 nm. Statistical Analysis: Data were expressed as mean \pm Standard Deviation. Analysis was performed by ANOVA, $p < 0.05$ was considered significant.

Results: Obtained results showed that the cytotoxic effects of GA were present at concentration values higher than 100 μ mol/L in PE/CA PJ15, while no cytotoxic effects were observed on HGFs; in particular, on fibroblasts, an intriguing proliferative effect (at the same concentration values) was

noted, even if not statistically significant.

Conclusion: In conclusion, this compound seems to be promising in oral cancer cells proliferation inhibition (although, its mechanism of action is not completely understood), the effect on healthy gingival fibroblasts will be further evaluated in future studies.

In vivo diagnosis of oral squamous cell carcinoma using optical coherence tomography: three cases reports

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Aim: Optical coherence tomography (OCT) is a new non-invasive biomedical optical technology that provides high resolution of the cross-sectional tissue images applied clinically in ophthalmology for diagnosis of retinal macular diseases.

Several studies have investigated the potential validity of OCT use in the assessment of oral lesions but, to date, there doesn't exist a bank of OCT in vivo data of oral tissues.

We report three cases of oral squamous cell carcinoma (OSCC), analyzing in vivo OCT use and comparing data with microscopic evaluation.

Methods: For these evaluations, we used in vivo VivoSight® OCT (Michelson Diagnosis). We decided to use the dermatological probe because clinical researches independently conducted have demonstrate the effectiveness of this new medical technology. Dermatologic diseases are very similar to the oral cavity diseases and also other ex vivo studies have used the technology of Michelson Diagnosis for the evaluation of oral cavity lesions.

A standardized protocol consisting of I) clinical examination and a classification of the lesions (exophytic, verrucous endophytic), II) an OCT analysis of the lesions; no preparation are necessary to performed OCT imaging. The operator obtained an OCT scan over a 6mm diameter central area where the surgeon then performed incisional biopsy. III) Then, incisional biopsy was performed. The biopsy specimens were processed routinely in 10% formalin and embedded in paraffin. Representative section of lesions were selected by pathologist and photographed under light microscopy.

Results: OCT assessment showed inhomogeneity of epithelial layers with epithelial cleavage, probably

associated with infection, and neoplastic epithelial tissue islands that invades the connective; also in endophytic lesion we can find hypo-reflective keratin layer or no layer due to structural damage from ulceration. All malignant lesions showed clearly the breakdown of the basement membrane.

Conclusion: Oral cancer is the eighth most common cancer worldwide. If detected at an early stage, survival is better than 90% at 5 years. To date, most oral cancers (60%) are diagnosed at advanced stages (III and IV). Also it is noteworthy that many OSCC develop from potentially malignant disorders (PMDs). Then, correct diagnosis and timely treatment of PMDs may help prevent malignant transformation in oral lesions. The current approach to detecting the transformation of leukoplakia/erythroplakia to OSCC is regular surveillance combined with biopsy or surgical excision. However, biopsy techniques – the current gold standard – are invasive and unsuitable for regular screening of high-risk sectors of the population. With the recent developments in optical engineering and biomedical imaging, several studies have investigated the potential validity of OCT non-invasive use in other medical specialization. It could be a new approach that will help improve the diagnosis and the follow up of oral lesions. The validity of OCT in ex vivo oral lesions is confirmed in literature, while in vivo OCT validity should be supported by comparison of data of several PMDs: further researches are needed.

Medical-related osteonecrosis of the jaw or actinomyces-related osteonecrosis of the jaw? A retrospective study and review of the literature

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Aim: The American Association of Oral and Maxillofacial Surgeons (AAOMS) Special Committee recommended in 2014 a changing in the nomenclature from BRONJ to the term Medication-Related Osteonecrosis of the Jaw (MRONJ), because of the growing number of osteonecrosis cases associated with other antiresorptive (denosumab) and antiangiogenic therapies. The histopathological findings include, in most cases, a combination of necrotic bone areas with devitalized trabecular bone and empty lacunae, with evidence of osteoclasts apoptosis without inflammatory infiltrate, and surface colonies of Actinomyces. The aim of the

study is to report on Actinomyces prevalence among the cases of MRONJ, taking into consideration also antiresorptive and antiangiogenic therapies, according to the new classification of the AAOMS 2014. Another aim is to report on the presence of those bacteria in our own MRONJ cases, higher than the prevalence found by other authors, in order to support the hypothesis of an infectious aetiology of these lesions.

Methods: The systematic review was performed using the database Medline, using the keyword "Actinomyces", in combination with "osteonecrosis" and one of the following terms "bisphosphonates", "denosumab", "sunitinib", "antiresorptive therapy", "antiangiogenic therapy". The results included clinical studies, case series, case reports, from 2004 and 2014. No publications have been excluded, except in-vitro studies, animal studies and studies about the linkage between Actinomyces infection and osteoradionecrosis. No studies have been excluded because of the language, on the contrary an appropriate translation has been performed. In many studies about ONJ, the presence of Actinomyces colonies was detected, but the exact percentage was not calculated: those studies have been excluded. The retrospective study was conducted on 36 patients, referred to the Dental Clinic, University Hospital of Padua, between May 2005 and January 2016. Those patient presented clinical and radiological manifestations of MRONJ and referred a history of bisphosphonates, antiresorptive or antiangiogenic therapies. Patient with a history of head-and-neck radiation therapy were excluded. Patient with a non-confirmed diagnosis of ONJ were excluded too. All the patients underwent surgical excision of the lesion and a biopsy was sent for histological examination. Criteria used for the diagnosis of Actinomyces colonies include the presence of filamentous bacteria, aggregated to constitute a mass, with color shades between the center and periphery on the colony, visible with H&E stains (the so called "sun-ray" effect) and with the Gram stain.

Results: A total of 42 articles were found. A total of 33 publications have been taken into consideration for the systematic review. 9 studies have been excluded due to the previously listed exclusion criteria. A total of 600 patients affected by ONJ have been taken into consideration, 438 showed the presence Actinomyces (73%). As far as our patients are concerned, 34 of 36 patients showed the presence of Actinomyces colonies at the histological examination (94.4%).

Conclusions: The reason why many drugs, characterized by different molecular reactions and administered for such different diseases, induce the same condition affecting the bones is still unclear.