SHORT COMMUNICATION

SC11 - MOLECULAR INTERACTION BEETWEEN BISPHOSPHONATES AND HUMAN BONE: A PRELIMINARY STUDY ON OSTEONECROSIS SEQUESTERS OF THE JAW.

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KEYS WORDS
Bone, Mineralization, Bisphosphonates, Raman microscopy, Molecular interactions.

INTRODUCTION
Bisphosphonates (BP), as anti-resorptive therapeutic agents, are used since many years to prevent bone loss and fractures. Their pharmacological efficiency is due to their high affinity for biomineral (1) which permits a selective localization and prolonged storage within the bone (2).
The cellular action into their biochemical processes is well-known: after their delivery to target bone
resorption sites, BP affect osteoclasts. BP seems to be stored in the skeleton for long periods of time, as inactive form (3). However information about molecular mechanisms on BP and bone interaction are lacking. Different hypotheses have recently been proposed (4, 5, 6), but the nature of binding and resulting effects on bone behavior remain unclear. Knowledge on bone modifications, especially those related to bone physico-chemical changes, is needed. These interactions in human bone are crucial for better understanding BP properties and clinical use.
The report of bisphophonate-related osteonecrosis of the jaw (BRONJ), identified as a complication with
long-term BP therapy, may provide a model for BP accumulation in biological conditions and their effects on bone composition and structure. The aim of this study is to examine effects of BP on bone quality and composition by Raman microspectroscopy approaches, which has been successfully used to characterize bone in physiological and pathological conditions (7, 8).

MATERIALS AND METHODS
Specimens: 13 bones sequesters were obtained from male (n=1) and female (n=12) subjects treated for osteoporosis, myeloma or bone metastasis with different BP (Zoledronate (ZO), Alendronate (AL), Pamidronate (PAM) or Clodronate (CLO)). The duration of BP therapy varied from 12 to 60 month. As control, 11 human mandibular samples from male (n=4) and female (n=7) cadaver were collected.
Raman microspectroscopy: A Labram confocal microspectrometer (Horiba Gr, Jobin Yvon, Lille, France) was used to acquire spectra. Raman spectra were excited using helium-neon laser (λ = 632.82 nm) and an objective x100 (NA=0.80). The set of acquisitions was performed in a range of 800–1750 cm–1. Raman spectra were acquired over 50 μm along a radial line by steps of 2 μm from the Haversian canal to the rim of the osteon. For each point, the integration time was 60 s and 10 accumulations. For each bone samples, 3 osteons in bone cortical were studied. Three physico-chemical parameters were extracted from Raman spectra: mineral to organic ratio, carbonate to phosphate ratio and cristallinity

RESULTS
For the control group (n=11), mean values of mineral to organic ratio, carbonate to phosphate ratio and cristallinity were: 7.93 +/- 0.68; 8.74 +/- 0.60 and 0.0554 +/- 0.0019 respectively. Preliminary results suggest an increase of mineral to organic ratio (fig. 1A) for ZO and

AL treatments (10.73 +/- 0.92 (n=5) and 9.99 +/- 0.53 (n=3)). PAM and CLO seems have minor effects on this parameter.

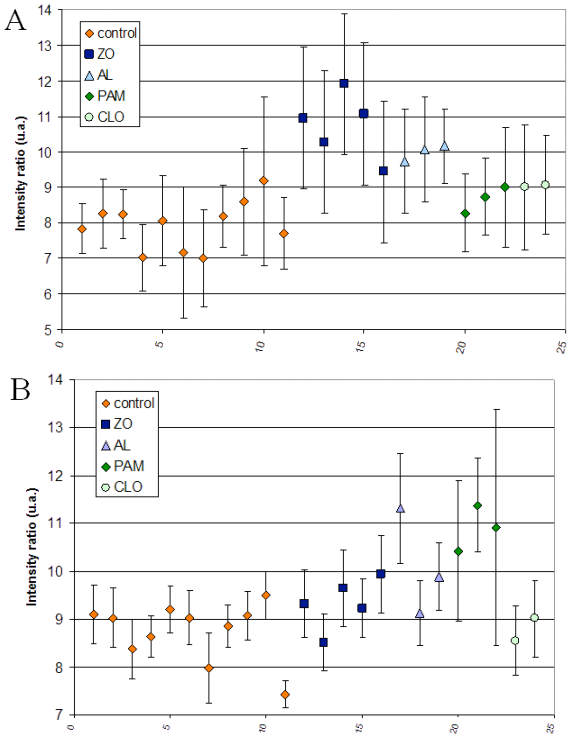

Figure 1 : Comparison of bone "mineral to organic ratio" (A) and "carbonate to phosphate ratio" (B) between control group and BRONJ group order by type of BP (ZO, AL, PAM and CLO). Means and standard deviations are notified for each group.

For carbonate to phosphate ratio (fig.1B), only the PAM treatment tended to increase it (10.90 +/-0.48 (n=3)). Cristallinity was not changed by any of the 4 BP treatments (data not shown).

DISCUSSION

Only few studies have been carried out about the BP molecular effects on bone. Some of them used vibrational spectroscopic techniques and evaluated the physico-chemical parameters. Both studies, Gamjaeger et al. (9) and Durchschlag et al. (10), showed an increasing of mineral to organic ratio compare to placebo in human trabecular bone with 3-years of ZO and 5-years Risedronate treatment, respectively. Boskey et al. (11) reported in the same way, significant increases of mineral content in human cortical bone with 3-years AL treatment. Others studies (12, 13) were carried out on animal models. Results shows similar trend such as greater mineralization. In our analyses, only cortical bone is investigated. Inside BRONJ group, differences are observed. The principal increase of mineral to organic ratio is observed in ZO and AL treatments. So, differences could be linked to the type of BP.

CONCLUSION

This study confirms the interest of spectroscopy Raman to study the influence of BP impregnation on bone. Additional data treatment (Chemometrics analysis) is in progress to increase sample number to further confirm these trends.

ACKNOWLEDGEMENTS

We would like to thank the Institut Français pour la Recherche en Odontologie (IFRO) for its financial support which allowed the realization of this works, and Pr. Francke of Anatomy Laboratory of Medicine University (Lille, France) for control samples collected.

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