SHORT COMMUNICATION

O-19. EFFECTS OF ZOLEDRONATE ON TWO- AND THREE-DIMENSIONAL OSTEOBLAST CULTURES

F. Thibaut[1,2], P. Pellen-Mussi[2], F. Pérez[2], D. Chauvel-Lebret [2,3].

[1]Pôle d'Odontologie et de Chirurgie Buccale, UF de Chirurgie et Pathologie Buccales, Centre Hospitalier Universitaire, Rennes, France [2]Laboratoire de Biomatériaux en Site Osseux - UMR CNRS 6226 - Sciences Chimiques de Rennes, Faculté d'Odontologie, Université de Rennes 1, Rennes, France [3]Pôle d'Odontologie et de Chirurgie Buccale, Consultation de Dysfonctionnements de l'appareil manducateur, Centre Hospitalier Universitaire, Rennes, France flora.thibaut@univ-rennes1.fr

Key words
Zoledronate, Osteoblasts, Spheroids, in vitro cytotoxicity

Introduction
Bisphosphonates (BPs) are drugs widely used to treat bone diseases in which bone resorption is in excess. Studies of action of BPs on bone have been mainly limited to their effects on osteoclast cells. However, the mechanism of bisphosphonates action on osteoblasts is not fully understood although various hypothesis have been proposed. In this report, the direct actions of Zoledronate, an amino-BPs, on human fetal osteoblasts (hFOB) were examined in two-dimensional (2D) and three-dimensional (3D) culture models. We focused on in vitro viability and cytotoxicity, spheroids morphology, genes expression and histological stain.

Materials and Methods
Cell culture :
The hFOB 1.19 (ATCC ®) is a conditionally immortalized human cell line stably transfected with a gene encoding a temperature-sensitive mutant (tsA58) of SV40 large T antigen. The cells grown at a permissive temperature of 33.5°C, whereas little cell division occurs at a restrictive temperature of 39.5°C. Cells were allowed to proliferate in basic culture medium or medium containing Zoledronate at various concentrations (10^{-5}M to 10^{-7}M), in 2D and 3D culture models. Experiments were performed at day 3 (D3) and day 10 (D10).

Viability/Cytotoxicity :
The methyl-thiazolyl-tetrazolium (MTT) assay and acid phosphatase (APH) assay were applied respectively in 2D and 3D cultures to determine cell viability in various culture conditions.
Spheroids morphology :
- Volume growth kinetics: To analyse spheroid diameter, phase-contrast images were collected with a camera during 21 days in various culture conditions.
- Scanning electron microscopy (SEM): Spheroids were fixed in 2.5% glutaraldehyde, dehydrated and sputtered with gold palladium to be examined using SEM.
RT-PCR analysis :
Total RNA was extracted from samples in 2D and 3D cultures. Reverse transcription from 1 μg of total RNA was performed and the amplification by PCR used specific primers for the selected genes : Runx 2, type 1 collagen, osteonectin, biglycane, and actin.
Histological stain:
Spheroid structure was analysed using Masson Trichrome staining (detection of collagen, cytoplasm and nuclei). Mineralization was detected using Von Kossa staining (detection of calcium deposition).

Results
High concentration of Zoledronate (10^{-5}M) reduces cell proliferation in 2D and 3D cultures, disturbs spheroid growth and morphology.
10^{-6} and 10^{-7}M Zoledronate respectively reduced or did not change cell proliferation in 2D

culture at D10; however, these concentrations promoted cell proliferation and did not disrupt the growth kinetics in 3D model.
Gene expression analysis shows that 3D cultures promoted osteoblastic marker genes expression. RT-PCR and mineralization study did not show real changes with Zoledronate at D3 and D10.

Discussion
This study is the first work to consider the effects of Zoledronate on osteoblasts in 3D cultures.
Spheroid model more accurately mimics the tissue environment in vivo than 2D cultures, better reflects the physiological response pattern and promotes osteogenic differentiation. This work shows the interest of hFOB spheroid which could be useful to study mechanism of action of BPs on osteoblasts and for assessment of innovative bioactive materials in regenerative medicine.
Results of this study show an anti-proliferative or cytotoxic effect of 10-5M Zoledronate; lower concentrations can enhance osteoblast proliferation in 3D model. These data are in accordance with previous studies showing the influence of Zoledronate on osteoblast proliferation in a dose-dependent manner. Regarding mineralization analysis, this work did not show real differences between culture conditions, probably related to too short evaluation times, while other authors suggest an increased mineralization with 10-5M Zoledronate (Reinholz et al, 2002). Future studies will highlight effects of BPs on osteoblastic marker genes expression and mineralization using increased evaluation times.

Conclusion
This work reveals a differential effect between 2D and 3D cultures. Zoledronate has an effect on the cell proliferation-differentiation balance which provides opportunities for application in bone tissue engineering, regenerative medicine and implantology. The hFOB 3D-spheroid culture model is an interesting tool in regeneration medicine which may be a strong contribution to the development of 3D systems for the evaluation of bone substitutes.

Acknowledgements
The authors acknowledge Dr Alain Fautrel and Mrs Pascale Bellaud (Plateforme d'histopathologie H2P2, Université de Rennes 1, Rennes, France) and Mr Joseph Le Lannic (Centre de microscopie à balayage et microanalyse CMEBA, Université de Rennes 1, Rennes, France) whose help and cooperation aided in the completion of this study.

References

1. Andersson T et al. (2010) J Mater Sci Mater Med, 21 (11), 3029-37.
2. Borromeo GL et al. (2011) Aust Dent J, 56 (1), 2-9.
3. Corrado A et al. (2010) Clin Exp Rheumatol, 28 (6), 873-9.
4. Ebert R et al. (2009) Bone, 44 (5), 858-64.
5. Friedrich J et al. (2009) Nat Protoc, 4 (3), 309-24.
6. Frith JE et al. (2010) Tissue Eng Part C Methods, 16 (4), 735-49.
7. Giner M et al. (2011) Eur J Pharmacol, 15 (2-3), 682-7.
8. Koch FP et al. (2011) Head Face Med, 7 (1), 4.
9. Morelli S et al. (2011) Arch Biochem Biophys. 15 (2), 248-53.
10. Reinholz GG et al. (2002) Breast Cancer Res Treat, 71 (3), 257-68.
11. Sharma DA et al. (2011) J Periodontol, 24.
12. Wang CZ et al (2010) Biomaterials, 31 (33), 8674-83.
13. Zhou Q et al. (2011) Biochem Biophys Res Commun. 7 (1), 127-32.