Proceedings of the XXIX GIRI Meeting; 2015 June 3 – 5; Verona (Italy).
International Journal of High Dilution Research 2015; 14(2): 35-39.
Available online at www.highdilution.org

Conference Presentation

Effect of Zincum metallicum and LPS treatments on femoral bone density in pregnant mice

Silvio Leite[1], Maristela Dutra-Correa[2], Claudio Costa[3], Vanessa Gallego Arias Pecorari[2], Emiko Saito Arita[3], Maria Martha Bernardi[2], Carla Holandino[4], Leoni Villano Bonamin[2]

[1]IF Sudeste MG, Brazil
[2]Universidade Paulista (UNIP)
[3]Faculdade de Odontologia, Universidade de São Paulo (USP)
[4]Universidade Federal do Rio de Janeiro (UFRJ)
Email: silvioleite@gmail.com

Abstract

Background: The simulated bacterial infection is a classical experimental model, by the injection of purified gram negative bacteria wall lipopolysaccharide (LPS) which produces effects similar to real infection, but without presence of the microorganism. The LPS is a stress factor as during the pregnancy and delivery, and pilot experiments have shown that ultra-high dilutions (UHD) may have a beneficial role in reducing the LPS stress. *Zincum metallicum* (Zm) has a potential to influence several physiological aspects of a living organism. It was chosen by a team of researchers from GIRI, to explore the effect of Zm on various experimental model (Zincum project).

The optical density is a radiographic method for the measurement of the amount of hard bone tissue, and is an accessible, simple and non-invasive method. It is hypothesized that UHD Zm may influence the Zinc metabolism, therefore, bone tissues were collected to evaluate the zinc concentration.

Methods: This project was previously approved by the animal ethical committee vide protocol#156/13 by CEUA-UNIP. 28 parental female generation (Pf) BALB/c mice from Universidade de São Paulo (Brazil) were divided in four blinded groups (n=7 for each). The mice were treated by the vehicle *Lactosis* 6cH (potentized lactose) as control; *Zm* 6cH, 30cH and 200cH separately through drinking water *ad libitum* during 31 days: 21 from the mate to the delivery and more 10 days of lactation. The potencies were chosen to cover a classical range of common use. The intraperitoneal LPS injection was made in Pf between 9th and 10th day of gestation day (n=5 to 6 per group). Some Pf gave birth and all its F1 sons were separated by sex, grown up to two months till adulthood, and exposed to LPS a day prior to right femur collection for analysis.

All the 71 collected samples were conserved cold and were labelled blind. They were exposed using a portable X-ray machine (Nomad® Aribex, USA). Images were analysed by an indirect digital scanner (Soredex Digora® Optime) with photostimulable phosphor plates (PSP) (**Figure-Ia and**

Cite as: Leite S, Dutra-Correa M, Costa C, Pecorari VGA, Arita ES, Bernardi MM, Holandino C, Bonamin LV. Zincum metallicum and LPS treatments of pregnant mice changing the femoral bone density. Proceedings of the XXIX GIRI Meeting; 2015 June 3 – 5; Verona (Italy). *Int J High Dilution Res.* 2015; 14(2): 35-39

Ib). The optical density was determined by numbers in an 8bits grayscale (0-255) and was measured by the software Digora for Windows® (Soredex, Finland) at 13 standardized points in four femur areas by two independent observers (**Figure-Ic**).

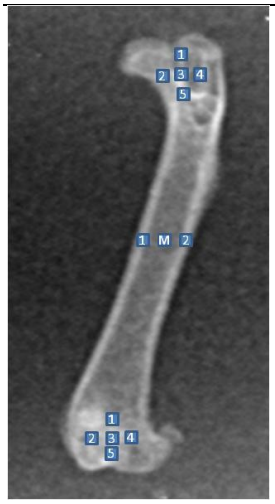
The proximal femur area for some animals and for Pf was discarded because of many missing area due to collecting artefact. The F1 data was analysed by a four-way ANOVA (IBM® SPSS® Statistics) to identify differences and interaction amongst mother's Zm treatment; mother's LPS treatment; sex; and four bone regions. For the Pf the data was analysed by a three-way ANOVA as above for Zm treatment; LPS treatment and delivery; and 3 bone regions. p-value < 0.05 was considered significant.

Figure-Ia. Portable X-ray machine.

Figure-Ib. Scanner, PSP and the data acquisition process.

OPEN ACCESS

Cite as: Leite S, Dutra-Correa M, Costa C, Pecorari VGA, Arita ES, Bernardi MM, Holandino C, Bonamin LV. Zincum metallicum and LPS treatments of pregnant mice changing the femoral bone density. Proceedings of the XXIX GIRI Meeting; 2015 June 3 – 5; Verona (Italy). *Int J High Dilution Res.* 2015; 14(2): 35-39



Proximal (1 to 5), Central cortical (1 and 2), Central medullar (M), Distal (1 to 5)

Figure-Ic. Mice femur acquisition areas: proximal (top); central medullar; central cortical; distal (bottom).

Results

The statistical factors were:

F1 (four-way ANOVA)

- mother's Zm treatment (4 variables: no-Zm; Zm200cH; Zm30cH; Zm5cH)
- mother's LPS treatment (2 variables: no-LPS; LPS)sex (2 variables: male; female)
- four bone regions (4 variables: Proximal; distal; central cortical; central medullar)

Pf (three-way ANOVA)

- Zm treatment (4 variables: no-Zm; Zm200cH; Zm30cH; Zm5cH)
- LPS treatment and delivery (4 variables: no-LPS and no-Delivery; no-LPS and Delivery; LPS and no-Delivery; LPS and Delivery)
- 3 bone regions (3 variables: distal; central cortical; central medullar)

OBS. the LPS/Delivery factors are seen like this by the graphics: LPS-D-, LPS-D+, LPS+D-, LPS+D+)

The selected factors are presented with the "*" to refer to that sub-groups.

The F1 four-way ANOVA identified that there was a significant difference for the factors region and sex (p-value < 0.05, p=0.000 and p=0.040 respectively). The F1m (mean=148.528 SD=2.872 n=8) had lower values in relation to F1f (mean= 162.073 SD=2.271 n=15), independently of the region, Zm and LPS mother's treatments. The region had the proximal (mean=171.40 SD 14.33 n=23) and distal (mean=177.58 SD 12.57 n=23) areas significantly higher than the central medullar

Cite as: Leite S, Dutra-Correa M, Costa C, Pecorari VGA, Arita ES, Bernardi MM, Holandino C, Bonamin LV. Zincum metallicum and LPS treatments of pregnant mice changing the femoral bone density. Proceedings of the XXIX GIRI Meeting; 2015 June 3 – 5; Verona (Italy). *Int J High Dilution Res.* 2015; 14(2): 35-39

(mean=144.47 SD 13.36 n=23) and central cortical (mean=130.20 SD 11.32 n=23). There was no interaction between the F1 studied factors.

The parental female tree-way ANOVA demonstrated that there were significative differences for the region (p=0,000), double interaction between region*Zm treatment (p=0,001), and triple interaction for region*Zm treatment*LPS and Delivery (p=0,025). The Zm treatments had n=6, 6, 6 and 7 for the Control, Zm200cH, Zm30cH, Zm6cH respectively; the LPS and Delivery n=3, 4, 12 and 6 for the LPS-D-, LPS-D+, LPS+D-, LPS+D+ respectively. The central-cortical*Zm6cH*LPS-D- (136) and the central-cortical*Zm30cH*LPS-D- (147) had lower density value in relation to the central-cortical*control*LPS-D- (169); the Central-cortical*Zm6cH*LPS+D+ had higher density value (193) in relation to the Central-cortical*Control*LPS+D+ (156), and also in relation of the Central-cortical*Control*LPS-D- (169). The distal*Zm6cH*LPS+D+ had the higher density value (220) in relation to the distal*control*LPS-D- (192), and also in relation of the Central-cortical*Control*LPS-D- (169). The central-medullar*Zm6cH*LPS-D- (118) had lower values in relation to the central-medullar*control*LPS-D- (148); the central-medullar*Zm6cH*LPS+D+ (169) had the highest values in relation to the central-medullar*Control*LPS+D+ (148), The Central-medullar*Zm6cH*LPS-D- (118) had lower values in relation to the Central-medullar*control*LPS-D- (148). The Central-medullar*Zm6cH*LPS+D+ (169) had the highest values in relation to the central-medullar*Zm6cH*LPS+D+ (148) and to the Central-medullar*Control*LPS+D+ (114). The Central-medullar*Control*LPS+D+ (114) is lower than the Central-medullar*Control*LPS-D- (148).

Discussion: The F1 generation presented density changes by sex only, and by region only, but neither by any treatments nor delivery. The sex difference at the F1 BALB/C generation can be explained due to the hormonal effect, but the literature has divergent results for C57BL/6 mice.[1, 2] The BALB/C mice were separated by sex before the puberty, had food *ad libitum*, no gonadectomy, no treatment were applied to the F1 and no perinatal effect at F1 was identified at that time. The femurs of male mice, overall, had lower density than females grown in same condition. The region difference was expected because of the morphology and the projected image from the x-ray over the photostimulable phosphor plates, showing different mineral deposit and development of the bone tissue of each region for the applied methodology.

The Zm 6cH Pf group showed consistent opposite density in all femur regions comparing the LPS+D+ to the LPS-D-, and lower than the control in two regions among three in the control LPS-D- treatment. When neither LPS nor delivery interference is present, there is reduction of bone density, but when there were both LPS simulated infection and delivery simultaneously, the bone density increases. The first phenomenon may be compatible with the traditional knowledge of homeopathy called proving, since all Pf received exclusively drinking water with Zm 6cH. The second phenomenon is compatible as therapeutic effect of Zm 6cH when the bones minerals were more requested for gestation process and influence of inflammation response to LPS. The traditional knowledge clearly recommends a low potency like 6cH to a body occurrence like inflammation with pregnancy model in contrast with the higher ones (30cH to 200cH or more), that

Cite as: Leite S, Dutra-Correa M, Costa C, Pecorari VGA, Arita ES, Bernardi MM, Holandino C, Bonamin LV. Zincum metallicum and LPS treatments of pregnant mice changing the femoral bone density. Proceedings of the XXIX GIRI Meeting; 2015 June 3 – 5; Verona (Italy). *Int J High Dilution Res.* 2015; 14(2): 35-39

are in general recommended to mental, behavioral and psychological issues. The low sample size may impact the precision of the fine effect range, but could not avoid its detection by the three or four-way ANOVA. The samples were blinded and the treatments groups were revealed only after the statistical analysis.

Conclusion: The murine BALB/c USP model has a sex dimorphism with the male femoral right bone density lower than the female. The *Zincum metallicum* 6cH treatment significantly changed the femoral bone optical density in female mice according to the LPS and delivery. Further research is warranted to explore the role of Zm on bone density.

References:

1. Zanotti S, Kalajzic I, Aguila HL, Canalis E. Sex and genetic factors determine osteoblastic differentiation potential of murine bone marrow stromal cells. *PLoS One*. 2014;9(1):1–13.
2. Glatt V, Canalis E, Stadmeyer L, Bouxsein ML. Age-related changes in trabecular architecture differ in female and male C57BL/6J mice. *J Bone Miner Res*. 2007;22(8):1197–207.

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) PROSUP#1187970 for doctorate allowance and PDSE#6225/14-4 PhD sandwich fellowship; Universidade Paulista (UNIP) for facilities support; Federal Institute of Education, Science and Technology from Southeast of Minas Gerais (IF Sudeste MG), Rio Pomba *campus* for general support; Federal University of Rio de Janeiro (UFRJ) for drug supply; Faculty of Odontology, University of São Paulo, for technical support. Professor Paolo Bellavite (UNIVR) for strategy of data analysis.

Keywords: perinatal; BALB/C; mice; Zincum metallicum; LPS; bone; femur; density; dimorphism

OPEN ACCESS

Cite as: Leite S, Dutra-Correa M, Costa C, Pecorari VGA, Arita ES, Bernardi MM, Holandino C, Bonamin LV. Zincum metallicum and LPS treatments of pregnant mice changing the femoral bone density. Proceedings of the XXIX GIRI Meeting; 2015 June 3 – 5; Verona (Italy). *Int J High Dilution Res.* 2015; 14(2): 35-39