Determination of Fluor Citotoxicity in Combination with Colecalciferole

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The objective of this study is to determine the mutagenic potential of fluoride. A group of 70 newborns was divided into two: group A (35 subjects) received solely 1,000 I.U. colecalciferol (vitamin D3) per day for 6 months, considered the control group whereas group B (35 subjects) were given a combination of 1,000 I.U. colecalciferol and 0.25 mg fluoride - 1 tablet of Fluor Vigantoletten 1000 (MERCK KGAA - GERMANY) daily.

At the end of the time of observation there was a significant difference between the plasma concentrations in the two groups. Fluoride is obviously available to infants when administered in combination with colecalciferol. We studied comparatively the cytotoxic potential to fluoride. After 1, 3, 6 month of fluoride administration we collected from all 70 children each a quantity of 500 microliter blood for determining the values of TNF-alpha (Tumor Necrosis Alpha-Factor) using the method ELISA (Enzyme linked immunosorbent assay). After determining there are no signs of cytotoxicity, the obtained values for TNF-alpha were lower than those specific to inflammatory reaction.

Keywords: fluoride, colecalciferol, TNF alpha, cytotoxicity

The work method is: the standard of cytokine is reconstituted with thinner of calibrator RD 6-21 and thus the stock solution is obtained; the stock solution is left at least 15 min, during which it has to be shaken gently; polypropylene tubes are used for the preparation of the standards; pipette 500μL of calibrator thinner RD 6-21 in each tube; in the first tube pipette 500 μL of stock solution; in each case the concentration of the obtained mixture is calculated; mix well and transfer 500 μL of the next tube, calculating again the concentration of the reagent in the tube; similarly then prepare the standards for different concentrations (500 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.6 pg/mL); the stock solution (undiluted standard in series) is used as a high standard (1000 pg/mL); the calibrator thinner RD 6-21 serves as the standard 0 (0 pg/ml). Prior to use, all the kit reagents are brought to room temperature, as well as the samples (culture supernatant or serum).

The strips which are not used are removed from the backing, by putting them into the protective envelope which then is sealed. Pipette 100 μL thinner RD 1-51 in each well of the plate, which is a conserved basic protein concentrate. Pipette 100 μL of each standard in the following order: 0 pg/mL; 15.6 pg/mL; 31.2 pg/mL; 62.5 pg/mL; 125 pg/mL; 250 pg/mL; 500 pg/mL, 1000 pg/mL on the strip 1. Starting with strip 2, pipette the well with 100 μL of thawed samples (culture supernatant or serum) within 15 min. Cover the plate with adhesive film and incubate 2 h at room temperature. At the end of incubation, each well is aspirated and automatically washed 4 times with 400 μL prepared washing solution. At the end of the washing operation to remove any traces of washing solution, the plate is placed on an absorbent paper and slightly tapped to remove debris from the wells without contaminating the samples. Add 200 μL of cytokine conjugate in each well (mixture of polyclonal anti-cytokine antibodies, conjugated with horseradish peroxidase, with preservative). Cover the plate with adhesive film and incubate 2 h at room temperature. At the end of the
incubation time, remove the adhesive film and wash the plate 4 times to remove the unbound conjugate, following the procedure described above. Add 200 μL of substrate solution to each well, cover the plate with a new adhesive foil plate and incubate for 30 min at room temperature in the dark. After the incubation time expires, add 50 μL of stop solution to each well. At the stopping of the reaction, the color in the wells changes from blue to yellow. If the color of the wells is green or does not modify homogeneously, shake the plate slightly for a complete homogenization. Then determine the optical density of the samples with an automatic reader at a wavelength of 450 nm, with a reference filter of 540 nm or 570 nm (for correction) within 30 min from the stopping reaction. Reading the samples at only 450 nm without correction can affect the accuracy (higher or lower accuracy).

Results and discussions

After the TNF-α measurements, we obtained the following values:

At 1 month: We identified three value ranges: 15 children have the serum value of cytokine between 6.30 to 9.10; 10 children have the TNF-α value of 9.11 to 11.70; 10 children have this index values from 11.71 to 17.90 pg/mL (fig.1).

Group A: all the children have the serum value of cytokine between 2.3 to 4.8 pg/mL.

At 3 months: 10 children have the serum value of cytokine between 6.30 to 9.10; 10 children have the TNF-α value of 9.11 to 11.70; 15 children have this index values from 11.71 to 17.90 pg/mL (fig.2).

Conclusion

The obtained values for group B compared to the control group A were minimally elevated but not enough to reach those values of inflammatory reactions. There are few subjects with slightly elevated values but considering that the subjects are children in the first months of life, the inflammatory reactions may have multiple causes including early tooth eruption and adaptation mechanisms of the baby to the new environment.

References

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Fig.1 Group B versus group A at 1 month with Fluor Vigantoletten1000 (MERCK KGAA - GERMANY)

Fig.2 Group B versus group A at 3 month with Fluor Vigantoletten1000 (MERCK KGAA - GERMANY)

Fig.3 Group B versus group A at 6 month with Fluor Vigantoletten1000 (MERCK KGAA - GERMANY)