Inhibition of osteogenic differentiation of mesenchymal stem cells of the offspring of rats treated with caffeine during pregnancy and lactation

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Abstract

Caffeine is an alkaloid widely consumed bacause it is present in medications, coffees, teas and chocolates. This compound goes through the placenta and is found in the milk that feeds the offspring, which can cause teratogenic alterations and reduce the formation, growth and bone mass. Considering thath mesenchymal stem cells (MSCs) are responsible for originating the entire skeleton, we hypothesize that these cells are the target of caffeine. The objective of this study was to evaluate the osteogenic differentiation of MSCs from offspring of rats treated with caffeine during pregnancy and lactation. 24 adults Wistar rats were divided randomly and equally into four groups: one group without caffeine (control) and three groups with caffeine in following doses: 25, 50 and 100mg/kg. Caffeine was diluted in distilled water and administered to mothers by an oro-gastric tube throughout pregnancy and lactation. The control group received distilled water as placebo. At weaning, three 21 days old puppies of each dam and group were euthanized for extraction of bone marrow cells. On the third passage and before osteogenic differentiation, the phenotypic characterization of cells by flow cytometry using the following antibodies: anti--CD11, anti-CD90, anti-CD34, anti-CD73, anti-RT1A and antiCD54. Then, the MSC from all groups were cultured in osteogenic differentiation. In 7, 14, and 21 days of osteogenic differentiation, MTT tests and the activity of alkaline phosphatase by BCIP/NBT were performed. In 21 days the quantification of number of mineralization nodules stained by Von Kossa and quantitative assessment of the expression of gene transcripts for osteocalcin, osteopontin, sialoprotein, type I collagen, alkaline phosphatase, and Runx-2 by RT-real time PCR were performed. Data were subjected to analysis of variance with comparison of means by t test after logarithmic transformation of the data. Differences were considered to be significant if $p \leq 0.05$. The extracted bone marrow cells of all groups showed phenotypic features consistent with MSC. The doses of 50 and 100mg/kg of caffeine significantly reduced the activity of alkaline phosphatase in all periods and the expression of collagen I in 21 days. The expression of gene transcripts for alkaline phosphatase, RUNX-2 and bone sialoprotein and synthesis mineralization nodules decreased significantly in all groups treated with caffeine. The expression of osteocalcin decreased significantly

only in the group treated with 50mg/kg of caffeine. It is concluded that caffeine passing from mother to offspring during pregnancy and lactation inhibits osteogenic differentiation of mesenchymal stem cells. It is postulated that this reduction in the osteogenic potential of mesenchymal stem cells may be involved in the genesis of bone changes observed in the offspring of mothers who received caffeine.

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