MELATONIN MODULATION OF RADIATION AND CHEMOTHERAPEUTICS-INDUCED CHANGES ON DIFFERENTIATION OF BREAST FIBROBLAST

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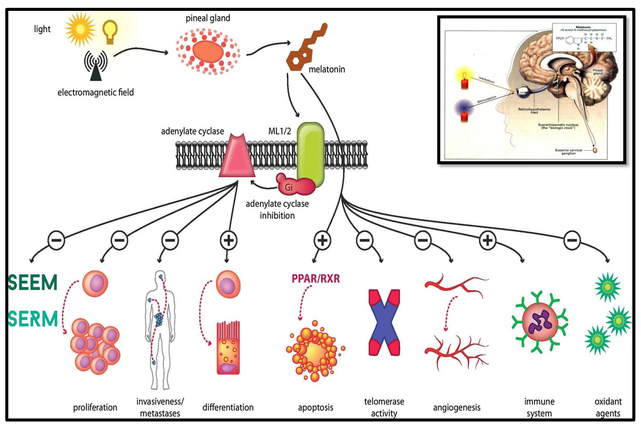
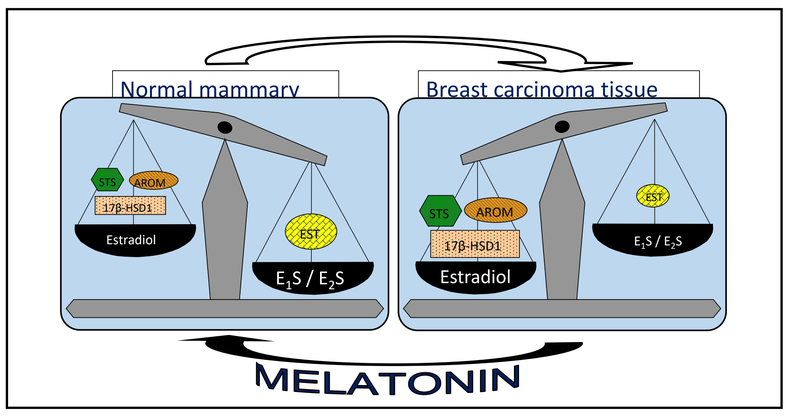
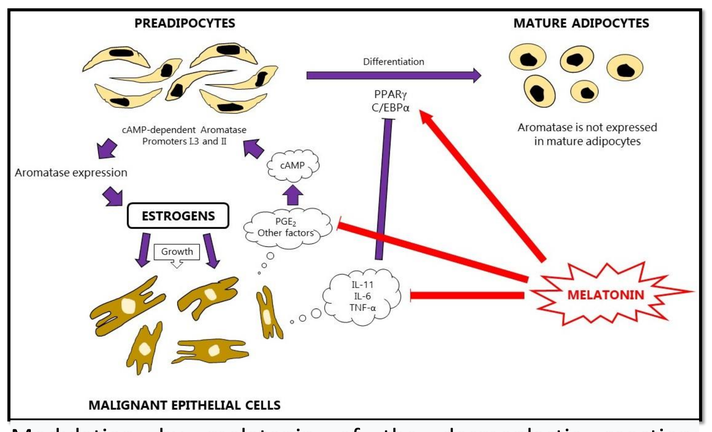
INTRODUCTION



Melatonin is the main hormone secreted by the pineal gland. Melatonin production is regulated by photoperiod insofar as its synthesis and secretion are repressed by light but induced at night in response to darkness.



Estradiol, the main biologically active sex hormone present in breast tissue, plays a very important role in the genesis and progression of the tumor in women. For this reason, it is also possible to consider estradiol as a "breast carcinogen".



Modulation by melatonin of the desmoplastic reaction: stimulates the differentiation of fibroblasts to adipocytes, adipogenesis, decreases the aromatase activity of adipocytes, inhibiting the expression of antiadipogenic cytokines.

OBJECTIVE

The main objective of this work is to study the effects exerted by chemotherapeutics, docetaxel and vinorelbine, and radiation on the differentiation of human mammary preadipocytes and the modulation of these effects by melatonin.

MATERIALS & METHODS

CULTURE CONDITIONS

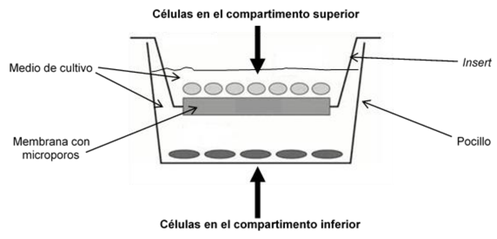
Human breast preadipocytes (BRF) were cultured until reaching confluence in preadipocyte Medium (PM-1) supplemented with penicillin and streptomycin in a humid atmosphere and they were differentiated into adipocyte with Adipocyte Differentiation Medium (DM-2).

IONIZING RADIATION TREATMENT

Both cell lines were exposed to X irradiation using a model YXLON SMART 200 tube at room temperatura. We used 8 Gy radiation as the optimal radiation dose.

MEASUREMENT OF CELLULAR PROLIFERATION

Cell proliferation was measured by the MTT Method. We used the reduction of tetrazolium salts and, at the end, we read absorbance at 570 nm.



Co-culture diagram

QUANTITATION OF TRIGLYCERIDES

We used the Oil Red O staining method to quantify the accumulation of intracytoplasmic triglyceride.

AROMATASE ACTIVITY

We used the tritiated water release assay, based on the formation of tritiated water during aromatization of a labeled androgenic substrate.

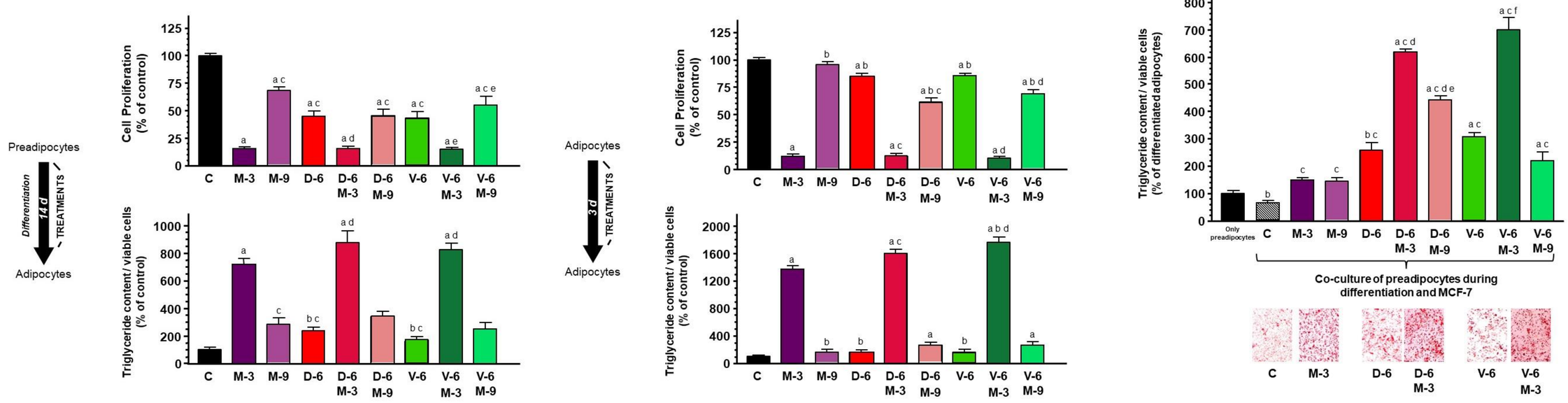
MEASUREMENT OF SPECIFIC mRNA GENE EXPRESSION

We used RT-PCR at the end of the experiments. Total RNA was purified with NZY Total RNA Isolation Kit and cDNA was obtain with NZY First-Strand cDNA Synthesis Kit.

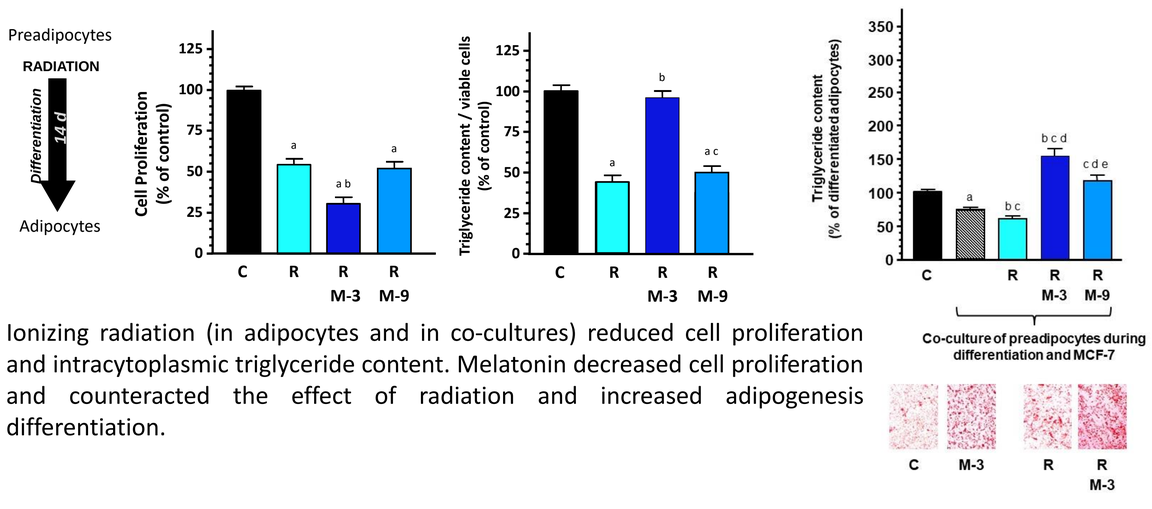
STATISTICAL ANALYSIS

We used GraphPad Prism software. Differences between groups were analyzed by using ANOVA, followed by the Student-Newman-Keuls test

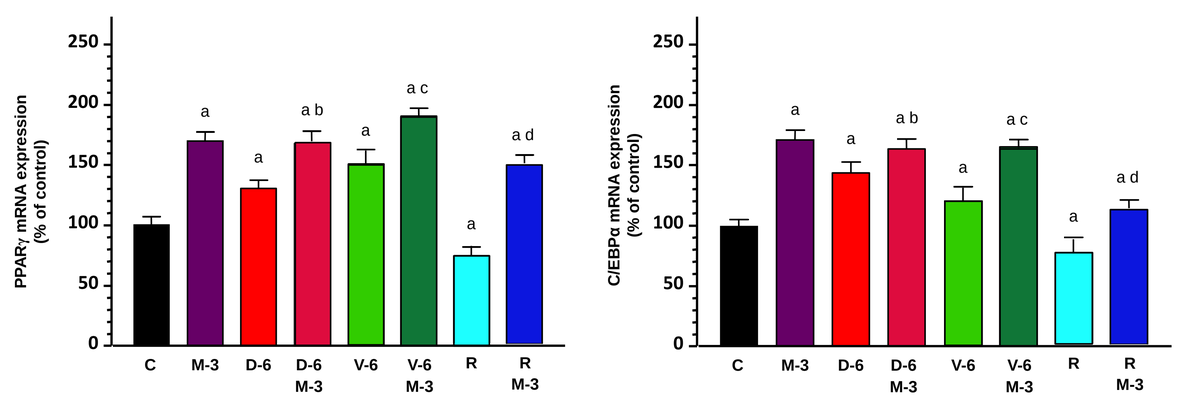
RESULTS & DISCUSSION



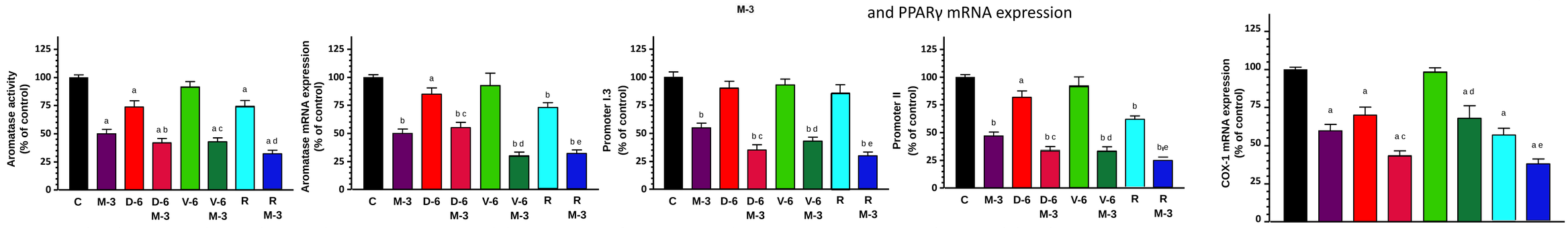
Docetaxel and vinorelbine reduced cell proliferation and increased the triglyceride content of adipocytes (treated or not during its differentiation), an indicator of adipogenic differentiation. In both cases, melatonin potentiated the effects of docetaxel and vinorelbine. The presence of malignant epithelial cells decreased triglyceride content. Docetaxel and vinorelbine increased adipogenic differentiation. Melatonin 1 mM stimulated adipogenesis differentiation and potentiated the stimulatory effect induced by docetaxel and vinorelbine .



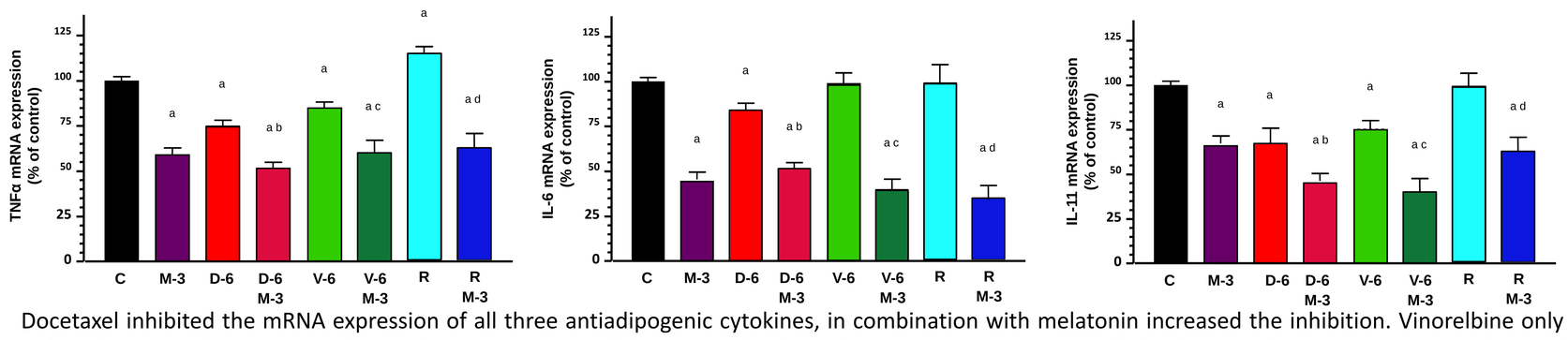
Ionizing radiation (in adipocytes and in co-cultures) reduced cell proliferation and intracytoplasmic triglyceride content. Melatonin decreased cell proliferation and counteracted the effect of radiation and increased adipogenesis differentiation.



Docetaxel and vinorelbine significantly increased C/EBPα and PPARγ mRNA expression. Radiation decreased C/EBPα and PPARγ mRNA expression. Melatonin in both treatments increased C/EBPα and PPARγ mRNA expression



Docetaxel and ionizing radiation decreased aromatase activity and the expression of aromatase and aromatase promoter II. Melatonin inhibited aromatase activity and the expression of aromatase, aromatase promoter I.3 and II and even more in the presence of docetaxel and radiation.



Docetaxel inhibited the mRNA expression of all three antiadipogenic cytokines, in combination with melatonin increased the inhibition. Vinorelbine only reduced TNFα and IL-11 expression, and in the presence of the melatonin the reduction was even more. Radiation increased TNFα mRNA expression and the melatonin counteracted the stimulatory effect. Melatonin alone induced a reduction of the three cytokines

Docetaxel and radiation inhibited COX-1 and COX-2 mRNA expression. Melatonin alone inhibited COX-1 and COX-2 mRNA expression and in combination with docetaxel or radiation

CONCLUSIONS

- Melatonin potentiates the stimulatory effect of chemotherapeutics on the differentiation of preadipocytes in mature adipocytes by increasing their stimulatory effect on C/EBPα and PPARγ and by increasing the downregulation of antiadipogenic cytokines, TNF-α, IL-6 and IL-11.

- In combination with radiation, melatonin counteractes the inhibitory effect of radiation on differentiation of preadipocytes, by increasing C/EBPα and PPARγ expression and by decreasing the TNFα expression induced by radiation.

- Docetaxel and ionizing radiation reduce the activity and expression of aromatase in breast fibroblasts. Melatonin potentiate that inhibitory effect by increasing the downregulation of aromatase promoter II and cyclooxygenases expression.