ARTICLE IN PRESS
+ MODEL

ELSEVIER

Cell Biology International xx (2006) 1–9

Cell Biology International

www.elsevier.com/locate/cellbi

# Activation of bone marrow cells treated with *Canova in vitro*

Ana Paula Ressetti Abud, Beatriz Cesar, Luiz Felipe Moscaleski Cavazzani, Carolina Camargo de Oliveira, Juarez Gabardo, Dorly de Freitas Buchi*

*Setor de Ciências Biológicas, Universidade Federal do Paraná (UFPR), Curitiba, PR, Brazil*

Received 24 March 2006; revised 26 April 2006; accepted 9 June 2006

**Abstract**

*Canova* is a Brazilian complex homeopathic medication produced from *Aconitum, Thuya, Bryonia, Lachesis and Arsenicum.* Previous studies demonstrated that *Canova* induces up-regulation in numbers of leukocytes. The bone marrow microenvironment is composed of growth factors, stromal cells, extracellular matrix, and progenitor cells that differentiate into mature blood cells. As it is the major site of blood cell formation, we studied *in vitro Canova* effects on bone marrow cells of mice. Swiss mouse femurs were dissected, cleaned, and the marrow was flushed. The cells were plated, treated or not, incubated for different times and processed for light, scanning electron, and confocal microscopy, and also flow cytometry. The treatment did not modify the expression of the analyzed surface markers or cytokine production. All microscopy techniques showed that a monocytic lineage ($CD11b^+$) and stromal cells (adherent cells) were activated by treatment. *Canova* also increased cell clusters over adherent cells, suggesting proliferation areas.


*Keywords:* Bone marrow cells; Stromal cells; Activation; *Canova* medication

## 1. Introduction

The medullar cavity of long bones and the interstices between trabeculae of spongy bones house the soft, gelatinous, highly vascular, and cellular tissue known as marrow. It has a unique anatomic structure that allows survival, proliferation, and differentiation of progenitor cells. Marrow stromal cells, extracellular matrix, growth factors and progenitors cells (which differentiate into mature blood cells) constitute this microenvironment (Kondo et al., 2003). This system allows interactions between stromal cells and haematopoietic stem cells (HSCs) (Haylock et al., 1994). This interaction is dependent, at least in part, on direct cell-to-cell contact or cellular adhesion to extracellular matrix proteins (Paul et al., 1991; Kameoka et al., 1995).

The adherent microenvironment of long-term murine bone marrow cultures is an extensive extracellular network of collagen, glycoproteins, fibronectin, and laminin, as well as the cellular stroma, including endothelial cells, macrophages, fibroblasts, adipocytes and reticular cells (Zuckerman and Wicha, 1983). The stromal cells may provide a microenvironment adequate for rapid expansion of the progenitor cells or by preventing apoptotic cell death of the progenitors (Kameoka et al., 1995). The establishment and maintenance of the extracellular matrix correlates with the production of haematopoietic cells in long-term murine bone marrow culture. According to Zuckerman and Wicha (1983), the extracellular matrix is essential for maintenance of *in vitro* haematopoiesis.

HSCs are defined as cells that are capable of both self-renewal and multilineage reconstitution of the haematopoietic system (Domen and Weissman, 1999). They have the capacity to circulate in the blood and can colonize irradiated haematopoietic tissue (De Gowin and Gibson, 1976). These cells are able to form the myeloid blood cellular lineages (granulocytes, monocytes/macrophages), erythroid (erythrocytes and megakaryocytes), and lymphoid (plasmocytes, T cells, B cells and

* Corresponding author. UFPR, Centro Politécnico, SCB, Depto de Biologia Celular, sala 215, Laboratório de Estudo de Células Inflamatórias e Neoplásicas, Jardim das Américas, Curitiba, PR, Brazil CEP 81531-980. Tel.: +55 41 3361 1770; fax: +55 41 3361 1568.
*E-mail addresses:* buchi@ufpr.br, labbiocel@ufpr.br (D.de F. Buchi).

1065-6995/$ - see front matter © 2006 International Federation for Cell Biology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.cellbi.2006.06.011