Effects of a membrane-metallopeptidase blocking agent thiorphan in long-term cultures of human bone marrow

SILVANA STANOVIĆ JANDA 1,*, MILIVOJ BORANIĆ 1, MIRNA SUČIĆ 2, MLADEN PETROVEČKI 3, BRANKA GOLUBIĆ-ČEPULIĆ 4, IGOR AURER 5 and BORIS LABAR 5

1 Ruđer Bošković Institute, Department of Molecular Medicine, POB 1016, Bijenička cesta 54, HR-10001 Zagreb, Croatia
2 University Hospital, Department of Cytology, Kišpatićeva ulica 12, HR-10000 Zagreb, Croatia
3 Department of Computer Science, Rijeka University School of Medicine and Dubrava University Hospital, Zagreb, Croatia
4 University Hospital, Department of Blood Transfusion, Kišpatićeva ulica 12, HR-10000 Zagreb, Croatia
5 University Hospital, Department of Internal Medicine, Kišpatićeva ulica 12, HR-10000 Zagreb, Croatia

Abstract—Thiorphan [(DL-3-mercapto-2-benzylpropanoyl)-glycine], a drug blocking the activity of membrane metalloendopeptidase EC 3.4.24.11 (CD10, CALLA), was added to long-term cultures of human bone marrow. Progression of the cultures was assessed by cell counts, cytology and clonogenic (GM-CFU) ability of the non-adherent cells in the supernatant and by morphology of the adherent stromal layer. A stimulatory effect on hematopoiesis was noted.

Key words: Long-term bone marrow culture (LTBMC); GM-CFU; macrophages; membrane metalloendopeptidase (CD10, EC 3.4.24.11); thiorphan; ovarian cancer.

ABBREVIATIONS

CALLA — common acute lymphoid leukemia antigen, CD — cluster of differentiation, DMSO — dimethylsulfoxide, FCS — fetal calf serum, GM-CFU granulocyte-macrophage colony forming unit, GM-CSF, M-CSF, G-CSF — granulocyte-macrophage, macrophage and granulocyte colony forming factors, IMDM — Iscove’s modified Dulbecco medium, INF — interferon, LTBMC — long term bone

*To whom all correspondence should be addressed. Silvana Stanović Janda, Ruđer Bošković Institute, POB 180, Bijenička 54, HR-10002 Zagreb, Croatia. Phone: (385) 1-4561111 ext 1558, Fax: (385) 1-4680094, E-mail: stanovic@rudjer.irb.hr
marrow culture, LTC — long term culture, MNC — mononuclear cells, TNF — tumor necrosis factor.

INTRODUCTION
Thiorphan, (DL-3-mercapto-2-benzylpropanoyl)-glycine, is a drug which acts by blocking the metalloproteinases present on the cell membranes [1]. One of those enzymes, neutral endopeptidase (enkephalinase, EC 3.4.24.11) is also known as the CD10 cell surface marker or the common acute lymphoid leukemia antigen (CALLA) [2]. It processes various neuropeptides and related signal molecules influencing cell activity, proliferation and differentiation [3–5]. In the lymphohematopoietic system, CD10 is expressed by immature B-lymphoid cells, mature neutrophils and bone marrow stromal cells in normal hematopoiesis as well as by the blasts in acute lymphoid leukemia, chronic myeloid leukemia and some non-Hodgkin’s lymphomas. Some non-hematopoietic tumors also express CD10 [2, 5–7].

Thiorphan and related CD10 inhibitors have been used in experimental and clinical studies for the treatment of pain [8–10], asthma [11, 12], hypertension [13, 14] and diarrhoea [15, 16]. A more popular group of metalloproteinase inhibitors can be found in drugs which act by blocking the angiotensin-converting enzyme (ACE).

We have studied the effects of thiorphan on hematopoiesis in short and long-term cultures of mouse and human bone marrow [17–19]. In long-term cultures of mouse bone marrow, thiorphan increased the cellularity, promoted the generation and maturation of myeloid elements and, in high concentrations, increased the GM-CFU content [17]. In clonal (short-term) cultures of the bone marrow of patients with non-Hodgkin’s lymphoma or acute leukemia in remission it has been shown that thiorphan stimulated GM colony formation [18, 19].

Continuing that work, we explored the effects of thiorphan on human hematopoietic cells grown in long-term bone marrow cultures (LTBMC). A stimulatory effect was expected.

LTBMC consists of an inductive micro-environment provided by a marrow-derived adherent cell layer (stroma) that promotes replication and differentiation of pluripotent stem cells [20, 21]. The initial number of suspended (non-adherent) hematopoietic cells rapidly declines in normal LTBMC and hematopoiesis begins after the establishment of an adherent stromal cell layer. That takes place in the second week of cultivation, and proceeds in foci attached to the stroma. Mature cells detached from the foci and liberated into the medium form the non-adherent cell population [20, 21]. By two to three weeks the number of non-adherent cells achieves 10–30% of the initially seeded cell population and the number of GM-CFU drops to 8–10% of the input value [20]. Macrophages constitute an integral part of the LTBMC and after the 2nd week their content in the adherent population of the normal LTBMC is usually 10–30% [22]. The macrophage