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Growth promotion by homocysteine thiolactone and its alpha-alkylated derivative – A role for excess growth in homocystinurias?

Short Communication

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Summary. It is controversial whether homocysteic acid or other homocysteine derivatives show growth promoting effects. In a clonogenic assay we could show that homocysteine thiolactone and its alpha alkylated derivative increased colony formation significantly. Our work favorizes previous observations showing growth promoting activity of homocysteine derivatives and encourages further studies on that subject with implications for growth in physioogy and under pathological conditions.

Keywords: Amino acids – Homocysteine – Homocysteic acid – Growth – Clonogenic assay – Growth promotion

Introduction

Homocysteine thiolactone is a potent radiation protector and excellent hydroxy-radical scavenger (Mao et al., 1993). Its use is limited due to toxic effects. The side effects known so far could be omitted by alpha-alkylation of homocysteine thiolactone and we therefore extended our literature searches and intensified our toxicological studies on these compounds.

A publication by Skovby in 1993 (Skovby, 1993) has drawn our attention to homocystinurias, human models of homocysteine biology, pathology and toxicity: increased length of long bones is an essential finding of this entity. Skovby did not explain this finding but referred to a publication by Clopath (Clopath et al., 1976). Clopath found growth promotion of hypophysectomized rats and assigned that effect to the activity of homocysteic acid. This effect, however, could not be reproduced by others, using larger numbers of animals (Chrzanowska et al., 1979).

Investigating into toxicity of homocysteine thiolactone and its alpha-alkylated derivative, we performed clonogenic assays and found growth promotion by both compounds.

Materials and methods

Cell line

The HT 29 human colonic carcinoma cell line was purchased from ATCC (American Type Culture Collection, Rockville, MD, USA).

Cell culture and clonogenic assay

The HT 29 human colon carcinoma cell line was grown in RPMI 1640 medium supplemented with 10% heat inactivated FBS (GIBCO, Grand Island, NY, USA), penicillin (100 U/ml) and streptomycin (100 ug per ml). The cells were incubated at 37 C in a humidified atmosphere of 5% CO2 : 95% air.

For subculture, monolayer cells were dispersed with .25% trypsin plus 1 mM EDTA at 37°C for 30 min. After centrifugation the cells were resuspended in fresh medium.

For the clonogenic assay exponentially growing cells were seeded in 24 well plates in medium containing 10% FBS at a density of 150 cells per well. Six hours after seeding, various concentrations of the test compounds were added. The cells were then maintained in the medium for ten days. Triplicate samples were used for each compound concentration. Colonies consisting of more than 50 cells were counted (Szekeres et al., 1992).

Results

Colony counts of controls were 68 $\pm$ 4 (means = $-$SD of three determinations) colonies per well. When cells were incubated with various concentrations of L-homocysteine thiolactone (Sigma) from 50 μg per ml increased colony counts by 20 and 36% were noticed (Fig. 1). Cells incubated with alpha-methyl-homocysteine thiolactone (Mao et al., 1993) 25 μg per ml increased colony

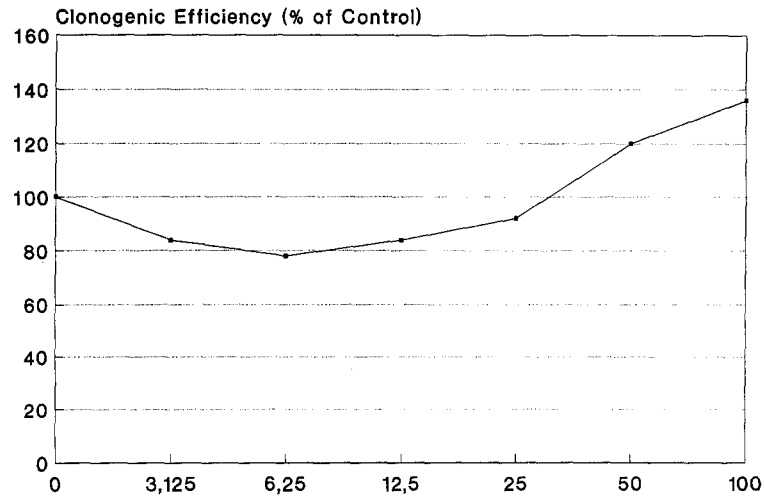

Fig. 1. Clonogenic efficiency in percent of controls vs concentrations in micrograms per ml by homocysteine thiolactone

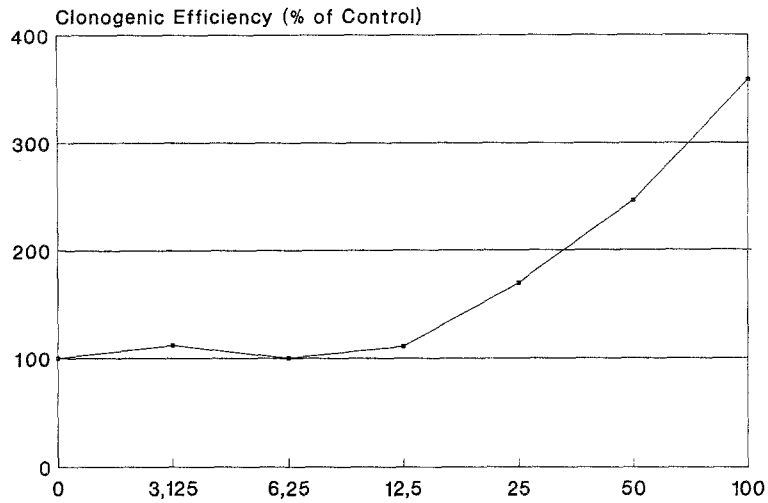

Fig. 2. Clonogenic efficiency in percent of controls vs concentrations of alpha-methyl-homocysteine thiolactone in micrograms per ml

counts by 70%, 50 μg per ml by 246% of untreated controls (Fig. 2). 100 μg per ml stimulated colony formation to a number of 223 $\pm$ 8 colonies per well which makes up 360% of untreated control cells.

Discussion

Homocysteine thiolactone and its methylation product increased colony formation significantly. As homocysteine thiolactone is cleaved in no time to homocysteine, this compound is able to exert growth promotion itself. We cannot, however, rule out that oxidation to homocystine, homocysteic acid, homocysteine sulfinic acid had taken place within the incubation period. The fact that alpha-methyl-homocysteine thiolactone has been more active a growth promotor can be explained by the observation that alpha-alkylated amino acids are not being metabolized by mammalian cells (Lubec et al., 1991).

Clopath and coworkers used a complex biological assay for growth promotion, which made us decide to work with a simple cell culture assay that works nonsophisticatedly and can be readily interpreted. Clopath and coworkers claimed the involvement of growth hormones. Although we have no evidence yet, we speculate that in our system the mechanism of action is rather at a promotor level. Studies with other analogues and in human studies in patients with homocystinurias are in planning and design.

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