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L-ergothioneine level in red blood cells of healthy human males in the Western province of Saudi Arabia

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Abbreviations: LER: L-ergothioneine, RBC: red blood cell, 2-Py-S-S-2-Py: 2,2'-dipyridyl disulfide and Py-2-SH: 2-thiopyridone

Abstract

Ergothioneine is widely distributed in biological systems, particularly in red blood cells of animals. However, it's functional role in human body is not well understood. In order to investigate the biochemical effect of L-ergothioneine, its concentration changes in human blood with respect to ages in healthy individuals was first investigated. L-ergothioneine concentrations in the blood of Saudi males from western province at different stages of life were measured by the procedure of Carlsson *et al*., 1974. At early stages of life (1-10 years), the concentrations of LER is 1.5-2.0 mg/100 ml. It increases gradually at the age of 11-18 years where it reaches the maximum value of 3.7 mg/100 ml. Then, it declines gradually to 3.0-2.3 mg/100 ml during the period of 19-50 years. An increase in the level of LER (2.8 mg/100 ml) was seen at the age of 51^{+}.

Keywords: L-ergothioneine, RBC

Introduction

L-Ergothioneine, the betaine of 2-mercapto-L-histidine was first isolated by Tanret from Ergot, the fungus infection of rye grain (Tanret, 1909). The interest in this compound was greatly stimulated by its subsequent discovery in blood, as a free form (Kawano *et al*., 1982). It was also found to occur in semen and various mammalian tissues, principally liver and kidneys (Melville, 1958; Mayumi *et al*., 1978). It is also found in the whole blood of pig (Heath *et al*., 1953) and rat (Kawano *et al*., 1982). The biosynthesis of LER *via* histidine from *Claviceps purpurea and Neurospora crassa* cultures were studied by different researchers (Heath *et al*., 1953; Melville *et al*., 1956)

Despite a considerable amount of work on this compound, its exact mode of action remains to be established and also the question of its origin in the animal body to be resolved. The contradictory results that characterize LER (Briggs, 1972; Hama *et al*., 1973) and the lack of significant progress in delineating the role of this compound probably derive in part from the lack of a convenient and specific assay for determination of this compound in biological materials.

Carlsson *et al*., reported a relatively rapid and convenient direct spectrophotometric assay of LER (Carlsson, 1974). The assay is based on the resistance of LER to oxidation in alkaline media by Cu^{2+}-catalyzed reaction and the very rapid reaction of LER with 2,2' dipyridyl disulfide (2-Py-S-S-2-Py) at pH 1.0 to provide essentially stoichiometric release of the chromophoric 2-thiopyridone (Py-2-SH).

In this report the Carlsson spectrophotometric assay for the determination of LER in solutions which include deproteinized human "male's" haemolysate has been adapted. This part of the study will be followed by similar determination for LER in human females blood in order to resolve its exact mode of action, the biochemical effect of LER in human body and to resolve the question of its origin in the animal body.

Materials and Methods

Materials

2,2'-dipyridyl disulfide and L-ergothioneine were purchased from Sigma Chem. Co. (St. Louis, MO, USA). All other chemicals are of reagent grade.

Blood samples

The blood samples (n=400) of healthy males, 50 from each group, were selected from different hospitals in Jeddah district of Saudi Arabia. Detailed history, mental, physical status and clinical examination showed that the selected group were completely healthy individuals and not subjected to any therapeutic drugs during the past 3 months before sampling.

Preparation of blood samples

Blood samples in citrate tubes were centrifuged at 1600 *g* for 45 min. The precipitate which include red blood cells (RBC) is used for LER analysis.

Precipitate which include with an equal volume of 0.9% NaCl solution and centrifugation was repeated.

Haemolysation and deproteinization by heat precipitation at alkaline pH were followed. Packed blood cells were mixed with 5 volumes of deionized water and the pH was adjusted to 8.0 by the addition of 1 M NaOH.

After 25 min, the haemolysate was heated in a boiling water-bath for 10 min. The resulting precipitate that had settled out was resuspended by stirring, and the suspension was heated for another 5 min. Solid material was removed after cooling by centrifugation for 15 min and the supernatant was then filtered. The filtrate was used for determination of LER (Carlsson *et al.*, 1974).

LER Determination

The spectrophotometric method for determination of LER was used according to Carlsson and his group (Carlsson *et al.*, 1974).

Results and Discussion

Ergothioneine is widely distributed in biological systems, particularly in red blood cells of animals (Stowell, 1961), which present in high concentrations as a free form (Kawano *et al.*, 1982) and in striated muscles of various animals (Hartman *et al.*, 1988). To investigate the biochemical effect of LER in human body, we have first examined its levels in healthy human blood and its distribution pattern with respect to ages. Figure 1 represents the concentration of LER among males with different age groups. It starts with low concentrations (1.5-2.0 mg/100 ml) in male children (1-10 years). While in males aged of 11-50 years forms an almost "sigmoid curve". Firstly, at the age of 11-18 years, the level of LER is rapidly increased (3.6-3.7 mg/100 ml) which is the age of most rapid physiological changes in man, concerning maturity. However, above this age, the level remains fairly constant (3.0-2.3 mg/100 ml), indicating a steady state of growth. The gradual increase of LER level in RBC's of older persons is due to the accumulation of the compound with aging. The values obtained for LER in healthy individual males are within the ranges of reported values (Hunter, 1928; Jocelyn, 1958).

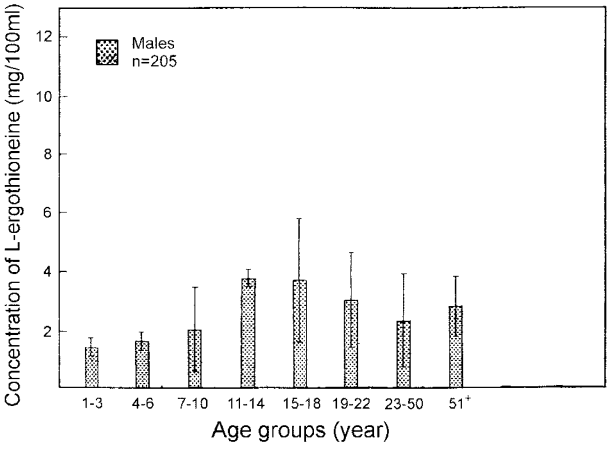

Figure 1. L-ergothioneine concentration in the blood samples of healthy individuals.

There are considerable variation in the standard deviation (SD) for the values of LER which may be explained by the fact that the normal age of RBC is around 120 days. During the early life of RBC, the LER content is high in young human erythrocytes and decline as the erythrocytes age. Since its discovery at the turn of the century, attempts to define a physiological function of LER have been unsuccessful, especially from when Sigma Company no longer produce it. However, different physiological function for LER or its metabolites had been suggested in the literatures which include transport of cations or carbon dioxide, catalysis of carboxylation or decarboxylation reactions, mediation of thyroid or anticholinergic action (Brummel, 1985), protect oxyhemoglobin from oxidation (Smith and Reed, 1992) and scavenger of hydroxyl radicals or an inhibitor of their formation, and perhaps of singlet oxygen (Han, 1992).

No conclusion about its physiological function can be withdrawn from this study alone. The data presented here might be helpful when compared with future study of LER in females. It might indicate accumulation or *in situ* biosynthesis depends on the similarity and dissimilarity.

Acknowledgments

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