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Amyloid-beta peptide affects the oxygen dependence of erythrocyte metabolism: A role for caspase 3

M. Elisabetta Clementi [c], Bruno Giardina [b,c], Deborah Colucci [d], Antonio Galtieri [d], Francesco Misiti [a,*]

[a] *Health and Motor Sciences Department, University of Cassino, V.le Bonomi, 03043 Cassino (FR), Italy*
[b] *Biochemistry and Clinical Biochemistry Institute, Catholic University, School of Medicine, L.go F. Vito n.1, CAP 00168 Rome, Italy*
[c] *CNR, Istituto di Chimica del Riconoscimento Molecolare (ICRM), L.go F. Vito n.1, CAP 00168 Rome, Italy*
[d] *Organic and Biological Chemistry Department, University of Messina, Messina, Italy*

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Abstract

Human erythrocyte metabolism is modulated by the cell oxygenation state. Among other mechanisms, competition of deoxyhemoglobin and some glycolytic enzymes for the cytoplasmic domain of band 3 is probably involved in modulation. This metabolic modulation is connected to variations in intracellular NADPH and ATP levels as a function of the oxygenation state of the cell, and, consequently, it should have physiologic relevance. The present study investigates the effect of amyloid-beta peptide exposure on this metabolic modulation and its relationship with the activity of erythrocyte caspase 3. Metabolic differences between erythrocytes incubated at high and low oxygen saturation disappear following to 24 h exposure to amyloid-beta peptide. Western blotting analysis shows that caspase 3 is concurrently activated. Pre-incubation of amyloid-beta peptide-treated erythrocytes with a specific inhibitor of caspase 3, partially restores the oxygen-dependent modulation. This finding suggests that human erythrocytes following to exposure to amyloid-beta peptide show a complete loss of the oxygen-dependent metabolic modulation, which is partially restored by caspase 3 inhibitor-treatment.

Keywords: Band 3; Pentose pathway; Erythrocyte; Caspase 3; Amyloid-beta peptide

1. Introduction

Alzheimer's disease (AD) is a brain pathology characterised by the presence of senile plaques in several regions of the central nervous system (CNS), especially in those areas where neurodegeneration occurs (Selkoe, 1994). A major protein component of the plaques is the amyloid β-peptide (Aβ), a 39–43 amino acid peptide derived from a larger transmembrane protein, Amyloid Precursor Protein (APP). It is now well established that Aβ possesses neurotoxic activity (Watt, Pike,

Abbreviations: Aβ, amyloid beta (1–42); cdb3, cytoplasmic domain of band 3; PPP, pentose phosphate pathway; Hb, hemoglobin; MetHb, methemoglobin; AD, Alzheimer's disease; Z-DEVD-FMK, benzyloxycarbonyl-Asp(OCH_3)-Glu-(OCH_3)-Val-Asp(OCH_3) fluoromethylketone; PFK, phospho-fructokinase; G6PD, glucose-6-phosphate dehydrogenase

* Corresponding author: Department of Health and Motor Sciences, University of Cassino, V.le Bonomi, 03043 Cassino (FR), Italy.
Tel.: +39 776 2994423; fax: +39 776 2993902.
E-mail address: f.misiti@unicas.it (F. Misiti).

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Walencewicz-Wasserman, & Cotman, 1994; Yankner, Duffy, & Kirschner, 1990). Aβ neurotoxicity has been associated to peptide self-aggregation, which leads to the formation of amyloid-like fibrils (Pike et al., 1995) and eventually to neuronal cell death through apoptosis. However, recent studies have shown that soluble forms of beta amyloid exhibit stronger neurotoxicity and in monomeric form, Aβ may be responsible for neurodegeneration in AD (Kim et al., 2003; Pillot et al., 1999).

In addition to Aβ peptide deposition in neurons, Aβ peptide is generated at high levels in platelets (Chen, Inestrosa, Ross, & Fernanadez, 1995) and it is present at nanomolar concentration in blood (Seubert et al., 1992). Red blood cells are also exposed to amyloid peptides on the luminal surfaces of cerebral microvessels (Ghisa et al., 1993; Grammas, Yamada, & Zlokovic, 2002).

Red blood cells have been reported to sequester monomeric Aβ (Kuo et al., 2001). Previous studies showed that important parameters indicative of red blood cell function and integrity might be negatively affected in red blood cells in AD patients. In particular, in AD red blood cells have been described changes in the physical state of membrane proteins (Kay & Goodman, 1994), alteration of Ca^{++} permeability (Engstrom, Ronquist, Pettersson, & Waldenstrom, 1995), alteration of the antioxidant enzyme activities (Clementi, Martorana, Pezzotti, Giardina, & Misiti, 2004; Delibas, Ozcankaya, & Altuntas, 2002; Rossi et al., 2002), morphological perturbations (Jayakumar et al., 2003) and an increased ratio of RBC/plasma choline levels (Blass, Hanin, Barclay, Kopp, & Reding, 1985).

In line with these alterations, in AD erythrocytes it has been reported an abnormal cellular aging, increased IgG binding and breakdown of band 3 protein (Bosman, Bartholomeus, de Man, van Kalmthout, & de Grip, 1991). Band 3, and in particular its cytoplasmatic domain (cdb3), plays a crucial structural role in linking the bilayer with the spectrin based skeleton network. It functions as an anchoring site for the membrane associated cytoskeletal proteins, such as ankyrin (Bennett & Stenbuck, 1980), proteins 4.2 and 4.1 (Han, Nunomur, Takakuwa, Mohandas, & Jap, 2000; Korsgren & Cohen, 1986), several glycolytic enzymes (Harris & Winzor, 1990; Jenkins, Kezdy, & Steck, 1985), glucose transporter 1 (Jiang et al., 2006), $p72^{SYK}$ protein tyrosine kinase (Harrison, Isaacson, Burg, Geahlen, & Low, 1994), hemoglobin and hemicromes (Walder et al., 1984; Waugh, Walder, & Low, 1987). The binding sites of these enzymes and proteins were more accurately investigated recently (Chu & Low, 2006). All these interactions regulate key elements of red blood cell structure and function, including cell flexibility (Goodall et al., 1994; Low, Willardson, Mohandas, Ross, & Shohet, 1991) and glucose metabolism. In particular, competition between deoxy-Hb and glycolytic enzymes, mainly phospho-fructokinase (PFK), for binding with cdb3 suggests an oxygen-dependent modulation of erythrocyte metabolism (Messana et al., 1996). Thus, upon Hb deoxygenation, ATP production increases, due to the activation of glycolysis following deoxyHb binding to cdb3. On the other hand, upon Hb oxygenation, PPP flux increases, due to the inhibition of glycolytic enzymes following to their binding to cdb3. Recently, it has been demonstrated that N-terminal cytoplasmatic domain of band 3, is degraded by red cell caspase 3 in stressed human mature erythrocytes (Mandal, Moitra, Saha, & Basu, 2002).

On this basis, we have asked the question whether activation of caspase 3 occurs in human mature erythrocytes following to Aβ exposure and if this event might induce metabolic alterations.

2. Materials and methods

2.1. Chemicals

[2-^{13}C]-glucose was purchased from Cambridge Isotope Laboratories (CIL, Andover, MA). All other reagents were of the highest grade and obtained from Sigma Chemical Co. (St. Louis, MO, USA). Aβ(1–42) peptide was obtained by Peptide Speciality Laboratories GmbH (Heidelberg, Germany). Analysis of the peptide by reverse phase high performance chromatography (HPLC) and mass spectrometry revealed a purity >98%.

Buffer A: (Hepes 25 mM, NaH_2PO_4 1 mM, NaCl 110 mM, KCl 5 mM and 2 mM $MgCl_2$) at pH 7.4 and 290 ± 5 mOsm kg^{-1}, measured by an Osmostat OM-6020 apparatus (Daiichikagakuco Ltd., Kyoto, Japan).

Buffer B: (Hepes 25 mM, NaH_2PO_4 1 mM, NaCl 110 mM, KCl 5 mM and 2 mM $MgCl_2$) at pH 7.3 and 290 ± 5 mOsm kg^{-1}, measured by an Osmostat OM-6020 apparatus (Daiichikagakuco Ltd., Kyoto, Japan). The control experiments were performed replacing Aβ peptide with a solution by an equal volume of Hepes buffer (untreated red blood cells).

2.2. Preparation of red blood cells

Heparinised fresh human blood was obtained from informed healthy donors in accordance with the ethical standards and immediately processed. Plasma separation was obtained by centrifuging at $2500 \times g$ for 5 min. Mature human erythrocytes were isolated by density