



Advanced glycation end products overload might explain intracellular cobalamin deficiency in renal dysfunction, diabetes and aging

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ARTICLE INFO

Article history:

Received 5 May 2011

Accepted 2 August 2011

ABSTRACT

Advanced glycation end products (AGEs) contribute to aging. Cobalamin (Cbl) is required for cell growth and functions, and its deficiency causes serious complications. Diabetics and renal patients show high concentrations of Cbl, but metabolic evidence of Cbl deficiency that is reversible after Cbl treatment. Cbl might be sequestered in blood and cannot be delivered to the cell. Megalin mediates the uptake of transcobalamin–Cbl complex into the proximal tubule cells. Megalin is involved in the uptake and degradation of AGEs. In aging, diabetes or renal dysfunction, AGEs might overload megalin thus lowering Cbl uptake. Transcobalamin–Cbl might retain in blood. Shedding of megalin and transcobalamin receptor under glycation conditions is also a possible mechanism of this phenomenon.

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Introduction

Accumulation of advanced glycated end products (AGEs) may accelerate aging of functional proteins and other biomolecules. The metabolic dysbalance of diabetes may contribute to the development of the long-term complications of this disease, including retinopathy, nephropathy and vascular disease [1]. For example, AGEs modified beta2-microglobulin is a component of amyloid plaque in amyloidosis [2] and AGEs enhance formation of beta-amyloid protein in the brain [3]. The biochemical basis of these complications are largely not known, but might be related to glycation of key proteins leading to loss of function, altered function, or even pathological by-products [4]. This review focuses on evidence suggesting a role for glycation in vitamin B12 resistance in aging and diabetes.

Maillard reaction is an early step towards cell aging

AGEs are a heterogeneous group of compounds that are produced by nonenzymatic, sequential glycation and oxidation reactions of sugars with free amino (–NH₂) groups on proteins, peptides, or amino acids. A relatively stable Amadori product is formed. The progress of the reaction depends on the half life of the protein and concentrations of glucose. The final irreversible stage of the reaction ends up with forming AGEs after dehydration and hydrolysis of the intermediate compound. This sequence of

events is known as the Maillard reaction or browning. Maillard reaction has been documented as early as 1912 in vitro especially in term of food science. AGEs may be produced via other pathways like oxidation of sugars, lipids, and amino acids to create aldehydes that covalently bind to proteins.

In an advanced step, the Amadori product undergoes complex rearrangements, cleavage and covalent binding reactions, which lead to the formation of AGEs [5]. The chemistry of glycation, the Maillard reaction and resultant protein modification might be an early step towards cell aging. During the last two decades, it has become increasingly clear that nonenzymatic glycation occurs ubiquitously in vivo. Glycation occurs also at physiological glucose concentrations and is thus not restricted to diabetes. Discovery of HbA1c [6] and the increased amount of this compound in the blood of diabetic patients [7] can be viewed as a revolutionary improvement in diabetic research. Measurement of HbA1c in blood is currently the best available marker for long-term diabetes control. Approximately 5–6% of total HbA1 are glycosylated in healthy individuals.

The presence of Amadori compounds formed with hydroxylysine and reducing sugars in aged connective tissues has been documented as early as 1973 [8]. Later on, Mester et al. suggested the formation of Amadori compounds in the blood from glucose and lysine rich protein or serotonin [9]. There are several common AGEs markers like N-carboxymethyllysine, pentosidine, and methylglyoxal derivatives [10]. Markers of AGEs are independently associated with chronic kidney disease [11]. Inhibitors of AGE formation or AGE breaker (ALT-711) [12,13] prevented AGE accumulation in the kidney of diabetic rats, thus suggesting that anti-AGEs might play a magnificent anti-aging role [14]. Several vitamins have been used in vitro as anti-AGEs. Pyridoxamine,

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benfothiamine, and aminoguanidine exert an anti-AGEs effect largely independent on their classical vitamin function via cross link breaking or inhibiting glycation [15]. Surprisingly, these agents improved also albuminuria without changing glycemic control in a rat model of diabetes [12] suggesting that AGE is causally involved in albuminuria and renal complications in diabetes. Other examples of glycation in vivo are found in structural proteins of the lens [16] or α -crystallin, a molecular chaperone protecting other lens-proteins [17].

In vivo glycation is an important source of reactive oxygen species. Protein damage by fragmentation and crosslinking has been linked to glycoxidation state. Glycation of lipoproteins promotes lipid peroxidation reactions, leading to speculation that glycation and glycoxidation may accelerate oxidative modification of lipoproteins in diabetes and promote the development of atherosclerosis [18]. In line with this, LDL isolated from diabetic patients is susceptible to oxidation in vitro and oxidation correlates with the extent of glycation of the protein [19]. Furthermore, a role of glycation of neurofilaments has been suggested in diabetes related peripheral neuropathy [20,21].

Metformin, an insulin synthesizer drug, has been shown to reduce the expression of receptor for advanced glycation end products (RAGE) in endothelial cells incubated under high or normal glycation conditions [22]. In osteoblast-like cells, AGEs enhanced RAGE protein expression and metformin reversed this effect [23].

The cytotoxicity of the glycation progresses slowly and latently, thus probably explaining the slow progress of aging. Therefore, targeting this generalized mechanism might delay or reduce the burden of age-associated diseases.

Cobalamin physiology

Cobalamin (vitamin B12, Cbl) is of critical importance to growth and function of mammalian cells. Cbl deficiency can cause anemia and/or serious neurological symptoms. Foods of animal origin are rich sources of the vitamin in human diet. Food-bound Cbl must be released and it can then bind the Cbl transporter, haptocorrin (HC). Thereafter, Cbl is transferred to intrinsic factor (IF), and is internalized by the enterocytes via IF-receptor. The receptor for IF–Cbl complex, also called cubam, is a complex of two proteins, cubilin and amnionless [24]. Cubam has been shown to participate in the intestinal absorption of Cbl and reabsorption of Cbl and various proteins from the glomerular ultrafiltrate (for example albumin, transferrin, apolipoprotein A-I and vitamin D-binding protein). Mutations in the amnionless and cubilin genes are causes of Imerslund-Gräsbeck syndrome a disease characterized by proteinuria [25]. The exact pathway for Cbl efflux from the enterocytes is not known, but recent studies suggested a role for multidrug resistance protein 1 (MRP1/ABCC1) for cellular export of Cbl [26].

In blood, Cbl circulates bound transcobalamin (TC) or haptocorrin. Holotranscobalamin (holoTC) is Cbl-bound to transcobalamin and the vitamin form that can enter the cells via TC-receptor. In the cell, Cbl is released from TC in the lysosomes, and directed either into the cytosol or to the mitochondria [27]. In the cytosol, methyl-Cbl is a cofactor for methionine synthase that converts homocysteine (Hcy) into methionine. In the mitochondria, adenosyl-Cbl is a cofactor for methylmalonyl-CoA mutase that converts methylmalonyl CoA (MM-CoA) to succinyl-CoA (Suc-CoA). Many steps in the intracellular Cbl distribution and the recycling of TC-receptor have been identified. Disorders in any step of this complex system can cause severe symptoms that are associated with homocysteinemia and/or methylmalonyluria.

In contrast to IF and HC, TC is a non-glycated protein. The difference in the glycation status of the three proteins is one possible mechanism for the specificity of TC-receptor for TC–Cbl complex

[28]. The protein sequence of TC and the three dimensional form of holoTC have been identified [28,29]. As observed earlier, the location of Cbl conjugation affected binding with TC [30]. Synthetic Cbl-derivatives can be internalized and alter the endosomal/lysosomal recycling of the receptor and/or cobalamin derivatives. Several Cbl derivatives have been developed for the purposes of preparing bio-conjugates as pharmaceuticals to be used for intracellular trafficking of cancer medications or tumor labeling [30,31]. Some derivatives of Cbl prepared by conjugation with the corrin ring substituent show decreased binding to TC, especially those involving the corrin B-pyrroline ring with substitutes on the c- and d-side chains [30]. Conjugation with the pyrroline A ring on the b-side chain decreased the binding of TC to an intermediate level.

The utilization of Cbl is increased in growing cells. The receptor for TC is overexpressed in several malignant cells such as human leukemic cell lines [32], breast [33], ovarian [34], and methionine-independent glioma cells [35]. In attempts to arrest cell growth and differentiation, specific targeting of Cbl-cellular receptor has been suggested as an alternative approach to classical anti-cancer drugs targeting DNA synthesis [36]. Modifying Cbl or its cellular receptor have their limitations because of the wide spread distribution of TC-receptor in tissues and organs [37]. Physiological modifications on Cbl, its binding proteins, or accessory proteins participating in Cbl internalization have not been reported yet, but might be one mechanism of impaired Cbl internalization and cellular deficiency in aging cells.

Cobalamin status in elderly, diabetics and renal dysfunction

Patients with chronic diabetes and/or renal dysfunction develop peripheral neuropathy, nephropathy and/or retinopathy. Also Cbl deficiency can cause neuropathological manifestations, although this is not restricted to diabetic patients. Markers for diagnosing Cbl deficiency have been recently recommended as screening laboratory tests for patients with polyneuropathy (Level C) [38]. Elevated concentrations of methylmalonic acid (MMA) and total homocysteine (tHcy) (both are metabolic marker for Cbl deficiency) are common in patients with diabetes and distal sensory polyneuropathy [39,40]. Unexpectedly, normal to high normal Cbl were detected in this group of PNP patients.

Discrepancies in blood levels of Cbl-markers in renal patients, elderly people and those with slight degrees of renal insufficiency have been reported [41–43]. Patients on regular hemo- or peritoneal dialysis show rather high serum concentrations of holoTC (mean holoTC 100 pmol/L) and total Cbl that do not explain severely elevated MMA [41]. Nevertheless, MMA can be lowered by approximately 40% after Cbl supplementation [41]. MMA-lowering after stopping Cbl-therapy persisted for at least 5 months indicating a metabolic improvement and a pre-treatment deficiency. MMA can not be normalized in many elderly people with slight or advanced renal insufficiency, but the residual elevation in MMA is not related to Cbl deficiency.

Cbl deficiency is common in patients with diabetes mellitus and can participate in long-term disease complications. Low concentration of holoTC or total Cbl is not common in diabetics, despite elevated MMA. We anticipate that Cbl-congestion in blood is associated with intracellular deficiency of the vitamin. In one study, serum Cbl was measured in 396 patients with diabetes and vital status was assessed after a median follow up of 15.7 years [44]. Concentration of Cbl was positively associated with all-cause mortality (HRR for 100 pg/mL difference adjusted for age, sex and diabetes duration = 1.15, 95% CI 1.08–1.22) and death from diabetes/nephropathy (HRR = 1.27, 95% CI 1.10–1.46). Moreover, higher Cbl was positively associated with higher hemoglobin A1c (HbA1c) and 2 h glucose [44], suggesting that Cbl is trapped in plasma under

high glucose conditions. Interestingly, the mortality from diabetes or nephropathy was associated with higher tHcy and higher Cbl [44]. Therefore, in this risk group, higher tHcy was associated with higher Cbl, which is in contrast to the expected correlation between the two markers [44].

Polymorphonuclear cells isolated from patients with renal insufficiency internalized less amount of labeled Cbl (TC-B12Co57) when compared to cells from healthy subjects [45]. This was associated with a comparable surface binding capacity of the vitamin indicating that the receptor for holoTC was not quantitatively altered. Mechanisms responsible for altered Cbl homeostasis and markers in diabetics or renal patients should be further investigated. In one study on patients with diabetes, an increased shedding of megalin and cubilin in urine has been related to albuminuria [46]. Megalin is responsible for re-internalization of holoTC in the proximal tubule (PT). Therefore, megalin overload in case of albuminuria can enhance holoTC loss and cause Cbl deficiency in the kidney. Shedding of TC-receptor in peripheral tissues in patients with diabetes might also be a possible explanation for high holoTC in plasma, despite the increase in serum level of MMA.

A role for metformin in lowering blood concentrations of Cbl or holoTC has been shown. Compared to insulin, 16 week treatment with metformin reduced levels of folate (7%) and Cbl (14%), but increased tHcy (4%) [47], suggesting a role for diabetes medication in the absorbance or utilization of the vitamin.

Many studies have shown that higher doses of Cbl are required to induce metabolic improvements in elderly people compared to young people. Mechanisms of this phenomenon deserve further testing. Elderly people (mean age 81.4 years) with serum Cbl between 100 and 150 pmol/L were treated for 4 weeks using oral Cbl (10–50 µg). Cbl (50 µg) was sufficient to increase serum Cbl in the study population but no significant changes were recorded in tHcy concentrations [48]. The effect of three oral doses of Cbl (25, 100, 1000 µg) for 6 weeks were compared in elderly people with serum Cbl < 221 pmol/L and MMA > 270 nmol/L [49]. Doses less than 1000 µg were not effective in normalizing MMA concentrations in this study [49]. Elderly with elevated creatinine were

excluded, suggesting that MMA elevation was related to impaired Cbl status and not directly to renal insufficiency [49].

The lowest oral dose required to normalize biochemical markers of Cbl has been tested in a randomized, double blind, dose-finding trial ($n = 120$, mean age/SD = 80/7 years) [50]. Study participants with renal insufficiency (serum creatinine > 120 µmol/L) were excluded. A daily oral dose of 2.5, 100, 250, 500 and 1000 µg Cbl were administered for 4 months to people with MMA serum concentrations of >260 nmol/L and serum cobalamin of 100–300 pmol/L. Cbl doses of 647–1032 µg were associated with the highest decrease of serum MMA (mean 33%) in at least 80% of the study population [50].

Megalin and the protein overload theory

Megalin mediates Cbl uptake in the proximal tubule cells

Megalin is a multiligand single-spanning transmembrane glycoprotein belonging to the LDL-receptor family. It is abundantly expressed on PT cells, in the intestinal tract, testis, liver, pulmonary system, and it also facilitates the uptake of Cbl in the blood brain barrier. TC-Cbl complex [51], vitamin D-binding protein [52], retinol binding protein [53], parathyroid hormone [54], albumin [55], and insulin [56] are known ligands for megalin. Megalin mediates the uptake of TC-Cbl complex into the PT cells where Cbl is separated from TC in the lysosomes of these cells and re-enters the blood stream bound to a newly synthesized TC. Megalin has also been shown to mediate the endocytosis of the lysosomal enzyme, cathepsin B, thus providing one major source of lysosomal activity in the PT cells [57]. Tissues expressing megalin including the kidney play a major role in vitamin B12 homeostasis and deficiency.

Megalin role in AGEs uptake in the proximal tubule cells

Megalin is involved in the cellular uptake and degradation of AGEs [58]. Although this function seems not to be a direct

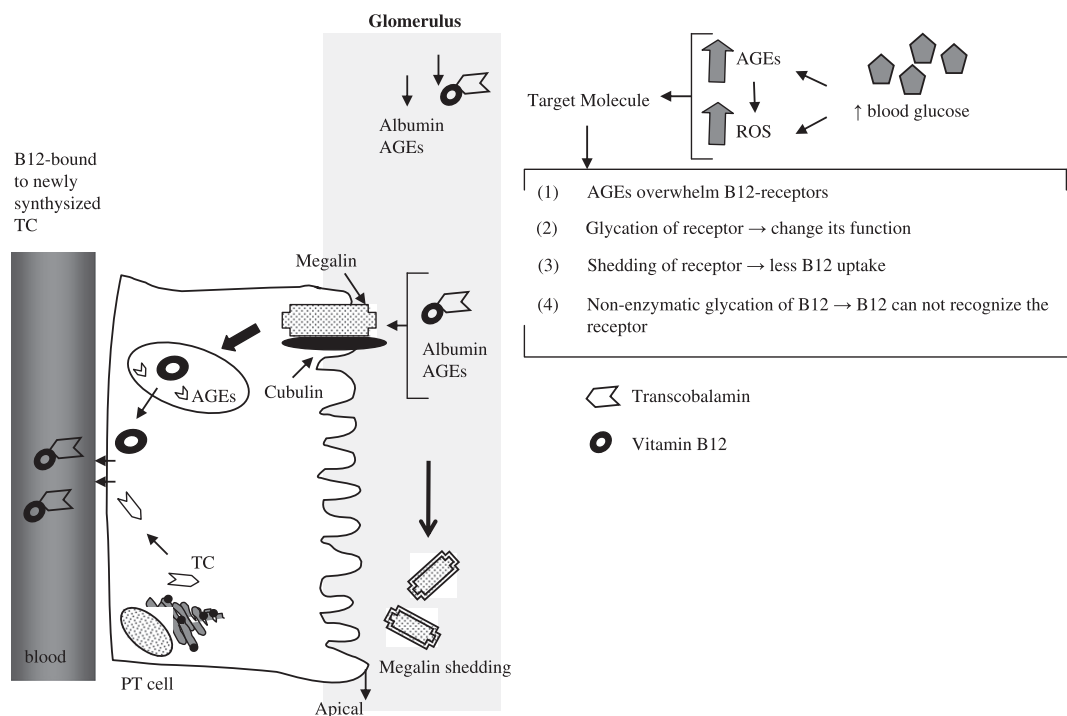


Fig. 1. Transcobalamin, albumin, AGEs and other ligands compete on megalin on the apical cite of PT cells. This can overwhelm the receptor and reduce B12 uptake. Receptor glycation can change its function or cause its shedding. TC; transcobalamin, PT; proximal tubule, ROS; reactive oxygen species.

interaction between megalin and AGEs, blocking megalin by its ligand (receptor-associated protein, RAP) can inhibit cellular internalization of AGE [59]. The AGE-megalín mechanism has a very important role in the pathogenesis of uremic and diabetic conditions and needs further investigation [59]. Over 95% of the albumin in the glomerular filtrate is reabsorbed in the PT via megalin–cubilín complex [60]. Inherited or acquired megalín dysfunction is a cause of megaloblastic anemia and has been related to increased urinary albumin excretion [61].

Patients with diabetes, elderly people, and those with renal dysfunction are likely to have elevated serum levels of AGEs that overwhelm the renal metabolism. Low- and high molecular weight AGEs are filtered by renal glomeruli and are metabolized by PT cells. AGEs are likely to be involved in the pathogenesis of diabetic nephropathy. Moreover, chronic renal failure, irrespective of diabetes, can cause reduced renal metabolism and accumulation of AGEs. The progression of renal diseases, including delayed protein turnover and accelerated oxidative stress, lead to enhanced formation and accumulation of AGEs [62]. This increase leads to the progression of AGEs resulting in a vicious cycle. AGEs might interfere with Cbl metabolism leading to intracellular Cbl deficiency.

The hypothesis: gathering pieces of the puzzle

We hypothesize that higher AGEs can compete with other ligands of megalín and prevents the re-absorption of Cbl in many organs (Fig. 1). Another possibility is that TC-Cbl will gain a different charge (glycated), can not be filtrated in the glomerulus and hence retain in blood. Glycation associated with aging can also enhance TC-receptor and megalín shedding. Therefore, Cbl deficiency in diabetes might be tissue specific probably restricted to tissues expressing megalín and TC-receptor and not necessarily associated with low blood concentrations of Cbl.

Conflict of interests

None declared.

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