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VISIONS AND REFLECTIONS

Age-related lysosomal dysfunction: an unrecognized roadblock for cobalamin trafficking?

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Abstract Vitamin-B_{12} is a generic term for corrinoid compounds that exhibit the biological activity of cyanocobalamin and are collectively referred to as cobalamins. Methylcobalamin and 5-deoxyadenosylcobalamin are the active cobalamins in human metabolism. Cobalamin plays a crucial role in the maintenance of homocysteine and methylmalonyl-CoA homeostasis and is required for erythrocyte formation and DNA synthesis. Data from human and animal studies indicate that cobalamin deficiency impairs neuronal function; a process that is thought to contribute to age-related cognitive decline and dementia. Cobalamin deficiency also results in dysfunction of the peripheral nervous system; among other disorders. Although there is a detailed understanding of the biochemical pathways that are perturbed in cobalamin deficiency, the mechanisms underlying age-related dyshomeostasis in such pathways remain to be addressed. Because cobalamin utilization is dependent on its efficient transit through lysosomes, and mounting evidence indicates that lysosomal function deteriorates in aging long-lived post-mitotic cells such as neurons, in the present article we review published data that supports the proposition that impaired lysosomal processing of cobalamin may play a significant role in age-related (neuro) degenerative diseases.

Keywords Aging · Lysosomes · Lipofuscin · Vitamin-B12 · Neurodegeneration

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Introduction

Vitamin-B_{12} is a generic term for corrinoid compounds that exhibit the biological activity of cyanocobalamin and are collectively referred to as cobalamins. Methylcobalamin (MeCbl) and 5-deoxyadenosylcobalamin (AdoCbl) are the cobalamins that are active in human metabolism. Cobalamin (Cbl) is required for erythrocyte formation and DNA synthesis and plays a crucial role in maintenance of neurological function. Data from human and animal studies indicate that cbl deficiency impairs neuronal function; a process that is thought to contribute to age-related cognitive decline and dementia including Alzheimer's disease [14, 37, 51, 71]. Cbl deficiency also results in dysfunction of the peripheral nervous system [46, 62]; among other disorders [30]. Although there is a detailed understanding of the biochemical pathways that are perturbed in cbl deficient states (see below), several questions remain regarding why such biochemical/metabolic perturbations increase with age. Prompted by the fact that cbl utilization is critically dependent on its efficient transit through the lysosomal compartment [10, 21], and the evidence that lysosomal function deteriorates in aging long-lived post-mitotic cells such as neurons [13, 19, 22, 55, 59, 61], in the present report we review the available literature in order to explore the idea that suboptimal lysosomal processing of cbl may play a role in age-related loss of neurological function.

Cbl absorption

Cbl (Fig. 1) is a water-soluble vitamin present in limited amounts in the diet, primarily in meat and dairy products. Clinically, total serum cbl concentrations are typically examined for deficiency status with serum cbl levels <150 pM and/or hematological anomalies treated as cbl deficiency. However, low serum cbl concentrations do not accurately reflect intracellular cbl status and thus serum homocysteine (Hcy) and methylmalonic acid (MMA) levels (>13 μM and >0.4 μM, respectively) are more reliable indicators of cbl deficiency [18]. These compounds accumulate in cbl deficiency as a result of reduced activities of two key enzymes, methionine synthase (MS) and methylmalonyl-CoA mutase (MMCM), that require cbl as a cofactor (discussed further below). Due to inadequate dietary intake and poor absorption, approximately 6% of the western population over the age of 60 has low serum cbl levels; with the prevalence of deficiency increasing with age [2].

Pernicious anemia accounts for approximately 15–25% of cbl deficiency. This disorder is characterized by a lack of intrinsic factor (IF), which limits the body's capacity to absorb cbl from dietary sources [18]. Pernicious anemia is an autoimmune disease that results in the destruction of gastric parietal cells which results in a lack of IF production. The resulting atrophic gastritis eventually leads to megaloblastic anemia and neurological disorders if left untreated. The majority of cbl deficiency (about 60–70%), however, is due to food-Cbl malabsorption, a problem of particular concern in the elderly [15]. This syndrome is characterized by the inability of the body to release cbl from food or intestinal transport proteins. These factors clearly make a major contribution to functional cbl deficiency.

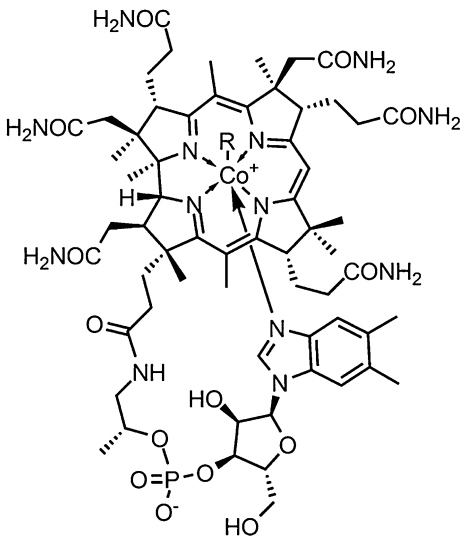

Fig. 1 Cobalamin structure. R = 5′deoxyadenosyl in 5′deoxyadenosylcobalamin (AdoCbl), R = CH_3 in methylcobalamin (MeCbl)

Dietary cbl undergoes carrier-mediated transport, first binding to salivary haptocorrin, from where it is released to IF in the duodenum. The intestinal absorption of IF-Cbl is mediated by cubilin receptors that are tethered to the surface of ileal mucosal cells via the amnionless protein [20, 25]. The IF-Cbl complex is sorted through lysosomes, the IF is degraded by lysosomal hydrolases, and cbl is freed and transported out of the lysosomal compartment. Cbl is then secreted via the basolateral membrane into the portal vein and transported to tissues as a complex with transcobalamin (TC) via the systemic circulation [43]. Plasma TC-Cbl is then internalized by the transcobalamin receptor (TCR) expressed on the cell surface (discussed further below). Plasma haptocorrin also binds cbl where it is thought to act as a store since it is not endocytosed by the TCR. It is noteworthy that intravenous cbl rapidly passes the blood–brain-barrier in humans, although the receptor(s) involved have not been definitively identified [63].

Cbl intracellular trafficking—the importance of lysosomes

Once the TC-Cbl complex is endocytosed by cells in the body, it is targeted to the lysosome where TC is degraded and the TCR is recycled to the cell surface (Fig. 2a) [3]. It is currently thought that cbl released from TC inside the lysosome is bound by another putative carrier protein that delivers cbl to a lysosomal transporter (Probable lysosomal cobalamin transporter/Limb region 1 protein homolog (LMBR1) domain-containing protein 1, LMBD1) that releases cbl to the cytosol [21]. Upon export from the lysosome, cobalamins are processed by the cblC gene product MMACHC (methylmalonic aciduria cblC type with homocystinuria) [24, 31, 32] and delivered to cytosolic MS and mitochondrial MMCM by the cblD gene product MMADHC (methylmalonic aciduria cblD type with homocystinuria) [16].

Mutations in the human *LMBRD1* gene that encodes the LMBD1 transporter cause combined methylmalonic aciduria and homocystinuria [49]. Mutations in *LMBRD1* represent one of eight complementation groups of inborn errors of cbl metabolism referred to as cblF. This genetic defect in lysosomal cbl release was discovered 25 years ago, well before the likely transporter involved was characterized [47]. These early studies showed that chloroquine (a compound that increases lysosomal pH above its physiological level of ~4.5 thereby inhibiting lysosomal protease activity) prevented the release of cbl from TC and also blocked the transport of lysosomal [57Co] Cbl to both MS and MMCM [47]. These studies provided crucial

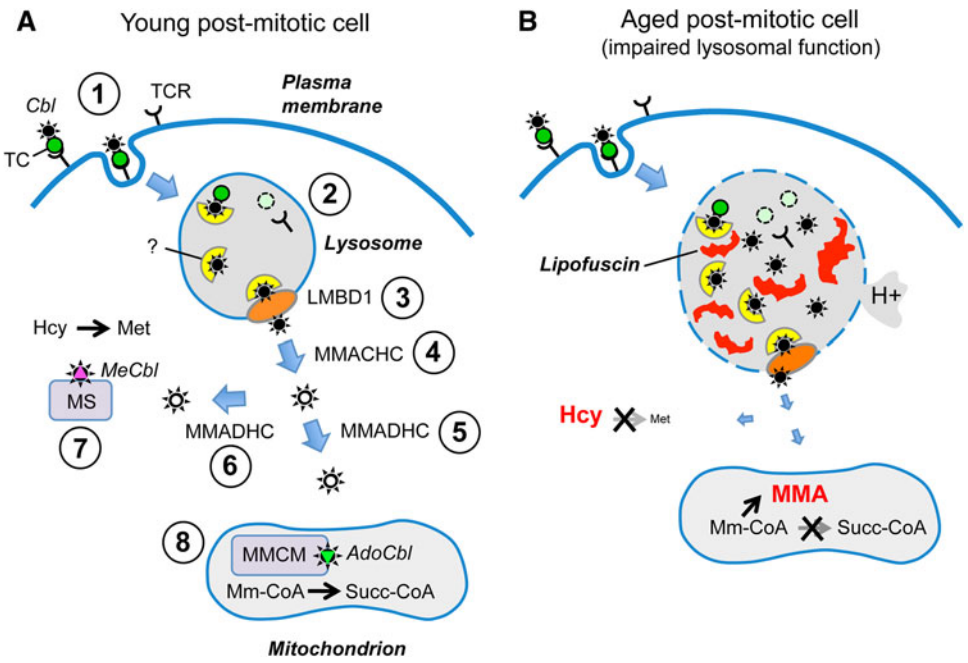

Fig. 2 Lysosomal trafficking of cbl in young and aged post-mitotic cells. **a** TCR-mediated endocytosis delivers cbl to the lysosome (*1*). Cbl is released from TC in the lysosome (*2*) and transported by an unknown carrier (?) to LMBD1 for delivery to the cytosol (*3*). Upon export from the lysosome, cobalamins are processed by MMACHC (*4*) and delivered via MMADHC (*5*) and (*6*) to cytosolic MS (*7*) and mitochondrial MMCM (*8*). Cytosolic MeCbl is required for conversion of Hcy to Met by MS (*7*). Mitochondrial AdoCbl is required for conversion of Mm-CoA to Succ-CoA by MMCM (*8*). **b** Aging processes (including lipofuscin accumulation) impair lysosomal enzyme function and induce proton "leakage" thereby increasing lysosomal pH. This is predicted to inhibit the release of cbl from TC (due to reduced activity and availability of acid hydrolyases) and impede the conversion of cbl to the "base-off" state (requires low pH). Overall, this will prevent delivery of cbl to MS and MMCM. As a result, Hcy and Mm-CoA homeostasis is disturbed leading to accumulation of Hcy and the Mm-CoA metabolite MMA that both contribute to cytotoxicity. *Cbl* cobalamin, *TCR* transcobalamin receptor, *TC* transcobalamin, *LMBD1* Probable lysosomal cobalamin transporter, *MeCbl* methylcobalamin, *Hcy* homocysteine, *Met* methionine, *MS* methionine synthase, *AdoCbl* 5′deoxyadenosylcobalamin, *Mm-CoA* methylmalonyl coenzyme-A, *Succ-CoA* succinyl coenzyme-A, *MMCM* methylmalonyl-CoA mutase, *MMACHC* methylmalonic aciduria cblC type with homocystinuria, *MMADHC* methylmalonic aciduria cblD type with homocystinuria

evidence that lysosomal dysfunction severely impedes intracellular cbl utilization.

In addition to the chloroquine-induced increase in lysosomal pH and concomitant inhibition of protease activity that prevents release of cbl from TC, it is now recognized that the acidic pH of the lysosome also influences the conversion of cbl from the so called "base-on" to "base-off" state that refers to the interaction of the dimethylbenzimidazole moiety of the cbl molecule with the central Co atom [9]. The cbl base-off state is speculated to be important for subsequent interactions with cytosolic cargo proteins [9]. There is strong evidence indicating that decreased lysosomal proteolytic activity and increased lysosomal pH occur as a consequence of aging in long-lived post-mitotic cells [36, 45, 59, 60]. It is therefore plausible that loss of lysosomal TC proteolysis and inhibition of the pH-dependent conversion of cbl to the base-off state accompany such age-related changes in lysosomal function. It is noteworthy that in addition to TC, at least one additional uncharacterized lysosomal cbl escort/transport protein is predicted to exist and this may present another point at which cbl transit could be disrupted when lysosome function is impaired [10].

Age-related impairment of lysosomal function—exquisite sensitivity of post-mitotic cells

Aging is accompanied by progressive cellular accumulation of macromolecular damage [59]. Although damaged macromolecules and organelles are continuously degraded by lysosomes through autophagy and replaced by newly synthesized biological structures, it is clear that some material progressively accumulates in post-mitotic cells. This is demonstrated by the accumulation of the "age-pigment" lipofuscin in long-lived non-dividing cells such as neurons, cardiac myocytes, skeletal muscle fibers, and retinal pigment epithelial cells [19, 58]. It is well known that cellular lipofuscin content positively correlates with oxidative stress and mitochondrial damage [55]. Furthermore, there is compelling data to suggest that lipofuscin accumulation impairs several lysosomal functions [13, 60].

Lysosomal enzymes are produced in the trans-Golgi network and are transported by secretory vesicles to late endosomes that acidify and mature into lysosomes. The continual fusion and fission of the lysosomal vacuoles ensures the distribution of acid hydrolases within the lysosomal compartment. Senescent post-mitotic cells contain large numbers of lipofuscin-containing lysosomes, to which a progressively greater proportion of lysosomal enzymes are directed in a futile attempt to degrade lipofuscin. These lysosomal enzymes are essentially lost for useful purposes (e.g., for the degradation of newly autophagocytosed material), resulting in a delayed turnover and the accumulation of waste products [59]. There is also evidence that lipofuscin accumulation may contribute to neurodegeneration [70] and that it may exacerbate neuropathological processes that, for example, may be caused primarily by an accumulation of toxic protein aggregates such as huntingtin [11, 56].

Even though lipofuscin-loaded lysosomes appear to be intact using microscopy techniques, it is now recognized that they are subjected to a high Fe-catalyzed oxidative stress [67] that compromises lysosomal membrane integrity leading to loss of the proton gradient [29]. The resulting increase in lysosomal pH significantly reduces protease action and if the lysosomal membrane is sufficiently damaged, cathepsins may be released to the cytosol and trigger apoptosis [68]. Although lysosomal heterogeneity exists both within cells and between cells it is quite clear that the net function of the lysosomal compartment is severely compromised with aging [64].

In addition to the impact of lysosomal lipofuscin accumulation, there are several age-related disorders in which lysosomal function is compromised. Neurodegenerative diseases including Alzheimer's disease, Parkinson's disease (and other Lewy body disorders) and Huntington's disease all involve the accumulation of aggregated proteins/peptides that eventually overwhelm lysosomal capacity for degradation [17, 34, 42, 48]. There is strong evidence that in these conditions the degradative capacity of the lysosome is impaired and the lysosomal membrane is destabilized [42]. In addition, lysosomal pH may be increased in specific lysosomal storage diseases (e.g., mucolipidosis Type IV), even in dividing cells [5].

Together these data indicate that lysosomal function deteriorates with age and in age-related degenerative disorders. Given the critical role that the lysosome plays in cbl metabolism (described above), it seems reasonable to suggest that cbl probably does not reach its intended intracellular targets in aged/lysosome-compromised cells, even though cbl supply to the cell may be adequate (Fig. 2b). An escalating cytotoxic trajectory would result due to the lack of both MeCbl and AdoCbl available to act as cofactors in the two important intracellular pathways described below.

Metabolic functions of MeCbl and AdoCbl—relevance to aging and neurodegenerative disease

Cbl has a complex chemical structure with a central cobalt atom tethered equatorially to four nitrogens donated by the corrin ring [27]. Although cbl exists in several forms, MeCbl and AdoCbl are the active forms in human metabolism. MeCbl and AdoCbl differ only in the functional group attached to the Co atom at the centre of the cbl corrin ring (Fig. 1). MeCbl is used to transform Hcy to Met via cytosolic MS. This reaction requires donation of a methyl group from *N*5-methyltetrahydrofolate to Hcy with the formation of tetrahydrofolate and Met. Met is utilized in the formation of *S*-adenosylmethionine (SAM), a universal methyl donor for numerous substrates, including DNA, RNA, hormones, proteins, and lipids. AdoCbl is required for the conversion of methylmalonyl-coenzyme A (Mm-CoA) to succinyl-coenzyme A (Succ-CoA) via mitochondrial MMCM. Succ-CoA then enters the Krebs cycle from where it may be utilized in many pathways including conversion to succinate which may be used as an electron donor or in the synthesis of porphyrins such as heme. In human cbl deficiency states, MS and MMCM activities are reduced which results in increased tissue and plasma Hcy levels and, subsequent to conversion of Mm-CoA to MMA, increased tissue and plasma MMA levels. Based on the roles of both MS and MMCM in methylation reactions and multiple pathways related to, for example, amino acid and lipid metabolism, it is not surprising that clinical cbl deficiency phenotypes are multifaceted [6]. In addition to the "loss of function" caused by impaired MS and MMCM activities, the accumulation of Hcy and MMA is cytotoxic which is thought to contribute to neuron death and to the loss of cognitive capacity [33, 40].

These findings are particularly important in the aging context as it is known that plasma levels of both Hcy and MMA increase with age and there is a positive correlation between plasma Hcy and MMA concentrations and cognitive decline [26, 50, 65]. Importantly, several studies have reported that plasma Hcy levels are positively correlated with brain atrophy in humans and this has led to the administration of cbl (both with and without folate) as a therapeutic agent for mild cognitive impairment (MCI) and Alzheimer's disease [50, 52, 65]. Although there is evidence that reducing Hcy levels in MCI patients can slow the rate of brain atrophy, the response appears to be linked to baseline Hcy levels [54]. Studies in rodents indicate that cbl supplementation significantly improves cognitive

performance [69, 71]. In human MCI and Alzheimer's disease cbl trials, however, the evidence for a positive effect on cognitive performance is not compelling [8, 35, 38, 39, 53].

Although food-Cbl malabsorption is an important cause of cbl deficiency in the elderly, this is not likely to account for the lack of efficacy regarding cognitive improvement in clinical trials since both oral cbl supplementation (that by-passes problems associated with release from food components) and parenteral delivery (that by-passes problems associated with both release from food as well as lack of IF) routes increase circulating cbl to the same degree in both young and aged subjects [4, 41]. This raises the possibility that other pathways that are independent of dietary malabsorption may contribute to suboptimal cbl utilization in aged individuals. One possibility that has not been previously recognized is that the lack of cognitive improvement may be due to impaired transit of cbl through lysosomes within neurons of aged individuals. Such a situation could arise due to the accumulation of lipofuscin in neurons (Fig. 2b), as we have discussed above. In addition, in neurodegenerative diseases including Alzheimer's disease and Parkinson's disease where lysosomal function is impaired [42], it is also likely that cbl release from lysosomal TC is compromised and conversion to the "base-off" state is suboptimal. Under these conditions, a localized cbl deficiency state would prevail despite the fact that plasma cbl levels may have been normalized by dietary supplements or intramuscular injections. Such problems with lysosomal function would help to explain why cbl supplementation has not provided consistent therapeutic benefits that have been hoped for in terms of improved cognitive capacity.

Concluding remarks

In conclusion, the available evidence points towards an impairment of lysosomal function as representing a novel "roadblock" that prevents cbl from reaching its target intracellular enzymes in long-lived post-mitotic cells such as neurons. This may represent a significant cause of "functional cbl deficiency" in aging and neurodegenerative diseases even when oral/parenteral cbl supplementation is used to maintain plasma cbl levels within a healthy range. This roadblock could contribute to the deleterious increases in Hcy and MMA levels that occur in the aging brain and thereby directly accelerate neurodegeneration. As there is already great interest in provision of dietary supplements of cbl and other B-group vitamins in the aging and neurodegenerative disease contexts [7, 23, 53], we feel that future detailed studies of intracellular cbl transport under conditions relevant to aging and impaired lysosomal function are warranted. Experimental approaches that may be useful in this context include assessment of cbl transport and Hcy/Mm-CoA homeostasis in cell culture models known to induce lysosomal lipofuscin accumulation [13, 44, 57] or impairment of lysosomal function due to pathogenic (e.g., amyloid-beta-induced) lysosomal membrane perturbations [28], as well as in mouse models of ageing, neurodegenerative diseases and certain lysosomal storage disorders [1, 12, 66]. It is conceivable that approaches to preserve lysosomal function or by-pass the dysfunctional lysosome roadblock may be explored in the future as novel strategies to escort cbl to its correct intracellular targets.

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