Mental retardation in Down syndrome: Two ways to treat

Pierre P. Kamoun
Biochemistry and Molecular Biology, Paris-Descartes University, 26 rue de Chartres, 92200 Neuilly, France

ABSTRACT
Mental retardation is a progressive condition in Down syndrome: intelligence starts to decline linearly within the first year. This phenomenon could be related to the overproduction of a toxic compound, hydrogen sulfide. Indeed, a gene located on chromosome 21 controls the production of cystathionine-β-synthase, an enzyme involved in hydrogen sulfide production in the central nervous system. It has recently been demonstrated that excess cystathionine-β-synthase levels are needed and sufficient to induce cognitive phenotypes in mouse models of Down syndrome. Thus, two therapeutic options might be used in Down syndrome patients: the use of a specific cystathionine β-synthase inhibitor and the use of an effective antidote to reduce hydrogen sulfide toxicity. Prenatal treatment of Down syndrome fetuses is also suggested.

Introduction
Apart from profound hypotonia, the behavior of newborns with Down syndrome (DS) may appear reasonably normal. Developmental delay generally becomes obvious during the first few months of life. The achievement of developmental landmarks is usually increasingly delayed over time. Thus, the average delay may be of about two months for the very early landmarks (e.g. rolling over, transferring objects) but delayed over time. Thus, the average delay may be of about two months for the very early landmarks (e.g. rolling over, transferring objects) but it lengthens gradually to reach one to two years for functions that normally appear around the age of two years [1]. Many studies assessing development during the first decade of life have shown that, even in DS children raised at home, there is a progressive, virtually linear decline in intelligence quotient (IQ) which starts within the first year of life [2]. By analogy with the toxic effect of phenylalanine in phenylketonuria, mental retardation in DS may also have a metabolic origin. It has been suggested that the metabolism of sulfur amino acids in the brain could lead to the production of a toxic compound, hydrogen sulfide [3].

Cystathionine β-synthase (CBS or EC 4.2.1.22) is an enzyme encoded by a gene located on chromosome 21 (21q22). CBS activity is increased by about 150% in fibroblasts from DS patients compared to normal subjects [4]. Also, CBS expression is 12 times higher in myeloblasts from DS children compared to normal subjects [5]. A polymorphism of the CBS allele is significantly under-represented in children with high IQ, suggesting that the level of CBS may influence cognitive functions [6]. In the brain of 34-week old DS patients, the level of CBS is about 3 times higher than in age-matched control subjects [7]. CBS is an enzyme involved in hydrogen sulfide production in the central nervous system (CNS) [8]. The role of hydrogen sulfide in different human models of cognitive defects has recently been described [9] as well as the role of polysulfides [10,11]. In rats, hydrogen sulfide is mainly synthesized by cystathionine γ-lyase (cystathionase) in the liver, kidney, enterocytes and vascular smooth muscle cells and by 3-mercaptopyruvate sulfotransferase in heart tissues [12]. It has recently been found that the brain of CBS-knockout mice produces hydrogen sulfide, suggesting the presence of another hydrogen sulfide-producing enzyme. This enzyme has been identified as the 3-mercaptosulfurtransferase (in combination with cysteine aminotransferase). Both enzymes are located in mitochondria [13,14].

Thiosulfate is the main product of hydrogen sulfide metabolism [12]. Due to its stability, thiosulfate has been used as an index for hydrogen sulfide poisoning [15]. Thus, the endogenous production of hydrogen sulfide can be estimated by monitoring thiosulfate excretion in urine (about 31 µmoles/day) in control subjects [16]. Following hydrogen sulfide poisoning, the urinary thiosulfate level significantly increases [17]. In a study assessing urinary compounds in 17 DS patients compared to 17 normal subjects, it has been shown that urinary thiosulfate excretion was two times higher in DS patients. In contrast, no differences have been observed for the other urinary sulfur compounds (cystine, taurine and inorganic sulfate) [18,19]. The volume of the cerebellum is significantly smaller in DS patients than in matched controls, even after adjustment for the total brain volume or total intracranial volume [20]. Interestingly, the chronic exposure of pregnant dams to low concentrations of hydrogen sulfide leads to abnormal growth of developing cerebellar Purkinje cells of pups [21]. However, the cerebellum of CBS-knockout mice is also smaller than that of wild-type mice [22]. Mouse models of CBS deficiency have been shown to be good, although not perfect, models for human CBS deficiency [23]. There are many discrepancies between mouse models and the disease in humans: (a) homozygous null mice, unlike humans, show a high degree of neonatal lethality due to liver failure; (b) homozygous null mice show a normal or subnormal methionine plasma concentration while...
hypomethioninemia is found in humans; (c) a lack of cerebellum abnormalities is found in homocystinuria [24].

Clinical and biological findings have suggested a relationship between DS and chronic hydrogen sulfide poisoning. In a recently published study [25], the major role of CBS in the cognitive defects observed in DS has been suggested. In a mouse model of DS, i.e. Dp(17Aabg1-cbs)1Yah also referred to as Dp1Yah, mice are trisomic for CBS and 11 protein-encoding genes. The region also encompasses 6 non-coding genes. These mice show a deficit in the novel object recognition test (NORT). To decipher the role of CBS in DS cognitive phenotypes, the authors have generated and characterized constitutive and conditional changes in CBS levels in the CNS of various mouse models. Thus, they have demonstrated that three copies of CBS are necessary to induce cognitive impairment in Dp1Yah mice and that an excess CBS level is sufficient to induce cognitive phenotypes in mouse models of DS.

**Potential therapeutic options for mental retardation in Down syndrome**

Two methods may be used to reduce hydrogen sulfide toxicity on the CNS: (a) inhibiting hydrogen sulfide production with specific CBS inhibitors, and (b) using hydrogen sulfide scavengers. In both cases, the chemical compounds must be able to pass the blood-brain barrier in order to target hydrogen sulfide levels in the brain.

**Use of CBS inhibitors**

CBS inhibitors have been widely investigated because CBS has recently been identified as a drug-target in several types of cancer [26]. Amino-oxyacetic acid (AOAA) is the most widely used CBS inhibitor, but it should be noted that it also acts as a GABA-transaminase inhibitor. AOAA has been administered to infants and children who were resistant to usual anticonvulsant medications [27]. It has been used at a dose of 200 or 300 mg/day without significant adverse events but its efficacy was not uniform. Indeed, AOAA is a general inhibitor of amino-transferases and it has been shown to inhibit CBS and cystathionine γ-lyase (cystathionase) (IC50 at about 8 and 1 µmole/L, respectively). AOAA improved learning and memory capacities in a chronic alcoholism rat model, and may be associated with reduced hippocampal hydrogen sulfide levels [28]. Thus, the use of AOAA as a CBS inhibitor seems safe and effective.

Benserazide is a decarboxylase inhibitor approved by the FDA for the adjuvant treatment of Parkinson’s disease associated with a very low toxicity. It has been combined with L-DOPA (Madopar®) for its action on the DOPA decarboxylase. Benserazide is a less potent CBS inhibitor than the reference compound, AOAA (IC50 at about 30 and 1 µM, respectively) [29]. An in vivo study has shown that the intraperitoneal injection of 300 and 600 mg/kg of benserazide inhibited cancer growth in tumor-bearing mice and no toxicity has been observed. Despite the low dose used in Parkinson’s patients (about 50 mg/day), a slight increase in plasma homocystine levels has been reported, confirming the inhibition of CBS [30]. However, benserazide cannot inhibit hydrogen sulfide formation in the brain because it is not able to pass the blood-brain barrier, although it is a very good inhibitor of CBS produced in extra-cerebral tissues (liver, kidney and others).

**Use of hydrogen sulfide scavengers**

Drugs counteracting the phenotypical consequences of CBS overexpression might also be used in DS. They have been developed to suppress the toxic effect of hydrogen sulfide in the brain. Sodium nitrite is the first compound developed to suppress hydrogen sulfide toxicity [19] and it has been used to treat acute hydrogen sulfide poisoning. Its action is mediated through the oxidation of hemoglobin to methemoglobin (metHb), a compound able to form a complex with hydrogen sulfide resulting in sulfolhemoglobin [31,32]. Another mechanism can be effective. Nitrite releases NO which reacts with hydrogen sulfide to produce polysulfides [33]. The administration of 4 mg/kg of sodium nitrite to human volunteers led to the formation of up to 7% of metHb. A significant difference has been observed in thiosulfate urinary excretion between DS patients and controls (21 diet-matched subjects): 5.36 ± 0.76 versus 2.23 ± 0.42 mmole/mole of creatinine (p < 0.0001) [19]. Thus, the daily thiosulfate production has been estimated at 74.5 µmoles in DS patients compared to 31.0 µmoles in normal subjects. Furthermore, a recent study [34] has shown that the reaction between hydrogen sulfide and metHb leads to the formation of a metHb-SH complex in intact red blood cells. This study has shown that the metHb-SH complex was stable in the long term, and that its slow decomposition leads to the formation of reduced oxyHb, thiosulfate and/or polysulfides as final products. Interestingly, nitrite-induced methemoglobinemia remains one of the best antitoxides available for the treatment of hydrogen sulfide poisoning [35]. Although the involvement of other mechanisms has been suggested in nitrite-induced hydrogen sulfide detoxification (including the effect of nitrite on mitochondrial enzymes), another mechanism might be the enhanced oxidative inactivation of hydrogen sulfide due to increased metHb levels in red blood cells. The safety use of oral sodium nitrite has been discussed [36] after its administration at a dose of 80 mg/day (1.1 mmoles) in patients with diabetes mellitus and active or healed foot ulcers where only a few adverse events (headache, nausea) have been observed. Thus, sodium nitrite may be used to reduce chronic hydrogen sulfide intoxication.

Disulfiram, a potent inhibitor of mitochondrial aldehyde dehydrogenase, has been used in human CBS transgenic mice at a dose of 10 mg/kg/day before testing them for the NORT [25]. The NORT paradigm was restored in disulfiram-treated transgenic mice but not in non-treated mice. However, a better understand of its mode of action and identifying disulfiram targets involved in the NORT are needed. The authors have suggested that this molecule may not directly inhibit CBS activity but probably rather act on the consequences of CBS overexpression [25]. Upon its absorption, disulfiram is rapidly reduced to diethyldithiocarbamate which then reacts with thiol groups. Disulfiram and its catebolate are potent copper chelators, so they might affect the activity of many copper-dependent enzymes. Disulfiram is a relatively non-toxic substance except for some cases of liver toxicity.

Cobinamide is the penultimate precursor of hydroxocobalamin (vitamin B12) produced by microorganisms. It has recently been shown that it readily reacts with hydrogen sulfide, by neutralizing two moles of sulfide [37]. The effects of three different types of ligand of cobinamide to reverse hydrogen sulfide toxicity have been investigated in a lethal rabbit model [38]. Rabbits received a continuous infusion of hydrogen sulfide donors (NaSH). Dinitrocoaminamide was among the most effective compounds able to reverse hydrogen sulfide toxic effects [38]. Cobinamide or various types of ligand must be administered intravenously or intramuscularly in case of acute hydrogen sulfide toxicity but it has been suggested that it could also be used in prophylaxis [38]. The significant superiority of cobinamide over hydroxocobalamin in both in vitro and in vivo studies could be due to the fact that cobinamide has a higher affinity for hydrogen sulfide and/or to the ability of cobinamide to neutralize reactive oxygen species [39]. The administration of cobinamide 2 min after mice exposure to hydrogen sulfide has been shown to significantly and dose-dependently reduce lethality [40]. On the other hand, cobinamide-treated mice experienced significantly fewer seizures and knockdowns compared to the hydrogen sulfide-exposed group. Moreover, cobinamide has also been shown to reverse hydrogen sulfide-induced weight loss, behavioral deficits, neurochemical changes, cytochrome c oxidase inhibition and neurodegeneration in a dose- and time-dependent manner. Also, cobinamide increases survival and is neuroprotective in case of hydrogen sulfide-induced neurological sequelae. Thus, cobinamide could be a perfect drug candidate to be used in DS. Unfortunately, cobinamide is not yet
marketed but it should be the upcoming years [41].

Conclusions

The best therapeutic option to treat mental retardation in DS might be to use a CBS inhibitor in combination with a hydrogen sulfide scavenger. Benserazide is of particular interest because it may decrease blood levels of hydrogen sulfide through its inhibitory effect on CBS overexpression in the liver, kidneys and in other peripheral tissues. DS patients treated with benserazide should present blood levels of hydrogen sulfide ranging from 2 to 4 mmol/L. The best therapeutic option to treat mental retardation in DS might be to use a CBS inhibitor in combination with a hydrogen sulfide scavenger. Benserazide is of particular interest because it may decrease blood levels of hydrogen sulfide through its inhibitory effect on CBS overexpression in the liver, kidneys and in other peripheral tissues. DS patients treated with benserazide should present blood levels of hydrogen sulfide ranging from 2 to 4 mmol/L.

References