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REVIEW ARTICLE

PROMISING THERAPEUTIC APPROACHES IN DOWN SYNDROME: ADDRESSING PARENTS' DOUBTS

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ABSTRACT

Background and Objective: New therapies are emerging to improve intellectual disability (ID) in Down syndrome (DS) and caregivers seek clarifications from medical providers. The aim of this study is to review pharmacological and non-pharmacological therapeutic targets in DS, with emphasis on postnatal therapies and their potential clinical use.

Data source: A narrative review was conducted including studies in English, published before January 2022, on patients under eighteen years of age. Search strategy was developed using the keywords of this review.

Data synthesis: Three major therapies have been targeted to improve intellectual disability (ID) in Down syndrome (DS): green tea extracts containing epigallocatechin-3-gallate (EGCG), other antioxidants and fluoxetine. EGCG, the only studied in humans, has shown beneficial effects in visual memory recognition, spatial working memory and executive functions. Its use can be considered if the recommended dose and careful monitoring are respected. The benefits of other antioxidants are controversial. Fluoxetine trials in mice models, either prenatal or postnatal, are promising.

Conclusions: There is still not enough scientific evidence to recommend, without reservations, any pharmacological therapy to improve ID in DS. The adverse effects associated with some of these emerging therapies underline the need for proper counselling of the caregivers by medical providers.

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Introduction

Down syndrome (DS), also known as the underling genetic defect – trisomy 21, is a disorder characterized by microcephaly, typical facial features and global development delay/intellectual disability (ID) of varying degrees. It is the most common genetic cause of ID in humans with an incidence of approximately 1:1000 live births worldwide. ID severity may differ considerably between people with DS.[1]

Trisomy 21 is also the most frequent genetic cause of Alzheimer's disease (AD) and about 50% will develop AD-related dementia.[1] At the moment, there are no pharmacological treatments available for ID or AD in DS.[2]

So far, cognitive functions in DS have no response to traditional therapies, leading to the emergence of alternative approaches, which represents a new hope for caregivers. However, the use and opinions regarding these therapies in the DS community are still not well documented.[3]

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An increasing number of traditional and alternative therapies have been proposed, tested in preclinical DS mice models and progressed to clinical trials in individuals with DS.[2] Over the last few years, very promising results have been obtained with a mouse model of DS, the Ts65Dn.[2] This model has been used because genetic substrate, biological and behavioral abnormalities and neurogenesis defects largely overlap with those of the human DS brain.[4]

As brain changes in DS are present prenatally, this period represents a great opportunity for therapeutic interventions. Considering brain development timing it is reasonable to hypothesize that: (i) adult therapies could modulate ongoing hippocampal neurogenesis and also already existing hippocampal and extrahippocampal circuits; it also may be used to prevent AD-linked neurodegeneration; (ii) neonatal therapies may substantially contribute to hippocampal and cerebellar development; (iii) prenatal therapies possibly have the biggest impact by affecting development of the entire brain.[5]

Since hippocampal-dependent learning and memory are severely affected in DS, the adult studies have been focused on this structure. At this life stage, the duration of treatment effects remains a matter of investigation.[5]

This paper aims to review pharmacological and non-pharmacological therapeutic targets in DS, with emphasis on postnatal therapies and their potential clinical use.

Methods & Materials

Literature search was performed between January and March 2022, through the following databases: MEDLINE via PubMed, Embase via OVID and the Cochrane Library. A narrative review was conducted and the search strategy was developed using the keywords of this review.

The literature search included all case studies, case series, case-control studies, retrospective and prospective studies in English, published before January 2022, on patients under 18 years of age. References from the retrieved studies were manually searched to identify additional papers.

Results

Three major therapies have been targeted in several studies and aroused the interest of the scientific community and caregivers: green tea extracts (GTE) containing epigallocatechin-3-gallate (EGCG), other antioxidants beyond EGCG and fluoxetine.

GTE containing EGCG

Evidence suggests that cognitive function can be improved by flavonoid-rich foods (e.g. green tea) due to antioxidant effects, which also have therapeutic benefits in AD.[6] Bain and colleagues described the non-competitive inhibition properties of EGCG, the major catechin in green tea leaves, on the kinase activity of the dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A (DYRK1A), encoded by the DYRK1A gene that is located in the DS critical region (i.e. on the long arm of chromosome 21) and thought to be a major contributor to cognitive phenotypes of DS.[7]

Improving the expression of DYRK1A in mice models was the major purpose of Jean-Maurice Delabar's group. Controlled correction of DYRK1A levels with EGCG, from prenatal to adult stages, induces a correction of synaptic markers associated with GABAergic and glutamatergic pathways. To get the most efficient rescue it seems therefore necessary to combine actions targeting these two pathways. Cognitive and behaviour testing revealed a significant improvement in novel object recognition memory on adult mice treated prenatally.[8]

The effectiveness of EGCG on the promotion of adult hippocampal neurogenesis has been reported[9] suggesting its potential clinical usefulness in neurodevelopmental and neurodegenerative disorders. A study evaluated EGCG effects on cognitive functions in adult DS mice and in DS subjects. Thirty-one young adults with DS (14 to 29 years old) were enrolled and EGCG solution was prepared from a green tea extract (Mega Green Tea Extract, Lightly Caffeinate® (0.8% caffeine) Life Extension R®, USA; EGCG content of 326.25 mg per capsule) every 3 days (EGCG concentration: 90 mg/mL for a dose of 2–3 mg per day). Participants assigned to the treatment or placebo group received one or two daily capsules depending on body weight, with a mean EGCG oral dose of 9 mg/kg/day (range 6.9–12.7). EGCG treated individuals have shown a higher accuracy in visual memory recognition and spatial working memory. Overall, the results are consistent with EGCG effects found in mice models and suggests that short-term EGCG treatment can improve cognition in young adults with DS. EGCG administration also led to improvements on both the quality of life and social functioning.[10]

EGCG has been found to be a safe substance. No liver dysfunction was observed and there was an improvement on the oxidative/antioxidative status combined with a healthy lipid profile.[10]

As occurred in mice, a clear relationship between memory improvement and homocysteine (Hcy) levels was found in humans, suggesting a direct dependence of cognitive improvement on DYRK1A activity. Also, after three months of EGCG discontinuation, cognitive effects declined along with a parallel decrease in plasma Hcy levels, suggesting that DYRK1A activity normalization induced cognitive changes through temporary neuronal modifications. This has functional implications, since DS learning and memory difficulties become remarkably more evident through childhood and adolescence and appear to be related to memory consolidation, which is a hippocampal system function.[10]

Through DYRK1A activity normalization, EGCG seems to promote learning and it is possible that a greater benefit could occur if associated with other interventions that also improve cerebral plasticity, such as cognitive stimulation.[10] Such assumption was studied in a double-blind, placebo-controlled, phase two, single centre trial.[6] The authors theorized that the effects of non-pharmacological cognitive rehabilitation in young adults with DS would be improved by administration of a green tea extract containing EGCG. For 12 months, young adults from 16 to 34 years old were enrolled to receive green tea extract supplement containing 45% EGCG (Life Extension Decaffeinated Mega Green Tea Extract®, 9 mg/kg per day) or placebo (rice flour) and cognitive training. Participants weighing 50–75 kg were given 600 mg/day of EGCG or placebo and those weighing 75–100 kg were given 800 mg/day of EGCG or placebo orally for 12 months and had a follow-up visit six months after treatment discontinuation. All patients received regular cognitive training sessions (30–50 min per session, three days per week) during the 12 months of treatment.[6] In this study, concerning the association of EGCG with cognitive training, beneficial effects were found both on memory and executive deficits, with enhancement of the everyday life skills after the 12 months of treatment. Moreover, sustained effects were observed after 18 months in memory and executive function. No adverse effects were reported. Nevertheless, cardiac toxicity at higher doses cannot be excluded and caution should be taken when EGCG is given along with cardiovascular drugs.[6] Although this study supports the EGCG use in clinics, more phase two and three clinical trials are needed.[6]

The therapeutic potential of EGCG in the mitigation of facial dysmorphologies associated with DS was also matter of evaluation. Starbuck and colleagues performed an experimental study with continued pre and postnatal treatment with two doses of EGCG supplementation in a mouse model and also an observational study with DS children that took EGCG as a green tea supplement. The effect of high (100 mg/

kg/day) or low doses (30 mg/kg/day) of EGCG, in the Ts65Dn mouse model, administered from embryonic day nine to post-natal day 29, was evaluated in the first study. The second one analysed a human sample of 288 children from zero to 18 years old, to whom available GTE containing EGCG obtained over the counter was administered. In this study, the onset and duration of treatment, dosage and brands were variable.11 The lowest tested EGCG dose improved the facial skeleton morphology in the mouse model. Similar results were observed in humans whenever treatment was administered during the first three years of life. However, higher EGCG dosing disrupted normal development and even increased facial dysmorphic features in both trisomic and euploid mice. This leads to the conclusion that EGCG modulates facial development with dose-dependent effects. Considering the potentially detrimental effects observed in mice, the therapeutic relevance of controlled EGCG administration towards reducing facial dysmorphology in young children with DS needs to be further investigated in clinical studies.[11]

Beyond DYRK1A overexpression, oxidative stress and mitochondrial dysfunction were also described in DS. EGCG has antioxidant properties and in vitro, reverted DS mitochondrial dysfunction. Scala et al.[12] found that decaffeinated EGCG plus omega-3 can be safely administered under medical supervision in DS children from one to eight years old with effective reversion of the mitochondrial I and V complexes activities deficits. In this study, a once a day 10 mg/kg dose of pure caffeine-free EGCG (EGCG > 90%, powder) was given during six months, suspending the powder in omega 3 (half or one teaspoon depending on the amount of EGCG: 250–500 mg EPA + DHA/day) to enhance EGCG bioavailability. No serious adverse events were reported. A reduction of plasma folates was observed in 3/14 (21.4%) of the patients who completed the study, with levels normalization after two months of levofolinic acid supplementation. As a secondary endpoint, it was explored whether EGCG could improve mental and psychomotor development in DS children applying GMDS-ER (Griffiths Mental Developmental Scales—Extended Revised), but results were inconclusive. This study emphasizes the importance of EGCG dose-effect relationship and the need of medical supervision in polyphenol supplementation. The evidence that treatment from postnatal day three (P3) to postnatal day 15 (P15) rescues numerous trisomy-linked brain alterations but does not elicit durable effects on the hippocampal physiology, suggests the need of prolonged EGCG administration.[13]

Another survey, "Attitudes about and usage of EGCG as a therapy in individuals with Down syndrome" was designed to characterize the DS community's knowledge, perception and experience with GTE and quantify its administration in patients with DS. GTE containing EGCG was provided by 18% of responding caregivers who were mostly younger, highly educated and used scientific sources and other parents feedback as information channels. Patients with DS who received EGCG were characterized as being less severely disabled. Most caregivers that did not administered this treatment reported concerns about its potential side effects and lack of effectiveness. Few caregivers consulted medical providers before taking their decision. Patients on treatment had between 0 to 27 years-old (M=6.5 years) and most received EGCG for over a year. The majority (57%) were over 10 years-old, but 72% had begun treatment before this age and a remarkable percentage (19%) was already receiving it in the first year of life. The main reasons for treatment discontinuation were side effects occurrence, lack of improvement and costs. A wide range of EGCG doses was referred, with a mean of 351 mg/day and a maximum of 2 g/day.[3] It should be noted that the small human studies administered GTE with 9 mg/kg/day EGCG for three months to individuals from 14-19 years of age[10] and 8-12 mg/kg/day for 12 months to individuals from 16-34 years.[6] EGCG treatment was linked to hepatotoxicity which is likely dependent on the dosage, route of administration and EGCG or other catechin content. Adverse effects occurred mainly when high concentrations of GTE or EGCG were taken in a single dose, with fewer adverse effects reported when it was taken as a beverage. Safe levels of EGCG in humans have been estimated in around 300 mg/day. Dosages from 150-800 mg/kg have been related to liver, kidney, thymus, spleen and pancreas damage in adults. The high risk of hepatotoxic interactions between dietary supplements and other drugs should be considered.[3] This aspect is particularly relevant when it is known that, on average, each child with T21 receives 3.3 supplements per day and their consumption is often not reported to the attending physician.[3] Also, the brand of the products used greatly varied, with only five individuals receiving the brand used in the small clinical trials conducted (Life Extensions®). This is noteworthy, given the lack of effective regulation of these products - which may not have the advertised amount of EGCG, as may contain polyphenols, possibly affecting the EGCG bioavailability.[3]

These results draw the attention to the need of a clearer communication between caregivers, medical providers and scientists about the potential benefits and risks of GTE, EGCG and other nutritional supplements intake in individuals with DS.[3]

Antioxidants (beyond EGCG)

Oxidative stress is associated with an imbalance between free radicals and reactive metabolites production and the antioxidant defenses. In DS, it has been associated not only with the trisomy of the 21st chromosome per se, but also with several of the organic morphological and immune disorders, as well as with the intellectual disability and premature aging.[14]

Acknowledging that, the possible use of oxidative stress markers in the prenatal screening of DS was studied, revealing both increased levels of DNA/RNA oxidative stress damage products and asprosin and decreased levels of vitamin D and alfa-1-antitrypsin. The role of these markers, especially asprosin, in prenatal DS screening seems promising.[15]

Studies examining effects of antioxidant elements on DS pathophysiology were not conclusive. Zinc (25-59 mg/day) administered for six months had no effect on lymphocyte functions but beneficed daily cough.[16] Selenium (10 µg/kg/day) administered for six months has increased the levels of IgG and decreased

infections.[17] Supplementation with megavitamin mixtures together with minerals was not beneficial and therefore its administration is not recommended. In a study of Lott et al.[18] a daily administration of α-tocopherol (900 IU), ascorbic acid (200 mg) and α-lipoic acid (600 mg) to 53 individuals with DS and AD for two years had no impact on cognitive functions compared to placebo. The same occurred in another study of 156 DS children who were supplemented with antioxidants, including reduced form of folic acid.[19]

Melatonin was also studied, considering its strong antioxidant properties, with neurodegenerative processes reduction and improvement of cognitive deficits in several animal models. Corrales et al.[20] found that it might improve the cognitive abilities of both Ts65Dn mice and control mice by reducing the age-related degeneration of basal forebrain cholinergic neurons. A human controlled trial evaluated serum melatonin and urine tryptophan metabolites in 15 children with DS and in non-DS children. Lower levels of serum melatonin and urinary kynurenine (metabolite of amino acid tryptophan) were found in DS patients, although the level of nocturnal secretion of melatonin was higher[21], suggesting a potential beneficial effect of melatonin treatment.

The Brain Derived Neurotrophic Factor (BDNF), a protein formed in the brain that promotes synaptic plasticity and neurons growth and survival was also studied as a potential DS therapeutic target. A study by Cotman and Engesser-Cesar found increased BDNF gene expression in animal experiments depending on enhancing physical activity during voluntary wheel running.[22] Cotman and Berchtold, found that exercise mobilizes expression of genes that affect brain plasticity processes, in addition to elevation of BDNF levels in animal experiments.[23]

A study with DS adults proved that lipid peroxidation, a marker of oxidative stress, decreased in saliva with aerobic exercise.[24] Plasma oxidative stress markers were reduced in DS adolescents who experienced a 12-week aerobic training program.[25]

Fluoxetine

Defective neurogenesis and severe dendritic pathology are important determinants of ID.[26] This knowledge generated interest in antidepressants as they enhance hippocampal neurogenesis[27] and fluoxetine, a selective serotonin reuptake inhibitor, was shown to increase neurogenesis in adult Ts65Dn mice.[28]

A mouse model by DS Bianchi et al.[4] demonstrated that treatment with fluoxetine in the early postnatal period P3-P15 restored neurogenesis and the total number of neurons in the dentate gyrus, which was accompanied by full recovery of a cognitive task.

Guidi's group studied fluoxetine effect on the dendritic development of mice aged 45 days, treated in the same early postnatal period. They found the restoration of the dendritic architecture and spine density of trisomic granule cells: not only the number of granule neurons was re-established but also their "quality" in terms of adequate maturation and connectivity. A short treatment period of 13 days was sufficient to rescue dendritic development and its effect remained for up to one month after treatment cessation. These findings strongly suggest that fluoxetine may be considered for the improvement of ID in DS and should lead to future clinical trials in children and adolescents with DS.[26] Moreover, another trial investigating the effects of fluoxetine during the embryonic phase, concluded that it fully rescued the abnormal brain development and the behavioural deficits typical of DS individuals.[29]

In practice and when applied to humans, the above data suggests that a simple pharmacological treatment during the earliest phases of development might improve neurogenesis and eventually, ID in infants with DS.[4] The outcome of fluoxetine treatment on adult DS patients would probably present a different outcome. Using a Ts65Dn mouse model of DS, Heinen et al.[30] studied the impact of an adult-start, chronic treatment with fluoxetine on behaviour without finding any beneficial effects. The hypothesis of the presence of genotype-dependent fluoxetine side effects was raised. Seizures and mortality were reported in treated Ts65Dn mice, but not in wild-type controls.

These studies highlight that early postnatal and adult-onset fluoxetine treatments may have distinct effects on behavioral impairments in Ts65Dn mice.

Discussion

The potential treatment of ID in DS has led to the emergence of several studies to evaluate both pharmacological and non-pharmacological therapies. However, it is a challenge to compare their outcomes since there are no common protocols: doses, route of administration and duration of treatments have been very different.[5]

Moreover, despite the promising results with some treatments, it is important to point out that most of the studies were performed in mouse models, precluding its prompt use in humans.[5]

Early therapies seem more promising in improving cognitive function, but postnatal period should not be neglected since slight contributions in learning or memory could represent a major achievement to patients and families.

Studies evaluating the effectiveness of EGCG are among the few performed in humans. Positive effects were demonstrated on cognition, memory, quality of life and social functioning. Nevertheless, the authors emphasize the importance of larger studies to establish the optimal dosage and timing for EGCG treatment while trying to minimize side effects. As EGCG is commercially accessible over the counter, it is extremely important to warn caregivers for the risks of its unsupervised administration.[11] Medical follow-up is essential not only to identify clinical benefits and to determine the proper dosage, but also to prevent and early detect side effects.[12]

Even though individuals with DS present with oxidative stress, antioxidant interventions, besides EGCG, have not shown clinical benefits. The choice of the antioxidants, doses and duration of the treatment may have been responsible for these unexpected results.[14] In the other hand physical activity and regular exercise, which antioxidative stress properties have been recognized,[31] seem to have a positive impact on cognitive functions and benefit people with DS and this should be emphasized to DS patients and their families.

Although fluoxetine is a very promising drug, so far, research has focused only on mice, thus lacking clinical trials to validate its effectiveness in humans.

As scientific evidence is still scarce, the potential risks of these therapies should be carefully weighed against potential risks and disease progression.

Another critical aspect that requires further investigation is the durability of the treatment effects after its cessation.[5] Prolonged treatment appears to be necessary even when started at an early stage.

Conclusion

New therapies are emerging for ID in DS and many caregivers are already administering them without medical advisement and lacking scientific foundations. EGCG is the only treatment studied in human trials and may be considered if the recommended dose is respected and careful monitoring is taken. Fluoxetine may be a very promising therapy if it replicates in humans the effects observed in mouse models. The adverse effects reported with some of the therapies underline the need for a close communication between caregivers and medical providers.

Authors Contribution

- Contributions to the conception or design of the work: Catarina Prior
- Acquisition, analysis and interpretation of data for the work: all authors
- Drafting the work and revising it critically for important intellectual content: all authors
- Final approval of the version to be published: all authors
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: all authors
- Study supervision: Inês Vaz Matos, Catarina Prior

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