The sooner the better: a “magic formula” to cure intellectual disability in Down syndrome?

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Down syndrome (DS) is characterized by brain development alterations that translate as intellectual disability (ID). Impaired brain development is mainly due to defective neuron generation and maturation, events that start from the earliest foetal life stages. Despite numerous efforts of the scientific community, there are currently no therapies for ID in DS. In the past decade, our group has demonstrated that early treatment with fluoxetine resulted in the restoration of neurogenesis and behavioural deficits in Ts65Dn mice, the most widely-used DS model. These findings set the basic idea of my therapeutic approach: “The sooner, the better”. Thus, the focus of my PhD project was on evaluating whether neonatal treatment with “unexplored” molecules restores the major neurodevelopmental defects (with particular interest with regard to the hippocampus) and cognitive performance in the Ts65Dn mouse. Based on different rationale, I have evaluated the effects of three molecules (ELND006, EGCG, and 7,8-DHF). Among these, the most promising is 7,8-DHF, a natural compound similar to BDNF (a protein that supports survival and growth of neurons), that activates its signalling pathway. Early treatment with 7,8-DHF stimulates a large improvement in hippocampal development and fully restores hippocampus-dependent memory, maintaining a safe profile. These results suggest an important translational impact of this molecule and highlight the role of timing in treatment efficacy.

What we face every day

Down syndrome (DS) is characterized by many phenotypic features, among which the most invalidating is intellectual disability (ID), that affects almost all those who have this pathology [1]. We have a wealth of information about the phenotypic causes of ID (i.e., neurogenesis and neuronal maturation impairment), but little knowledge is available concerning the molecular basis that regulates this aberrant process. DS is an extremely complicated pathology (around 300 coding genes are triplicated; [2, 3]) and this is the main reason why there is currently no effective therapy to restore brain development.

Neurodevelopmental defects in Down syndrome: what happens to neurons

The first question that arises when we talk about ID in DS is “Why do all individuals with DS show this phenotype?” From prenatal life stages, people with DS have an overall reduced brain volume (Figure 1) [1]. Brain volume reduction is, in turn, due to a low number of nerve cells, suggesting defects in proliferation potency starting from the earliest phases of neurogenesis (the process by which new nerve cells are born). In addition, this condition is worsened by alterations in neuron maturation, the mechanism that enables a nerve cell to cross-talk to another.

Timing for therapy is the big deal: “the sooner the better”

Neurogenesis and neuron maturation are early events in human foetal development [4], and neurodevelopmental alterations start just as early in DS. Thus, treatments should be administered as early as possible [5], [6]. In the past decade, our group has produced several studies in which we have shown that early treatments may restore brain development in DS. In our research we have used a mouse model of DS, the Ts65Dn mouse. This mouse is globally considered to be a good model for DS because it recapitulates the developmental alterations that characterize this pathology. In my thesis work I tried to stress the concept “The sooner, the better”. These four words highlight how important it is to choose the right temporal window for pharmacotherapy.

Is there a panacea for DS? More arrows in your bow

Our group has been studying the effects of fluoxetine (or Prozac, an antidepressant of SSRI class) on the Ts65Dn mouse for a long time, and demonstrated that a neonatal treatment resulted in the restoration of neurogenesis and behavioural deficits, and that these positive effects lasted after treatment cessation. We strengthened this evidence by treating Ts65Dn mice prenatally with fluoxetine. The treatment restored neurogenesis and cell density throughout the forebrain, with positive effects on learning and long-term memory [7, 8]. These results showed, for the first time, that the neurodevelopmental defects that characterize DS are reversible, provided that therapy is administered very early during the lifespan. After these promising results, the final step is the design of a clinical trial in order to evaluate whether fluoxetine could be considered as a “panacea” for ID in DS. A trial to verify the safety of fluoxetine in a paediatric population is currently underway in Italy at University Federico II of Naples. Despite these strong bases and our hopes, we cannot take it for granted that a therapy that is effective in a mouse model is similarly effective in humans. Thus, it is important to establish whether there are other molecules that are as effective as (or more effective than) fluoxetine.

My PhD goal and results. We tried to find a novel, effective, and safe pharmacological approach for ID in DS

In order to find other compounds capable of restoring brain development in DS, we administered three different molecules to the Ts65Dn mouse, based on the rational basis reported hereafter. In my work as a whole I focused on the hippocampus, a brain region that is fundamental for learning and long-term memory, and that is particularly affected in DS. The first molecule I tried was ELND006 (ELN). ELN is a selective inhibitor of a mechanism that brings about the formation of AICD, a small segment derived from the processing of Amyloid Precursor Protein (APP, a triplicated gene in trisomy 21), that, when accumulated, (in DS and Ts65Dn) can reduce neurogenesis. Therefore, the inhibition of AICD production in the early neonatal period may revert the trisomy-linked neurogenesis defects. We administered ELN to Ts65Dn mice in the first two postnatal weeks and examined the effects immediately after (short-term effects) and one month after (long-term effects) treatment cessation. We chose this temporal window because in the first two postnatal weeks the bulk of new neuron formation takes place in the mouse hippocampus. We found that ELN reinstated various neuroanatomical alterations in the hippocampus of Ts65Dn mice and that these effects were retained one month after treatment cessation. Importantly, treatment also restored the electrical functionality of the hippocampus. Unfortunately, treatment had long-term adverse effects on mouse viability. Another molecule we tried was the antioxidant epigallocatechin-3-gallate (EGCG), a natural inhibitor of DYRK1A, which is a protein produced by a triplicated gene in individuals with DS and Ts65Dn mice. It has been proposed that DYRK1A could play a pivotal role in neurogenesis impairment in DS. We administered EGCG to neonate Ts65Dn mice, as we had done for ELN, in order to examine short- and long-term effects on neurogenesis. Immediately after treatment cessation, we found that EGCG restored hippocampal neurogenesis and connections between neurons. These effects, however, disappeared in adolescent mice (after one month) which, in addition, showed no long-term effects on hippocampus dependent learning and memory. Thus, in order to see lasting effects, treatment with EGCG should not be discontinued. 7,8-dihydroxiflavone (7,8-DHF) is a natural molecule that has been shown to be able to activate an important endogenous protein system, the Brain-Derived Neurotrophic Factor (BDNF) signalling pathway. The BDNF/TRKB system, which plays a key role in neurodevelopment, is deregulated in DS, suggesting that this deregulation may contribute to trisomy-linked brain alterations. We treated Ts65Dn pups with 7,8-DHF (in order to mimic the action of BDNF) for two weeks. We found a large improvement in hippocampal neurogenesis and full rescue of neuron connections. (Figure 2). Importantly, Ts65Dn mice that were treated continuously for 45 days exhibited full rescue of hippocampus-dependent memory and no side effects (Figure 2). In the last part of the study, we treated 5-month old Ts65Dn mice with 7,8-DHF for one month and found no effects on brain architecture or cognitive functions. This puts the accent on the importance of timing of therapies.

Conclusions

In my thesis work I exploited the Ts65Dn mouse in order to evaluate the effects of three different molecules on hippocampus development in the neonatal period. A comparison of the three therapies indicates that all of them are able to rescue hippocampal development. Treatment with ELN, despite its strong effects, may pose some caveats for human use. It would be interesting to try other molecules with a common rationale, hoping for a safer profile. An ongoing clinical trial in young adults with DS shows that EGCG plus cognitive stimulation exerts some benefits on cognition. Our study suggests that treatment with EGCG alone may be effective in infants with DS, but should not be discontinued. Early treatment with the flavone 7,8-DHF fully restored hippocampus-dependent memory and had a safe profile, suggesting an important translational impact. In contrast, in another study that we published after completion of my PhD, we show how a discontinued treatment with 7,8-DHF does not have any positive impact on hippocampus dependent learning and memory. These results indicate that the timing of the administration of this molecule is critical for the attainment of positive effects on the brain [9]. The demonstration that it is possible to pharmacologically prevent brain alterations in a model of DS with 7,8-DHF may stimulate the design of clinical trials that could begin during pregnancy after diagnosis of trisomy 21, or in the early stages of postnatal life in children with DS. Numerous studies have shown that antioxidant therapy can have positive effects in mice, but when administered in humans, the effect was ephemeral [10]. Nevertheless, in a recent review by our group, we report how 7,8-DHF could be the right molecule for many pathologies, including several brain disorders [11]. Taken together, these findings give new hope for future interventions aimed at ameliorating the quality of life of people with Down syndrome and their families.

In the meantime…

It has been more than 2 years since I discussed my PhD thesis. Meanwhile, a lot of hard work has been done by colleagues all over the world in searching for new therapies for intellectual disability in Down syndrome. In 2020 alone, a year in which research was greatly stalled due to the Corona crisis, 10 articles were published on possible new pharmacological approaches for ID in DS with novel molecules tested in the Ts65Dn mouse [12]. I think we are on the right path to solving the complicated puzzle that characterizes Down syndrome.

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Take home message

Abnormal brain development, and in particular neurogenesis and neuron maturation defects, are key determinants of intellectual disability in Down syndrome. No therapies are currently available for ID in DS.

Good timing (early treatment), plus the right molecule may represent an effective combination with which to restore brain development disorders, and thus intellectual disability, in individuals with Down syndrome.

7,8-DHF, a natural flavone that mimics BDNF, restores hippocampus-dependent memory and has a safe profile, suggesting an important translational impact of this molecule.

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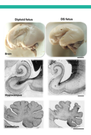

Figure 1. Brain hypotrophy in Down syndrome fetuses. Examples of the brain (scale bar 1 cm), hippocampus (scale bar 1 mm), and cerebellum (scale bar 2 mm) of a diploid (left) and a DS fetus (17–21 GW) (right). Images were taken and modified from [1], [13]. Abbreviation: DS, Down syndrome.

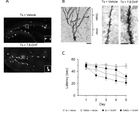

Figure 2. Main results of neonatal treatment with 7,8-DHF on P15 Ts65Dn mouse model. (A) Effects of treatment on the size of the population of cells in the S-phase of the cell cycle in the dentate gyrus of the hippocampus; (B) Effects of treatment on dendritic spine density in the dentate gyrus of the hippocampus. (C) Effect of treatment on spatial learning in young adult Ts65Dn mice. Abbreviations: DHF, dihydroxyflavone; Eu, euploid; Ts, Ts65Dn.