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Proceeding Paper

Antipsychotic Drugs Counteract Autophagy and Mitophagy in Multiple Sclerosis

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Abstract: Background: Multiple sclerosis (MS) is a neuroinflammatory and neurodegenerative disease characterized by myelin damage followed by axonal and ultimately neuronal loss. The etiology and physiopathology of MS are still elusive, and no fully effective therapy is yet available. **Objectives:** We investigated the role in MS of autophagy (physiologically, a controlled intracellular pathway regulating the degradation of cellular components) and of mitophagy (a specific form of autophagy that removes dysfunctional mitochondria). **Methods:** Our study has been performed by using in vitro, ex vivo and in vivo model of MS. Furthermore, experiments have been also conducted in human biofluids obtained from healthy and MS-affected individuals. **Results:** Three main findings emerge from the present work. First, autophagy and its selective forms occur in MS patients and in experimental models of MS; second, these phenomena play a causal role in MS be-cause their inhibition prevents myelin loss; third, two clinically used drugs can inhibit autophagy, prevent demyelination, induce remyelination, and revert MS behavioral deficits. **Conclusions:** Our findings suggest to repurpose Food and Drug Administration–approved drugs for the treatment of MS, at least in patients with MS variants that are more closely modeled by CPZ, like type III and IV. Such compounds may accelerate recovery from a demyelinating attack and prevent relapses.

Keywords: keyword 1; keyword 2; keyword 3 (List three to ten pertinent keywords specific to the article yet reasonably common within the subject discipline.)

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Autophagy is a fundamental cellular pathway present in all the cell of our body. Firstly identified as the mechanism responsible to face periods of nutrient deprivation, nowadays autophagy intervenes following several stimuli and can mediate the degradation of damaged and harmful molecules, pathogens and organelles [1–3]. In this last case, the autophagy towards mitochondria (named mitophagy) is the most important and studied selective form of autophagy [4]. Autophagy and mitophagy control the cell death and survival. Therefore, it is not surprising that these mechanisms are involved in almost all human diseases, including neurodegeneration. In this context, several works demonstrate that autophagy and mitophagy work to avoid the progressive loss of neural cell [5]. This mainly occurs for the neurological disorders characterized by an aberrant accumulation of misfolded protein. Consistently, autophagy and mitophagy are deputed to remove the intracellular protein accumulation and to preserve a health mitochondrial population. At the same time, however, excessive activities of these catabolic process may be deleterious for the cells and provoke the cell death. Multiple Sclerosis (MS) is not characterized by an aberrant accumulation of damaged and misfolded protein. MS is a multifactorial neuroinflammatory disease, in which immune cells promote the myelin degradation [6]. This immunological damage is further sustained by the proinflammatory cytokines secreted by macrophage and microglia that promote neuronal, oligodendrocyte, and vascular damage in vitro and in vivo models of MS [7]. In this picture, the role of autophagy and mitophagy in MS remains still elusive. Investigations demonstrate that autophagy is a protective

mechanism [8–10]. Opposite, some studies describe autophagy as a maladaptive process that support the progressive loss of myelin. Recently, we demonstrated that the loss of mitochondrial functions is fundamental to block the maturation of a precursor oligodendrocyte cell into a mature and myelinating oligodendrocyte. Furthermore, we and other research groups also found excessive levels of autophagy and mitophagy elements in the cerebrospinal and serum of MS-affected patients [11–14]. Interestingly, these increased levels also correlated with the active state of the pathology. At first sight, these studies suggest that autophagy and mitophagy are deleterious for a person affected by MS. However, our study lacked to effectively demonstrate that such increased autophagy and mitophagy levels were effectively due to an excessive activity of these processes and not be due to an aberrant accumulation of autophagy and mitophagy elements caused by a failure of degradation of autophagy vesicle. Taking account of this, we decide to perform deeper investigations to finally address about the role of autophagy and mitophagy in MS.

The first part of our investigation was performed in vitro, in which purified cultures of oligodendrocyte precursor cells and the more physiology cultured of mixed-glial (composed of astrocytes, neurons, oligodendrocytes and glia cells) were exposed to pro-inflammatory cytokines. Interestingly, we found that in our experimental models the progressive loss of myelin (induced by the inflammatory environment) was always accompanied by a progressive increase of autophagy and mitophagy. Most importantly, by conducting specific experiments aimed to verify whether the formation of autophagosome was always accompanied by its degradation, we finally demonstrated that the excessive presence of autophagy and mitophagy markers was due to an uncontrolled activity of these cellular mechanisms. Then, we asked whether these observations were also conserved in more complex MS-model, which are ex-vivo organotypic brain slices and an in vivo MS model (the mouse model of demyelination induced by cuprizone). Remarkably, all the compounds capable to induce demyelination in the brain slices (lysolecithin) and in vivo (cuprizone) triggered the autophagy and mitophagy pathways and provoked loss of function of the mitochondrial compartment.

Having found excessive activity of this catabolic pathways, we asked whether by using compounds capable to interfere with these mechanisms it could be possible to block the progressive loss of myelin and reactivate the myelinating process. Recent investigations demonstrated that anti-psychotic compounds may have an unexpected role to block autophagy. In particular, it has been suggested that haloperidol and clozapine may have these effects in neurons and act as similarly to the most known autophagy inhibitor chloroquine (CQ). CQ is also used in clinic to treat several pathologies. Furthermore, CQ has been also proposed as a potent anti-cancer and anti-inflammatory agent [15,16]. However (as also demonstrated by the recent efforts to use CQ to block the COVID-19), CQ has serious side effects. Considering that haloperidol and clozapine are commonly used by people, we tried to demonstrate that these agents may be also useful against MS, and we also tried to give a molecular interpretation. We verified whether this also occurred in our cellular in vitro cultures models and we found that both clozapine and haloperidol block autophagy at the late stages. But, undoubtedly, the most important readout of our experiments was that these compounds also reactivated the myelin production. Next, we moved to perform the experiments in the brain slices pre-treated with the demyelinating agent lysolethicin. Also in this model, clozapine and haloperidol improved not only the myelin production but also the myelination rates. We considered this aspect of particular importance since several compound which were proposed to improve the myelin levels, failed to promote the axon myelination. Finally, we verified the efficacy of haloperidol and clozapine to improve the myelination process in vivo. To this, we took advantage of the cuprizone moose model of demyelination, in which mice are fed with 0.2% cuprizone for 5 weeks. During this period, mice undergo to demyelination. After cuprizone withdrawal, the myelination process reactivates spontaneous. We performed two types of in vivo experiments. In the first, clozapine and haloperidol were administrated after the 5-

weeks of cuprizone treatment. In the other, antipsychotic compounds were given together cuprizone. The results obtained were surprising: clozapine and haloperidol were able to improve the remyelination process (in the first set of experiments) and, at the same time, block the demyelination as observed in the second set of experiments. But, most importantly, we observed that such compounds also improve the motor behaviors of mice.

In conclusion, our data suggests that haloperidol and clozapine can accelerate the myelinating process and prevent relapses, which are typical in patients with relapsing-remitting variant of MS.

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