Non-lysosomal β-glucocerebrosidase inhibition with ambroxol hydrochloride in an animal model of amyotrophic lateral sclerosis

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Title: Neuroprotection and axonal plasticity after inhibition of non-lysosomal glucocerebrosidase with ambroxol hydrochloride in an animal model of amyotrophic lateral sclerosis

***Introduction*:** Metabolomic and transcriptomic reports connect glucosylceramide and other glycosphingolipides to neurodegeneration and muscle denervation in amyotrophic lateral sclerosis (ALS) (1). Glucosylceramide is degraded by GBA1 and GBA2 β-glucocerebrosidases and their inhibition with conduritol B epoxide (CBE) is beneficial in an animal model of ALS (2). Ambroxol hydrochloride is a safe drug known to inhibit GBA2, a non-lysosomal form of β-glucocerebrosidase. The aim of the study was to investigate whether ambroxol hydrochloride could be a drug candidate for amyotrophic lateral sclerosis.

***Method*:** *In vitro* β-glucocerebrosidase activities were determined using an assay based on 4-Methylumbelliferyl-β-D-glucopyranoside (n=5-6/group). Adult FVB female SOD1^{G86R} mice and non-transgenic mice were used. *In vivo* experiments followed current European Union regulations and were approved by the local ethic committee (Aafis#4555). Ambroxol hydrochloride was given through drinking water (160mg/kg/day, vehicle: drinking water). Muscle innervation was assessed by histology (n=4 WT; n=7/group SOD1^{G86R}mice). Disease progression was monitored daily (n=14/group). *In vitro* axonal plasticity was studied with a co-culture of spinal cord explants and myoblast cells (n=3-7 spinal explant/group). Data was expressed as the mean±SEM. Difference among groups was assessed with ANOVA followed by two-tailed Fisher's LSD or Log-rank test. p-values<0.05 were considered significant.

***Results*:** Compared to controls, spinal cord of SOD1^{G86R} mice had higher GBA2 activity (100%±16 versus 301.9%±78.5, n=6). Ambroxol hydrochloride (100µM) did not affect GBA1 activity but inhibited 50% of GBA2 activity (Fig. 1A, B). In vivo, ambroxol hydrochloride improved muscle innervation (Fig. 1C) and extended survival (Fig. 1D) in symptomatic SOD1^{G86R} mice. In vitro, ambroxol hydrochloride (100µM) enlarged functional neurite network (Fig. 1E).

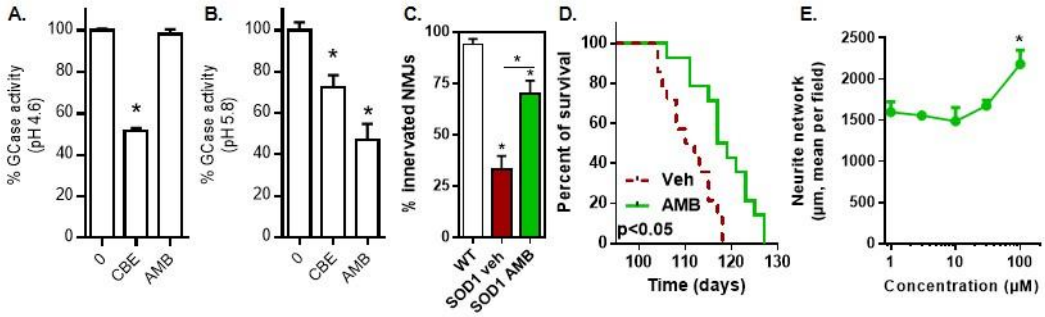

Figure 1. Ambroxol hydrochloride supports axonal plasticity and improves survival in SOD1^{G86R} mice. A-B. CBE and ambroxol hydrochloride's effect on in vitro GBA1 (A) or GBA2 (B) activities. C. AMB's effect on muscle innervation. D; Kaplan-Meier showing survival of SOD1^{G86R} mice. E. Neurite network of spinal explants. NMJs, neuromuscular junctions. AMB, ambroxol hydrochloride. *, p<0.05.

***Conclusions*:** Glycosphingolipids play an important role in muscle innervation, which degenerates in ALS from a very early disease stage. Ambroxol hydrochloride is a safe drug which may prove to be effective in this devastating disease.

References:

[1] Henriques A, et al. (2015). *Hum.Mol.Genet.* **25**:7390-7405
[2] Henriques A et al. (2017) *Scientific Reports.* **7**:5235