

The cysteine protease inhibitor, E64d, reduces brain amyloid-beta and improves memory deficits in Alzheimer's disease animal models by inhibiting cathepsin B, but not BACE1, beta-secretase activity

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The abnormal accumulation of brain amyloid-beta peptides (A $\beta$ ) is thought to cause Alzheimer's disease (AD) and compounds, which reduce A $\beta$ , are likely to be disease-modifying AD therapeutics. A $\beta$  are produced by a protease(s), functionally called beta-secretase, which cleaves amyloid-beta protein precursor (APP) at the beta-secretase site sequence, which in most AD patients is the normal wild-type (WT) sequence. Compounds, which inhibit WT beta-secretase activity and thereby reduce brain A $\beta$  production, are likely to be broadly effective AD therapeutics. Intracerebroventricular administration of the small molecule cysteine protease inhibitor E64d to transgenic mice expressing human APP containing the WT beta-secretase site sequence and the London mutant gamma-secretase site sequence (APPLon) reduces brain A $\beta$  and improves the memory deficits and brain amyloid plaque, which develop in those mice and mimic that seen in AD patients. Although E64d is thought to inhibit WT beta-secretase activity, it is not clear if it is inhibiting the cysteine protease cathepsin B (CatB) or the aspartyl protease BACE1 WT beta-secretase activity. Moreover, E64d has not been shown to be effective in an animal model when administered by a therapeutically acceptable route for treating AD patients. To evaluate these issues, we orally administered E64d, to normal

guinea pigs or transgenic mice, both of which express APP containing the human WT beta-secretase site sequence. In guinea pigs, oral E64d administration caused a dose-dependent reduction of up to 92% in brain, CSF and plasma of Abeta(40) and Abeta(42), a reduction of up to 50% in the C-terminal beta-secretase fragment (CTFbeta), and a 91% reduction in brain cathepsin B activity but increased brain soluble APPalpha (sAPPalpha) by 60% and BACE1 activity by 20%. In transgenic APP<sup>Lon</sup> mice, feeding E64d formulated chow improved memory deficits and reduced brain Abeta(40) and Abeta(42), amyloid plaque, brain CTFbeta, and brain cathepsin B activity but increased brain sAPPalpha and BACE1 activity. We conclude that E64d likely reduces brain Abeta and improves memory deficits by inhibiting CatB, and not BACE1, WT beta-secretase activity and that oral E64d administration has potential as a disease-modifying treatment for most AD patients.