Retrograde neuronal injury in response to glutaric acid in Glutaric Acidemia type 1 (GA-1) mouse model

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INTRODUCTION: Glutaric acidemia type 1 (GA-1) is an inborn error of lysine, hydroxylysine and tryptophan catabolism. This disorder is accompanied by accumulation of glutaric acid (GA) and 3-hydroxy glutaric (3-OH GA) acid in the tissue, serum and urine due to enzymatic deficiency of Glutaryl-CoA dehydrogenase (Gcdh). During the first few years of life, affected children can experience irreversible striatal injury leading to permanent disability. The striatal medium spiny GABAergic neurons are predominantly injured, with some sparing of the cholinergic neurons. Since the serum and urine GA levels often poorly correlate with the brain GA levels, it was proposed that GA accumulation in the brain is due to neuronal Gcdh deficiency [1]. In the brain, neurons appear to be the predominant cell type expressing Gcdh, however, these neuronal population are mainly localized in the thalamus, hippocampus and cortex (deep cortical layers) [1]. Nonetheless, the striatum receives input from many areas, including regions with high Gcdh expression such as thalamus and cortex. In this study we investigated whether the addition of exogenous GA to the thalamus or cortex can precipitate the striatal injury. To our surprise we discovered neurons of the substantia nigra to become affected and undergo vasogenic edema in response to exogenous GA in the thalamus.

METHODS: *Gcdh -/-* mice of mixed C57BL6/J X 129SvEv genetic background were used. MRI was performed on the 11.7 T Bruker, with the 2 mm birdcage coil. A calibrated micro-capillary tubes (~40 nm diameter) were used for all brain injections. The pH neutral solution of 5 mM GA was prepared and 3 nl were injected into the thalamus of 4 Gcdh-/- mice (ventral posteromedial nucleus, coordinates relative to Bregma: 1.7 MV, -1.5 AP, -3.4 DV), and cortex of another 4 Gcdh-/- mice (coordinates: 2.16 ML, 0.02 AP, -1.76 DV). Mice were imaged immediately after (1h post GA injection) and then again after 17 h. Prior to imaging mice were anesthetized with 3% isoflurane, and kept at 1-1.5% during the imaging. To determine possible cytotoxic edema at 1 h following GA injection a diffusion-weighted sequence was used (five 1 mm-thick slices, TR/TE=3000/27 ms, big delta=12 ms, small delta=5 ms, with three b-value=0, 500 and 1000, 169X217 μm^2 resolution, 2 NAX). To determine possible vasogenic edema following GA injections (at 1 h and 17 h) mice were imaged using T_2-weighted multi-echo spin echo sequence (ten 1 mm thick slices, TR/TE 3000/8.17-114.35 ms, 14 echoes, 156^2 μm^2 resolution, 2 NAX). The transverse relaxation time (T_2) was caluculated on a pixel-by pixel basis using ImageJ (plugin by Karl Schmidt). In addition we used Mn^{2+} enhanced MRI [2], to further dissect thalamic and cortical projection circuits in Gcdh-/- mice. A different set of Gcdh-/- mice was injected with 4 nl of 200mM $MnCl_2$ solution (4 mice were injected into the thalamus and 4 mice were injected into the cortex, same coordinates as for GA injections). We used 3D RARE imaging sequence (TR/TEeff=250/12 ms, RARE factor 4, 100^3 μm^3 voxel size, NAX=4) to visualize manganese enhancement at 1 h and 17 h post injections. Statistical analysis was performed using paired t-test.

RESULTS: In Gcdh-/- mice injected with GA into the thalamus, there was no evidence of cytotoxic or vasogenic edema at 1 h post injection, **Fig. 1 (A-C)**. A significant increase in T_2-relaxation time (94.3±18.4 *vs*. 48.8±4.3) was found in the substantia nigra in all 4 Gcdh-/- mice, and sparse (but significant) increase in T_2-relaxation time was found in thalamus (65.3±12.7 *vs*. 46.7±3.1) at the site of injection at 17 h post GA injections, **Fig. 1 (D, E)**. In contrast, in two Gcdh-/- mice injected with GA into the cortex, there was increased signal intensity in the cortex and the striatum on both T_2-weighted (**Fig. 2 B**) and diffusion-weighted images as early as 1 h following the injection that remained elevated at 17 h. There was no significant change in T_2-values in the affected striatal region between 1 and 17 h post injection (43.1±6.7 *vs*. 49.1±6.3). In the remaining two Gcdh-/- increased signal intensity on T_2-weighted image was only observed in the cortex (the injection site and additional site in close proximity to the injection site). The axonal tracing from the thalamus (**Fig. 3 A-F**) with Mn^{2+} in Gcdh-/- mice revealed increased signal intensity in the striatum, cortex and the hippocampus, but not the substantia nigra, **Fig. 3 (G-L)**. The axonal tracing with Mn^{2+} injected into the cortex, revealed increased signal intensity in the thalamus and the striatum.

DISCUSSION: This work represents the first *in vivo* evidence to implicate glutaric acid as neurotoxin and to demonstrate retrograde neuronal injury distal to the site of injection. The concentration of GA injected into the brain are similar to autopsy reports of brain GA concentration during the metabolic crisis in children with GA-1 up to 2 mM [3], and to what was found in Gcdh-/- mice fed high lysine diet, up to 4 mM [1]. Opposite to our expectations, addition of GA to the thalamus precipitated neuronal injury in the substantia nigra, while Mn^{2+} was preferentially transported to the striatum, cortex and hippocampus. Since it is unlikely that glutaric acid was retrogradely transported to the substantia nigra, our findings suggest "excitotoxic" type of injury to GABAergic neurons of the substantia nigra pars reticulata, known for extensive inputs to the ventral posteromedial nucleus of the thalamus. Our findings from cortical injections of GA, confirm the possibility that local increases in GA concentrations (presumably by neurons rich in Gcdh) can affect the synaptic terminals of vulnerable neurons to cause striatal injury. In conclusion, the evidence for retrograde neuronal injury by GA is presented, however the exact mechanism of this injury and clarification on the neuronal population involved will require further investigation.

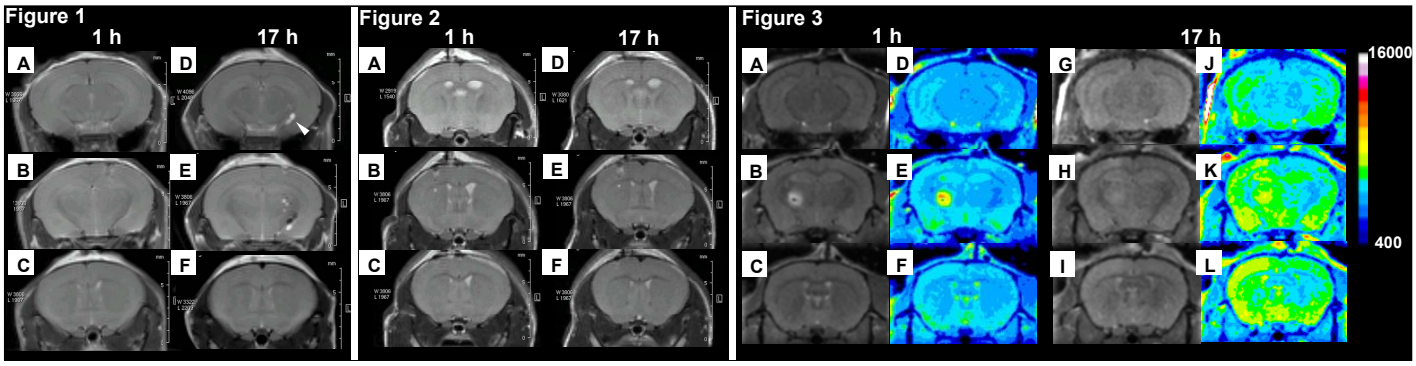

Figure 1. (**A-C**) T_2-weighted images at 1 h post thalamic GA injection into the Gcdh-/- mice show no evidence of neuronal injury. (**D-F**) T_2-weighted images at 17 h post GA injection, with noticeable vasogenic edema in the thalamus and substantia nigra (arrowhead). **Figure 2**. (**A-C**) Increased signal intensity was present at 1 h post cortical GA injection in the cortex and the striatum, and persisted at 17 h (**D-F**). **Figure 3**. Representative 3D RARE with corresponding colored intensity images at 1 h (**A-F**) and 17 h (**G-L**) post Mn^{2+} thalamic injection. Increased signal intensity corresponding axonal Mn^{2+} tracing was noticed in the cortex, striatum, while none in the substantia nigra (**J**). The colored intensity scale is shown on the right.

REFERENCES:

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