Relative Efficacy of Nicotinamide Treatment of a Mouse Model of Infantile Niemann-Pick C1 Disease

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Abstract

Nicotinamide delivered in drinking water at about 2g/kg/day significantly prolonged survival and showed a suggestive improvement on memory in the *Npc1^{nih}/Npc1^{nih}* mouse model of infantile NPC1 disease. It is likely that this role is due to its function as a histone deacetylase (HDAC) inhibitor although another HDAC inhibitor, valproic acid, was without effect. Nicotinamide could also work by preventing/reversing oxidative stress.

Statement of author's contributions

CAM and IAB performed the experiments which were devised by RPE who also authored the ms. with CAM.

Introduction

Niemann Pick Type C (NPC) is a rare autosomal recessive disorder and is characterized by impaired motor function, speech complications (dysarthria), inflammation of the liver and spleen, and cerebellar dysfunction (Heidenreich and Erickson, 1997). This is due to a mutation within the intracellular cholesterol transport system. There are two forms, NPC1, which consists of a mutation in the NPC1 protein located within the endosome and which makes up 95% of NPC human occurrences; the other form is NPC2, which is 5% of the human cases, and is caused by a mutation in the NPC2 protein. The NPC2 phenotype is more severe than the NPC1 mutant, (Patterson, 2001). We have extensively studied the disease in mouse models. The *Npc1^{nih}* mutant was created through a retrotransposon insertion that created a knock out of the NPC1 gene, effectively removing the protein (Loftus, et al, 1997). This mutant shows severe cerebellar dysfunction, and reduced survival when compared to wild-type (WT), and may represent a more severe, infantile form of the NPC1 human disease.

There has been an extensive search for drugs to treat the disorder and one, hydroxypropyl-beta-cyclodextrin (HPBCD; Camargo, et al, 2001; Liu, et al, 2009) is currently in phase II trials. However, to date, this treatment only slows the disorder and other treatment modalities are needed. In the meantime, various common medications and/or food additives may ameliorate symptoms. The anti-inflammatory agents ibuprofen (Smith, et al, 2009) and vitamin E (Bascunan-Castillo, et al, 2004; Xu, et al, 2009) have been beneficial in animal models while curcumin has not much (Borbon, et al, 2012). Recently, histone deacetylase (HDAC) inhibitors have been proposed as drugs for the disease (Pipalia, et al, 2011). We have tested two drugs with HDAC inhibitory

properties, valproic acid (Kim, et al, 2007) and nicotinamide (by inhibition of sirtuins, Jackson, et al, 2003) but have only found efficacy with nicotinamide which we present here.

Materials and Methods

Mice

The *Npc1^{nih}* mutant was originally found on the BALB/cNctr inbred strain and is a natural transposon-induced knockout (Loftus, et al, 1997). This is a substrain of BALB/c which was derived at the National Center for Toxicology Research. For the *Npc1^{nih}* mouse mutant, DNA was typed by PCR according to Loftus et al. (1997).

Drugs and delivery

Nicotinamide (Sigma, batch 079K1404) was presented to mice as a 1% solution in their drinking water. Given that mice drink about 4 ml. per day, this works out to about 2g/kg/day. Valproic acid was delivered as a 0.25% solution in drinking water, a dose of 500 mg/kg/day which had been effective in a mouse model of amyotrophic lateral sclerosis (Sugai, et al, 2004).

Memory test

We used the passive avoidance shock chamber test in order to test memory ([Ader et al., 1972](#)). Mice were 4 to 5 weeks old when trained. The training period consisted of 3 days followed by weekly testing. On day 0, mice were deprived of food overnight. On day 1, mice were kept in a dark area for at least 2 h; two pellets of food were placed in the dark side of the chamber; mice were placed head first into the left corner of the light compartment; they were allowed 300 s to explore the chamber and the latency time to enter the dark compartment was recorded. On day 2, mice were kept in a dark

area for at least 2 h; mice were then placed head first into the left corner of the light compartment; they were allowed 300 s to explore the chamber; 5 s after the mouse entered the dark compartment, a five second shock at 85 V to the grid in series with a 224 k Ω current limiting resistor was administered (about 0.6 mA, similar to a static electricity shock); if the mouse remained in the dark compartment, a second shock was administered 30 s after the initial shock; every time the mouse re-entered the dark compartment, the shock was administered; the latency time to enter the dark compartment was recorded. On day 4 or subsequent testing days, mice were kept in a dark area for at least 2 h; mice were then placed head first into the left corner of light compartment; they were allowed 300 s to explore the chamber; the latency time to enter the dark compartment was recorded, and they were considered to have failed the test at this point if they hadn't entered the dark compartment.

Statistical analysis

The Kaplan Meier Survival Statistic, a log rank test, was used to compare survivals of treated and untreated mice.

Results and Discussion

We did not find a prolongation of survival with the HDAC inhibitor, valproic acid (Table 1, p0.3 v.s. untreated) which had improved neuronal differentiation in cultured neural stem cells (Kim, et al, 2007). This drug is well known as an anticonvulsant but also is an HDAC inhibitor (Gottlicher, et al, 2001). In fact, weight gain of the valproic acid treated mice was significantly lower than in the untreated *Npc1^{nih}/Npc1^{nih}* mice (data not shown) which could suggest a toxic effect of this dose of valproic acid in these mutant mice. Of course, a very much larger cohort of mice might

have shown a statistically enhanced survival since the average survival was intermediate between the untreated and nicotinamide treated mice.

On the other hand, there was a significantly increased survival when the *Npc1* deficient mice were treated with nicotinamide. The mice treated with 2g/kg/day of nicotinamide survived significantly longer than did the untreated mice (Table 1). The 11 day mid-survival difference had a probability of $p=0.03$ compared to untreated *Npc1^{nih}*/*Npc1^{nih}* mice.

Table 1. Survival of *Npc1* -/- treated and untreated mice in days

Untreated (N=13)	Nicotinamide Treated (N=11)	Valproic Acid Treated (N=7)
61	68	60
65	77,77	66
66 67	78	73
68,68	80	82
72,72	82, 82	84
76	90,90	86
79	98	92
83, 83	99	
86		

Although not statistically significant, there were also suggestive changes in the memory status of the treated mice (Fig. 2). A higher percentage trained to the shock avoidance

paradigm (0.5% v/s/ 0.33%) and those that did, remembered it longer than the untreated mice (from an average of 2.5 to 5 weeks).

Fig. 1

These results extend the findings that HDAC inhibitors can ameliorate symptoms in mouse models of neurological disease. (Ferrante, et al, 2003; Hockly, et al, 2003). The extensions of survival fall in the class of what tamoxifen and vitamin E accomplished (expressed in failures of weight gain which translates into about a 3-fold greater survival; Bascunan-Castillo, et al, 2004) and are significantly better than curcumin's effect (barely significant in our hands; Borbon, et al, 2012). However, it is far less than what treatment with hydroxypropyl-beta-cyclodextrin, especially when delivered intrathecally, accomplishes (Liu, et al, 2009).

Nicotinamide is classified an inhibitor of the Class III HDACs which are members of the Sirtuin 2 family. These are nicotine adenine dinucleotide dependent (McLaughlin and

La Thangue, 2004). Importantly, nicotinamide was found to restore cognition in a mouse model of Alzheimers disease (Green, et al, 2008). NPC disease has frequently been called “juvenile Alzheimers” and breeding *Npc1* deficiency into a mouse Alzheimers model enhanced amyloid plaque formation (Borbon, et al, 2010). Green, et al (2008) did not find changes in amyloid levels but did find a reduction in phosphorylated tau. They correlated this with an increase in acetylated alpha-tubulin indicating inhibition of Sirtuin 2 which deacetylates tubulin (Green, et al, 2008).

It is also possible that the beneficial effect of nicotinamide is due to its role in ameliorating oxidative stress. Multiple studies showing elevated reactive oxygen species in NPC1 cells are reviewed by Fu, et al (2010). Particularly relevant are the gene expression profiles reported by Vasquez, et al (2011) finding differential gene expression of cerebellar genes associated with oxidative stress in *Npc1* deficient mice. Nicotinamide can prevent apoptosis in toxicity-induced neurodegeneration (Klaidman, et al 1996) and multiple roles are involved. It protects cells from apoptotic genomic DNA fragmentation and membrane phosphatidyl serine exposure to ROS (Chong, et al, 2004). These protective effects require activation of the Akt1 signalling pathway while alterations in mitochondrial potential and inhibition of FOXO3a phosphorylation are also involved (Chong, et al, 2004). The role of NAD^+ , for which nicotinamide is a precursor, in both pathways is recently and thoroughly reviewed by Canto, et al (2015).

While nicotinamide is clearly not a cure-all for NPC1, its addition to the therapeutic regimen could possibly enhance cognition and survival in patients receiving HPBCD or other therapies. It is tolerated in high doses (Knip, et al, 2000) and is readily available.

Compliance with Ethical Standards

Financial support was received from the Wings Like Eagles Foundation, Tucson, AZ. There are not conflicts of interest for any of the authors. Mice were handled according to approved protocol 14-502 of our institution.

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