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Research Article

Use of Nonclinical and Expanded Access Clinical Study data to Guide Development Programs Targeting Mitochondrial Diseases of Lipid Peroxidation

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ABSTRACT

Background: Many diseases of neurodegeneration associated with mitochondrial Lipid Peroxidation have poorly predictive animal models and present unique challenges for clinical trial design. RT001 is a dideuterated isotopomer of linoleic acid that inhibits Lipid Peroxidation. To correlate non clinical models with clinical response, we reviewed the effects of RT001 in disease models and clinical indications associated with Lipid Peroxidation.

Methods: We identified 10 specific target diseases known to be associated with Lipid Peroxidation. We evaluated the effects of RT001 in cellular models, animal models, and expanded access clinical study of these Lipid Peroxidation associated diseases.

Results: Significant beneficial effects were seen in 7 disorders. Clinical efficacy was seen in 6, no benefits were seen in 3, and data was inconclusive in 1. Inconsistencies between nonclinical and clinical experiences were seen; *in vitro* and animal models often demonstrate a lack of consistency with clinical response.

Conclusion: The response to RT001 in nonclinical models often demonstrated little concordance with the clinical effects in patients with the corresponding disease. The best predictor of RT001's beneficial effects in patients remains clinical exposure. Thus, because RT001 has efficacy in some diseases and not others, there is no substitute for performing definitive clinical trials disease by disease in order to determine clinical benefit.

Keywords: Expanded access; Mitochondrial disorder; Lipid Peroxidation; Neurodegeneration; RT001

INTRODUCTION

Research into neurodegeneration has often been limited by the unpredictable response of pre-clinical models of disease. Disease models that fail to express the full range of clinical pathology or that vary in phenotypic response may account for this lack of consistency. Early evaluation of promising drug candidates in patients is further complicated by the paucity of clinically meaningful biomarkers of drug response.

Mitochondrial neurodegenerative diseases pose specific challenges for drug development. These diseases typically have an insidious onset, a slow and variable clinical course, and symptom expression may be observed only after a significant proportion of neurons is impaired or already lost. The neurodegenerative process itself is often not amenable to direct observation and, thus, cannot be monitored in clinical trials. The optimal outcome measures, the sample size required and the treatment duration need to be defined in relation to the predominant clinical manifestations and the expected course of the disease. Typically, rating scales are used as outcome measures of clinically meaningful symptom severity. However, by the time that symptoms develop, the disease may have progressed beyond the point that a therapeutic intervention is likely to reverse or halt the progression of the disease. All of these factors can be

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further impacted by low prevalence of any specific disease as many of these diseases are very rare; the absence of well-defined knowledge base of the natural history of the disease, or of a population of sufficient size to allow for a randomized, placebo controlled clinical trials may further impair attaining clinical proof of concept and clinical trial design.

The discovery of several conjugated aldehydes that react with sulfhydryl and amino groups and inhibit vital metabolic processes led to the association of Lipid Peroxidation with numerous diseases [1]. Whether as a primary cause of disease or a secondary consequence, oxidative stress has been implicated in the onset and progression of a number of neurodegenerative diseases [2,3]. The pathophysiological role of Lipid Peroxidation in various neurodegenerative disorders may influence the onset, clinical manifestations, progression and the ultimate outcome of the disease. Mitochondria are particularly prone to Lipid Peroxidation associated malfunction due to the high concentration of PUFAs in mitochondrial membranes, as well as elevated oxygen levels, Reactive Oxygen Species (ROS) turnover, redox metabolism, and transition metal presence. Although prevention or treatment of Lipid Peroxidation represents a reasonable therapeutic target for many of these diseases, drug development programs in this area have been hampered by the futility of antioxidant agents that can never achieve concentrations in the mitochondrial and cellular lipid membranes required for a meaningful therapeutic benefit. Some of these compounds may even exacerbate the problem because they sometimes act as prooxidants under certain conditions.

RT001 is a deuterated homologue of linoleic acid that makes membrane PUFAs resistant to Lipid Peroxidation (LPO). A strong protective effect against Lipid Peroxidation (LPO) is seen when deuterated PUFAs replace non deuterated PUFAs in lipid bilayers at levels exceeding 20% [4]. Treatment with RT001 has shown early signs of efficacy in patients with Friedreich's ataxia, a disorder of intracellular free iron imbalance that initiates Lipid Peroxidation (LPO), resulting in increased oxidative stress and mitochondrial dysfunction [5]. This experience has led to optimism that RT001 may have therapeutic efficacy in treating a variety of other mitochondrial neurodegenerative diseases in which Lipid Peroxidation (LPO) is known to play a role. The therapeutic approach of treating Lipid Peroxidation, as the common downstream event, rather than focusing on targeted disease specific therapies also represents a paradigm shift in approach. In theory, the blockade of Lipid Peroxidation with RT001 combined with a disease specific agent might prove synergistic.

Because of the large number of mitochondrial and neurodegenerative diseases in which Lipid Peroxidation is implicated and the consequent broad range of indications for which RT001 may have efficacy, there is obvious need for strategic selection of neurodegenerative indications to be evaluated for treatment with RT001. In order to obtain initial signals regarding the safety, efficacy, and practicality of treating patients with a wide variety of neuro degenerative disorders, we evaluated RT001 in various *in vitro* cell culture systems, *in vivo* animal models, and expanded access open label clinical studies in order to define indications for which further clinical development efforts are warranted.

MATERIALS AND METHODS

Cell and animal models

We identified target diseases that are known to be associated with Lipid Peroxidation based on published data. As a result, diverse cellular and animal models of diseases associated with Lipid Peroxidation were evaluated for the effect of RT001.

Clinical studies

In parallel, patients with diseases linked to Lipid Peroxidation were selected for participation in expanded access study protocols. In all instances the study sponsor and disease experts agreed upon the rationale to treat the specific Lipid Peroxidation driven disease processes under consideration. Expanded access protocols were developed for each specific protocol, and local institutional review board approval was obtained. Each subject provided informed consent. Following baseline examinations, RT001 was prescribed at 2.88 g daily (960 mg Three Times a day) for pediatric indications, and 5.76 g daily (2.88 g Two Times a day) for adult indications. For subjects in recent expanded access protocols, a 1 month period higher dose drug loading was employed to enhance early exposure to the drug candidate (3.84 g daily for pediatric dosing, or 8.64 g daily for adult dosing) [6].

Validated rating scales for the various conditions were used when they were available (for example, Amyotrophic Lateral Sclerosis (ALS), Progressive Supranuclear Palsy (PSP). Subject performance scores while taking RT001 were compared to natural history scores over time. When scales for a particular condition were not available, clinically meaningful parameters and quality of life measures were included as endpoints. In the case of Infantile Neuro Axonal Dystrophy (INAD), this culminated in the development of a formal Infantile Neuro Axonal Dystrophy rating scale [7].

RESULTS

The effect of RT001 was tested in cellular and animal models of several different neurodegenerative diseases associated with Lipid Peroxidation (Table 1).

Indication	Model	Key readout(s)	Response (Lipid peroxidation reduction)
FA[1]	MCK-KO mouse model	Survival	No (N/A)
FA[1]	Yeast, murine, human cell culture models	Cell viability	Yes (Yes)

ALS[3]	G93A mt SOD-1 mouse model	Survival	No (N/A)
INAD[6]	Drosophila iPLA2-VIA KO	Behavioral tests	Yes (Yes)
INAD[6]	Human fibroblasts from INAD patients	Cell viability	Yes (Yes)
GPX[4] deficiency[7]	Erastin or (1S, 3R)-RSL3 induced ferroptosis in G-401 cells	Cell viability	Yes (Yes)
GPX[4] deficiency[7]	Gpx4 Neuronal Inducible KO (NIKO) mouse model	Survival	No (N/A)
Alzheimer's	Aldh-2 KO mouse	Behavioral tests	Yes (Yes)
Alzheimer's	APP/PSI double mutant transgenic mouse	Amyloid β-peptide levels/ Behavioral tests	Yes/No (Yes)
Tay Sachs	Sandhoff Hexb −/− KO Mouse	Survival	No (N/A)
Parkinson's	Mice treated with MPTP	Dopamine level & TH	Yes (Yes)
Parkinson's	Neuronal cell cultures from PD patients	Cell viability	Yes (Yes)
Parkinson's	A53T α-synuclein rat PD model	Behavioral tests, DA cell death, inflammation	Yes (Yes)
Huntington's	Q140 KI mouse	Behavioral tests	Yes (Yes)
Diabetic Retinopathy	Ins2Akita diabetic mice	Retinal ganglion cell survival	Yes (Yes)
Neurogenic inflammation	Sciatic nerve ablation rat chronic pain model	Pain reduction	No (N/A)
Atherosclerosis /Inflammation	Transgenic APOE*3-Leiden.CETP mice	Inflammation, atherosclerotic lesions	Yes (Yes)

Note: 1: Friedreich's ataxia, 3: Amyotrophic lateral sclerosis, 4: Late Onset Tay Sachs Disease, 6: Infantile neuroaxonal dystrophy, 7: Spondylometaphyseal dysplasia

Table 1: The indications, model description and the response to RT001.

In addition to the *in vitro* and animal models above, RT001 was administered clinically to 54 subjects with different diseases (Table 2).

Indication	n	Response
FA[1]	19	Yes
PSP[2]	4	Yes
ALS[3]	23	Yes
GM2 gangliosidosis[4]	1	Yes
SCA[5]	1	Inconclusive
ACOX1-GOF	1	No
Neuroserpinosis	1	No
INAD[6]	2	Yes
GPX[4] deficiency[7]	1	No
APO e[4] Alzheimer's	1	YES8
Late Onset Tay Sachs (LOTS)	1	Yes

Note: 1: Friedreich's ataxia, 2: Progressive supranuclear palsy, 3: Amyotrophic lateral sclerosis, 4: Late Onset Tay Sachs Disease, 5: Spinocerebellar atrophy, 6: Infantile neuroaxonal dystrophy, 7: Spondylometaphyseal dysplasia, 8: Initial results positive, further testing ongoing

Table 2: The indications, the number of subjects tested, and the response to RT001.

DISCUSSION

We reviewed the effect of RT001 on various cell culture and animal models, and the clinical experience with RT001 in 10 different indications associated with Lipid Peroxidation for which effective therapies are lacking. For the nonclinical data, significant beneficial effects were seen in full or partial form for 7 of the 10 disorders studied. In the clinical studies, early evidence of potential efficacy signals was observed in 6 indications, no apparent benefits of RT001 treatment were seen in 3 indications, and data was deemed inconclusive for 1 indication.

Significant inconsistency between nonclinical and clinical experiences exists; the *in vitro* and animal models to date demonstrate a lack of consistency with clinical response. For FA, the two nonclinical studies showed conflicting results [8,9].

While the clinical out-comes have been encouraging. In another example, no effects of Deuterated-Poly Unsaturated Fatty Acids (D-PUFAs) were seen in the mouse model of Late Onset Tay Sachs (LOTS), while the clinical response was positive. Conversely, D-PUFA treatment in a rat model of Parkinson's disease led to disease modifying beneficial effects against α-syn induced pathology [10], while benefits in the clinical setting have not yet been tested. Of the remaining 6 indications that demonstrated some nonclinical benefits, the clinical experience has shown an apparent lack of clinical benefit in 2 indications (GPX4 deficiency and non-Apolipoprotein E4 (APOE4) linked Alzheimer's disease). Development efforts for many of these indications are ongoing.

These apparent inconsistencies are aligned with previous experiences in for instance Alzheimer's disease. In a transgenic mouse model, Deuterated-Poly Unsaturated Fatty Acids (D-PUFAs) reduced amyloid beta protein, but had no effect on spatial learning and memory deficits [11]. However, in a study of aldehyde dehydrogenase 2 null mice, Deuterated-Poly Unsaturated Fatty Acids (D-PUFAs) decreased cortex and hippocampus F2 isoprostanes by approximately 55% and prostaglandin F2a by 20%-25%. These changes were accompanied by improved performance in cognitive/memory tests [12]. Numerous reasons exist for the apparent lack of preclinical animal model concordance and further confirm general inability of preclinical data to reliably predict clinical outcomes.

Cell culture studies are used extensively to test the potential role of drugs in mitigating the effects of oxidative stress. However, the methodologies under which these experiments are conducted may inflict a non-physiologic oxidative stress that may, under certain circumstances, lead to misleading conclusions. Many potential therapeutic agents that have previously shown remarkable effects when added to tissue culture cells have proven to be disappointing when tested *in vivo* [13]. This may be due to differences in drug bioavailability and achievable exposure of target tissue/cells in cell culture and within organism, Absorption, Distribution, Metabolism and Excretion (ADME) effects of drug administered *in vivo*, different sensitivity of the tissues relative to isolated cells due to differences in cell to cell interactions and microenvironment etc. Although the models may recapitulate the initial protein expression or other pathological features linked to the human disorder, the pathophysiologic events that occur in the human disease are rarely accurately displayed in part because of the differences in: disease etiology in animal models relative to human patients as well as in animal and human anatomy, organismal size, lifespan and physiology, and in particular in rodent and human brain anatomy and physiology, among other factors [14].

The appropriate use of a drug candidate to treat a specific medical condition invariably requires a balancing of the risks of treatment with the potential treatment benefit. The absence of toxicity with a drug candidate like RT001 strongly favors treatment, especially when being used to treat incurable, progressive diseases for which effective treatments do not exist. However, other factors may influence drug development and treatment decisions. Despite optimism based on pre-clinical models or understanding of disease mechanism, the actual clinical response to drug treatment cannot be predicted with certainty without a well-designed and conducted clinical trial. Limited drug supply may force prioritization of development programs. Prior to embarking on an in depth development program expanded access programs can provide highly informative initial clinical experiences in a variety of diseases, thus facilitating program prioritization. This approach has helped select disease targets for which subsequent rigorous, controlled clinical trials are warranted.

A statement of caution regarding the use of expanded access protocol experience to guide further drug development options deserves mention. The limited nature of the exposures in expanded access protocols may not be representative of larger groups of subjects for that specific indication. Hence, abandonment of a development program based on expanded access experience may lead to the premature rejection of an indication. Not surprisingly, only well designed and statistically powered clinical trials can provide a definitive answer to the activity of the drug candidate in a specific indication.

CONCLUSION

We examined the effects of RT001 on various neurodegenerative diseases in cell culture models, animal models, and in patients. Our results so far suggest responses to RT001 in nonclinical models demonstrate little concordance with the clinical effects in patients with the corresponding disease notwithstanding concentration levels consistent with putative efficacious results between the nonclinical models and human clinical studies. Although the response in nonclinical models may yield insight into the mechanism of action of RT001 in a specific indication, the cellular or animal model response may have limited benefits for selecting target disease indications as the data to date evidence that the efficacy in humans cannot be predicted solely on these cellular and animal responses.

Despite these limitations, aligning the outcomes in preclinical models with clear evidence of target engagement can facilitate clinical study design and obtaining efficacy readouts and enhance understanding of the Pharmacokinetic/Pharmacodynamics (PK/PD) relationship for particular drug candidate. Nevertheless, the unpredictability of RT001 therapy in human clinical trials for a broad spectrum of neurodegenerative diseases based on cellular and animal nonclinical models suggest that the underlying etiology of these diseases may be more complicated and the degree of involvement of Lipid Peroxidation in the initiation and propagation remains to be determined.

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