No clinical toxicity is seen in vivo from hyperpolarized PASADENA MR reagents or catalyst

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Introduction

Magnetic resonance spectroscopy provides the unique ability to assess the biochemical millieu of living cells, *in situ*. Molecules with hyperpolarized ^{13}C atoms can vastly enhance spectroscopic signals so as to trace metabolic transformations. The latest generation of tracer reagents are intermediaries in cellular respiration pathways, such as succinate, maleate, and fumarate. Earlier reagents were, by necessity, harsh, non-physiologic chemicals, such as 2-hydroxyethylacrylate (HEA) and 2-hydroxyethylpropionate (HEP) [1]. Rhodium norboradiene serves as the catalyst for the transfer of hyperpolarized magnetic spin orders from parahydrogen to the ^{13}C atom of these tracer reagents. This hyperpolarization method is called PASADENA (**p**arahydrogen **a**nd **s**ynthesis **a**llow **d**ramatically **e**nhanced **n**uclear **a**lignment) [2,3]. The utility of this method in cancer research is illustrated by our work with BALB/c + Colon -26 tumor mice (Fig. 1,2).

Purpose

With the goal of developing clinically useful assays of cellular metabolic processes, a number of promising tracer reagents have been investigated. Hyperpolarized ^{13}C compounds drastically increase the signal strength available for magnetic resonance spectroscopy and imaging of ^{13}C and ^{15}N containing compounds. As a prerequisite to clinical trials, the safety profile of any candidate tracer reagent, metabolite, or reaction catalyst must be characterized.

Methods

In vitro toxicity was inferred from the inhibitory effects of tracer reagents and the rhodium catalyst on liver cell cultures, as assessed by the AlamarBlue technique. Since metabolically active cells consume AlamarBlue, the less the cells grew (i.e. more inhibited by test substance), the more blue dye remained. Plotting blue dye intensity versus test substance concentration yielded inhibitory concentration (IC) values. Thus, the lower the IC_{10} or IC_{50} value, the more inhibitory the test substance [4,5]. The *in vitro* results led to the development of a cation-exchange filter system to remove the catalyst prior to any intravenous injection. The intact Sprague-Dawley rat was then selected to gauge filtration efficacy, as well as determine how cell culture results translate to the intact animal model. The "limit test/main test" method replaced obsolete, traditional LD_{50} determinations by testing for toxicity above and below a dose of interest [6,7]. If no untoward effects were seen at the dose of interest, then the LD_{50} must lie above this dose. If, however, toxicity occurred at this dose, then lower doses were tested. This method reduced the number of animals required to obtain an assurance of safety, at or below the dose of interest.

Results

First generation reagents, such as HEA and HEP, as well as the rhodium catalyst exerted inhibitory effects on liver cell cultures (low IC values, Table 1). Note the increase in IC_{10} (i.e. less toxic) after a solution of rhodium catalyst was filtered to remove the catalyst (0.1 vs 2.5). Metabolite-based PASADENA reagents (fumarate, maleate, succinate) had two to three orders of magnitude higher IC values than HEA had. *In vivo* results are shown in Table 2. All 25 rats survived without clinical signs of disease for the entire 14 days of the experiment after a single tail vein dose. One of these 25 rats exhibited hepatic congestion and nephrosis on histology.

Table 1: The *in vitro* effects of PASADENA reagents and rhodium catalyst on hepatic cell cultures

Test compounds	In vitro (mM)		In vivo **LD_{50}** (mmol/kg)
	IC_{10}	**IC_{50}**	
2-Hydroxyethylacrylate (HEA)	<0.1	0.1	1.3
2-Hydroxyethylpropionate (HEP)			50
Fumarate	5	100	50
Maleate/succinate mixture	30	70	18
Rhodium catalyst + **no** filtration	0.1	1	
Rhodium catalyst + filtration	>2.5		

Table 2: The *in vivo* effects of intravenous PASADENA reagents and rhodium catalyst on rats (N=25)

Test solution groups (N=5 each)	A	B	C	D	E
Filtered 2.5 mM catalyst	x				
Unfiltered 2.5 mM catalyst		x		x	x
9mM Succinate (4.6 mg/kg)	x	x	x		
100mM Succinate (50.6 mg/kg)				x	
300mM Succinate (151.7 mg/kg)					x
In vivo results					
Clinical disease	no	no	no	no	no
Histologic abnormalities	no	no	no	no	1*

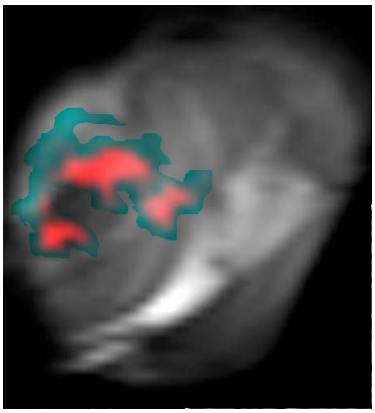

Figure 1: Carbon MRI of hyperpolarized ^{13}C-succinate (colored) superimposed on ^{1}H MRI of Colon-26 tumor on dorsum of BALB/c mouse. 22 mM ^{13}C-succinate was administered through a tail vein catheter. Tumor localization was achieved with a surface coil.

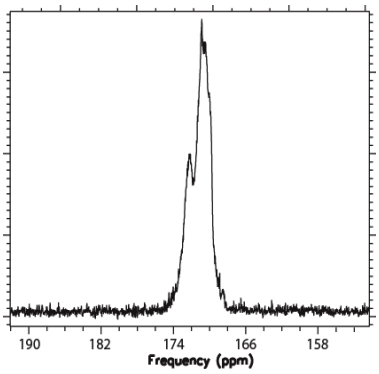

Figure 2: ^{13}C spectrum of Colon-26 tumor on BALB/c mouse. Broad peak appeared after infusion of 22 mM hyperpolarized ^{13}C-succinate. No metabolites seen, confirming colon tumor's lack of succinate oncogene.

Discussion

First generation PASADENA reagents, HEA and HEP, were quite toxic, *in vitro*. The rhodium catalyst also seemed toxic. From these data, we expected rats receiving intravenous solutions, filtered to exclude the catalyst, to do well clinically and histologically. The *in vivo* safety data of the catalyst, however, exceeded this expectation. Not only did filtered solutions fail to produce disease, neither did the unfiltered catalyst itself. All 25 rats survived, regardless of the presence of catalyst. 24 out of 25 rats exhibited no microscopic abnormalities in their liver or kidneys. No rat of the 300 mM succinate group appeared ill. This level is well above the usual 10 to 30 mM succinate dose used in PASADENA [8], as typified by the experiment depicted in Fig. 1 & 2. Thus, the newer, metabolite-based reagent, succinate, did not produce clinical signs of acute intravenous toxicity in rats**.** The rhodium hydrogenation catalyst produced only subclinical hepatic and renal toxicity, as evidenced by differences in its *ex vivo* and *in vivo* effects. Hence, toxicity appears to pose no barrier to the future clinical utility of the PASADENA method. Sterility testing or a "disposable" PASADENA polarizer needs now to be developed.

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