Rapid toxicity screening of novel PASADENA MRI contrast agents

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Introduction: Parahydrogen and Synthesis Allow Dramatically Enhanced Nuclear Alignment (PASADENA) provides subsecond ^{13}C MR imaging in vivo by hyperpolarization of 13C enriched contrast agents (1,2,3). However, successful use of the PASADENA technique *in vivo* MRI and MRS has been limited by the toxicity of the compounds used in the contrast agent (4). The present study defines a rapid toxicity screening procedure for potential PASADENA compounds, and defines IC10 and IC50 concentrations.

Methods: Cryogenically preserved Cl-9 rat hepatocytes (ATCC CRL-1439) were cultured as monolayers in growth media, incubated at 37°C in a 5% CO_2 atmosphere, and grown to log phase in 48 hours. Test chemicals were dissolved in growth medium at different concentrations and added to cells upon medium renewal. Cells were exposed to toxins for 24 hours at which time alamar blue indicator dye was added at 10%. Absorbancies at 570 nm and 600 nm were measured (5). Results were plotted against the log of the final concentration of test compound. The IC10 and IC50 were the concentration at which the dose-response curve intersected -10% and -50% growth, respectively. Using this *in vitro* assay, we determined the IC10 and IC50 for five PASADENA *in vivo* imaging reagents.

Results: HEP, (2-hydroxyethylpropionate), at 300mM illustrates sub-second hyperpolarized ^{13}C MRI *in vivo* (Fig.1). However, HEA, (2-hydroxyethyl acrylate), the precursor of HEP (Eq. 1), proved extremely toxic. *In vitro*, IC10<0.1mM and IC50=0.1mM, was comparable to published *in vivo* LD50 of 1.3 mmol/kg. The catalyst solution (Perfos) also proved highly toxic, IC10=0.15mM and IC50=1.0mM. Passage through a cationic membrane filter eliminated the cytotoxic elements of the catalyst solution and liver cells survived at all concentrations of filtered catalyst up to 2.5 mM, IC10>> 2.5mM. *Cis*-fumarate the product of the hydrogenation of acetylene dicarboxylate (Eq. 2), a novel PASADENA reagent developed in this Laboratory (6) was two-orders of magnitude less toxic than HEA (Table).

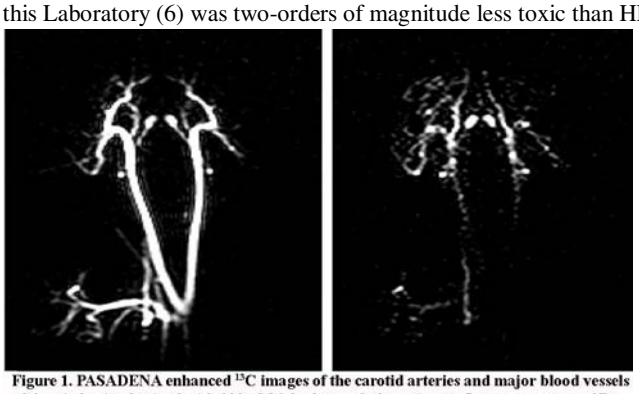
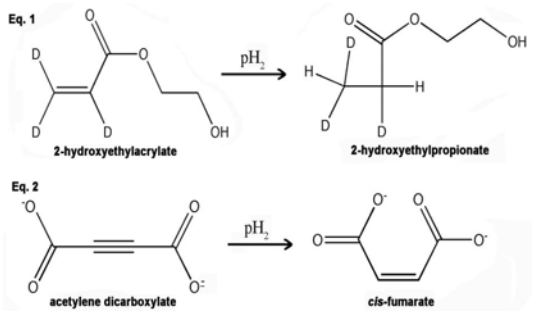


Figure 1. PASADENA enhanced ^{13}C images of the carotid arteries and major blood vessels of the pig brain obtained with 300mM 2-hydroxyethylpropionate. Images courtesy of Dr. Pratip Bhattacharya and colleagues at GE Healthcare: Amersham, Malmo.

Table 1. *In vitro* toxicity of potential PASADENA compounds.

Test Compound	*In vitro* Toxicity IC10 (mM)	IC50 (mM)	*In vivo* Toxicity LD50 (mmol/kg)	Effective PASADENA (mM)
2-hydroxyethyl acrylate (HEA)	< 0.1	0.1	1.3	
2-hydroxyethyl propionate (HEP)	-	-		300
Catalyst - Biphosphine Rhodium norbornadiene salt (Perfos)	0.1	1.0		2.5
Filtered Catalyst	> 2.5	-		2.5
Acetylene Dicarboxylate	1.0	3.0	4.4	
cis-fumarate	30	70	18.0	10
Fumarate	5.0	100	50	
Succinate			20	



Discussion: Despite the 10,000 fold enhancement in signal for *in vivo* MRI and MRS, the PASADENA method employs compounds that are biologically toxic. When used at concentrations above 300mM (See Fig 1) HEP exceeds in vitro !C10 one-thousand fold. The most promising result of the present study is the demonstration that a new custom-synthesized PASADENA contrast agent, *cis*-fumarate, is markedly less toxic than its predecessor, HEP. Nevertheless, still lower concentrations will be needed than those used hitherto. Further work is required to define the lower limit of detection of hyperpolarized ^{13}C *in vivo*.

Conclusion: Identification of non-toxic ^{13}C imaging agents improves the prospect of *in vivo* PASADENA imaging and spectroscopy in experimental animals and ultimately of clinical PASADENA in humans.

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