Toxicity of Intracellular MRI Contrast Agents Containing Gd(III)-DTPA

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The goal of our research is the imaging of protein expression. Targeted contrast agents can experience environments significantly different from those of standard contrast agents. We therefor examined the cellular toxicity of agents targeted to the high affinity folate receptor prepared from Gd(III)-DTPA and folate conjugated dendrimers. Receptor positive cells were treated with buffer, Gd(III)-DTPA, $CoCl_2$, folate dendrimer-DTPA-Gd(III), folate dendrimer-DTPA-Gd(III) with excess free folate, or folate-Gd(III)-DTPA for 72 hours. Viability was determined via dye exclusion. Folate containing compounds showed *very low* levels of toxicity that was inhibited by excess free folate. Therefor the observed *low* toxicity levels require *internalization.*

Introduction

Many different factors contribute to the toxicity of magnetic resonance imaging contrast agents. These include the thermodynamic, conditional thermodynamic, and kinetic stability constants of an ion-chelate complex in addition to the selectivity constant of that complex. Physiological parameters such as pH and endogenous ion concentrations effect these constants.[1] Thus the toxicity of an ion-chelate complex may differ depending on the biological environment experienced by the agent.

We recently developed an MRI contrast agent for use in detecting the molecular expression of high affinity folate binding proteins, with the specific focus on the high affinity folate receptor (hFR). This agent stains the extracellular membrane, intracellular organelles, and the cytoplasm.[2] This staining pattern is consistent with the mechanism of folate delivery into cells via the hFR.

Both the mechanism of folate accumulation via the hFR and the intracellular organelles that accumulate folate expose the complex to a pH < 5. The Gd(III)-diethylenetriaminepentaacetate (Gd(III)-DTPA) complex releases the Gd(III) with vastly different half-lives at pH 7.4 vs 5.0.[3] This release of gadolinium from the linear chelate could result in very high levels of cellular toxicity. We therefor tested the hypothesis that cellular internalization of Gd(III)-DTPA based agents via the hFR was toxic.

Materials and Methods

Folate-DTPA and Folate-DTPA-Gd, were kindly provided by Dr. Michael D. Kennedy from Dr. Philip Low's Group (Purdue University, West Lafayette, IN).

In Vitro Cytotoxicity

Folate-receptor positive cells, OVCA 432, were grown and plated as described elsewhere. Approximately $10^3 - 10^5$ cells were added (~500µl-1000µl) to either a 35mm Petri dish (Becton Dickinson Labware, Franklin Lakes, NJ) or (~250µl) an 8-well plastic chamber slide (Nunc Inc., Naperville, IL) followed by addition of folate-free media to a total volume of either 2ml or 500-250µl. The cells were allowed to attach in the appropriate dishes for 24hrs. Three replicates were performed for the control, 1mM $CoCl_2$, 5mM Gd-DTPA, and free-folic acid competition study (total folic acid in media = 19.9µM) on any single day. Two replicates were performed for the 0.70mM (Gd)/ 0.018 mM folate-PAMAM-TU-DTPA-Gd(III), g=4, and 0.70mM (Gd) Folate-DTPA-Gd on any single day.

Following the 24-hour cell attachment period, the media was exchanged for fresh media containing the appropriate agent, and the cells were incubated for 72 hours in a 37°C incubator with 5% CO_2. Following the incubation period, the cells were removed and the relative cytotoxicity of the agents was determined.

Cell cytotoxicity was determined using the trypan blue exclusion method. A hemocytometer was used to count stained and unstained cells to obtain percent viability:

% viability = Viable cells (unstained) / [Viable cells + Dead cells (blue)].

Each replicate was counted 2-3 times and an average of 2-3 dishes for each condition was used to represent the compound's performance per day. Each compound was tested on at least 3 separate days. An analysis of variance (ANOVA) followed by the Student's t-test was used to compare one condition to another to test for statistical significance.

Results and Discussion

The data in Table 1 indicate that hFR receptor positive cells treated with either buffer or Gd(III)-DTPA (negative controls) exhibited the same % viability, and the toxic agent $CoCl_2$ (positive control) showed a significantly reduced % viability ($P < 0.0001$). Treating the cells with the folate conjugated dendrimer containing Gd(III)-DTPA resulted in a *slight* toxicity, 68.3 ± 7.6 % viability, significantly different from both the negative and positive controls, $P < 0.03$ and 0.0001 respectively. This reduction in viability was inhibited by the addition of excess folate to the medium. Cells treated with both free folate and the folate conjugated dendrimer containing Gd(III)-DTPA had a percent viability of 80.1 ± 2.4 which was significantly different from the cells treated without free folate, $P < 0.02$, but not from the negative controls. Cells treated with folate-DTPA-Gd(III) lacking the dendrimer, but at the same [Gd(III)], expressed the same level of viability as those treated with the folate-dendrimer-DTPA-Gd(III), 68.4 ± 0.8 %. Dendrimers lacking the folate but with DTPA-Gd(III) did not reduce the % viability relative to controls.

Treatment	Percent (%) Viability
Buffer	85.0 ± 6.2
5 mM Gd(III)-DTPA	85.7 ± 5.0
1 mM $CoCl_2$	5.6 ± 2.7
Folate-PAMAM-TU-DTPA-Gd(III) 0.70 mM Gd(III)	68.3 ± 7.6
Free Folate plus Folate-PAMAM-TU-DTPA-Gd(III) 0.70 mM Gd(III)	80.1 ± 2.4
Folate-DTPA-Gd(III) 0.70 mM Gd(III)	68.4 ± 0.8

Table 1. Cell Viabilty following treatment.

The data presented above are consistent with the hypothesis that the internalization of the Gd(III)-DTPA results in a *slight* but significant level of toxicity. We can not rule out however an anti-folate like effect of the folate conjugated system. All we can say is that the *slight* increase in toxicity requires the internalization of the agents. While one might not worry about the *low* cytotoxic effect of a diagnostic agent specific for tumor cells, one must realize that the proximal tubules of the kidneys also accumulate these types of agents. Although, in vivo we may not see even this low level of toxicity as the in vitro studies were made at a relatively static concentration of the agent for 72 hours where as an in vivo system is dynamic with an elimination phase and a long receptor recycling rate. Thus while the in vitro data suggest a benefit from using a kinetically stabile Gd(III)-chelate, like DOTA, in vivo data may not have the same toxicity

[1] A.D. Watson, S.M. Rocklage, and M.J. Carvlin in D.D. Stark and W.G. Bradley (editors), *Magnetic Resonance Imaging*, Mosby Yearbook, St. Louis, 1992, p. 372.

[2] Wiener EC, Konda S, Shadron A, et al.. Invest Radiol 1997; 32: 748–754.

[3] Franano FN, Edwards WB, et al.. Magn. Reson. Imaging. 1995; 13: 201-214.