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13. ABSTRACT *(Maximum 200 Words)*
Folate receptors are overexpressed on a variety of human cancer cells, including breast cancer cells, but are highly restricted in normal tissues. Folate-conjugated radiopharmaceuticals have shown specificity for folate-receptor-bearing cells and promise in cancer imaging in animal models. We proposed to explore the coupling to folate of a series of MR contrast agents and work is in progess in this area. Folate is being coupled with a series of ligands for complexation with paramagnetic metal ions, including iron(III) and gadolinium(III) to yield a series of new MR contrast agents. Following completion of the synthesis and characterization of these novel contrast agent-conjugates, selectivity for breast cancer cells that overexpress the high-affinity folic acid receptor will be examined. Chinese hamster ovary (CHO) cells that have been modified to overexpress FR-beta have been cloned and grown. Western blot analysis has been performed to confirm the overexpression of FR-beta. The cells are easily visualized microscopically which will allow for qualitative analysis of the uptake of the dark red folate-hydroxypyridinone complexes of iron(III). Flow cytometry will be performed in order to measure the uptake of the gadolinium(III) complex of folate-DTPA.

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Introduction

Folic acid (Figure 1) is a vitamin that enters cells through either carrier-mediated or receptor-mediated processes. The former involves a trans-membrane protein that has a low affinity for folic acid (K_D ca. 1 – 5 μM) and that catalyses passive transport while the latter involves a high-affinity folate receptor (folate-binding protein, K_D ca. 100 pM) that promotes endocytosis of folic acid. Folate-drug conjugates are not substrates for the low-affinity protein but may be targeted to the high-affinity folate receptor. Use of the γ-carboxyl of folic acid as a site for drug conjugation through chemical modification does not measurably compromise the affinity for the folate receptor while modification of the α-carboxyl may cause essentially complete loss of specificity. (Reddy and Low 1998)

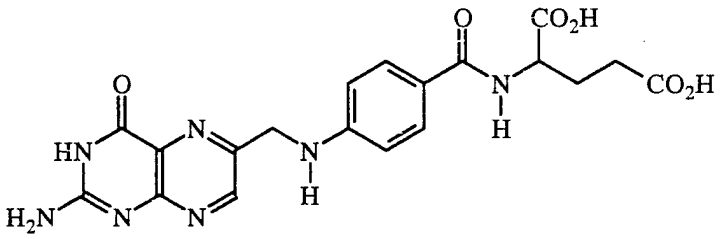

Figure 1

Folate receptors are overexpressed on a wide range of human cancer cells but are highly restricted in normal tissues. While a wide range of folate-drug conjugates and folate analogs have been examined as targeted therapeutics (Gruner and Weitman 1999), the targeting of diagnostic pharmaceuticals is only recently being explored (Reddy and Low 1998). Folate conjugates of ^{111}In (Wang, Luo et al. 1997; Mathias, Wang et al. 1998), ^{67}Ga (Mathias, Wang et al. 1996; Wang, Lee et al. 1996), and ^{99m}Tc (Guo, Hinkle et al. 1999) chelates have been synthesized and examined as targeted radiopharmaceuticals. An [^{111}In]-DTPA-folate (DTPA = diethylenetriaminepentaacetic acid) agent was shown (Wang, Luo et al. 1997; Mathias, Wang et al. 1998) to be specific for folate-receptor-bearing cells with uptake kinetics similar to those of free folate. In a preliminary mouse study, γ-scintigraphy allowed visualization of a folate receptor-positive KB cell tumor following i.v. administration of the radiopharmaceutical. Conjugation of [^{67}Ga]-deferoxamine to folate through a procedure that generated a mixture of isomers via amide bond formation at the α- and γ-carboxylates of folate has been described (Wang, Lee et al. 1996). Separation of the isomers and competitive binding studies showed that only the γ-modified folate is recognized by the folate receptor on KB cells. A [^{99m}Tc]-6-hydrazinonicotinamido-hydrazido-γ-folate conjugate (Guo, Hinkle et al. 1999) allowed ready visualization of subcutaneously implanted folate receptor-positive tumors in mice.

Targeting of contrast agents for magnetic resonance imaging (MRI) presents an additional problem because the concentration of contrast agent required for tumor imaging by MRI is much higher than the concentrations required when radiopharmaceuticals are employed as imaging agents. Attempts to conjugate MR contrast agents to antibodies suggested (Eckelman, Tweedle et al. 1988) that 100 to 1000 gadolinium ions per receptor would be required for useful image contrast. However, this estimate is not applicable to the high-affinity folate receptor as recycling occurs (Wiener, Konda et al. 1997) which will lower the demand dramatically. A Gd-dendrimer complex, conjugated to folate, was shown (Wiener, Konda et al. 1997) to increase the longitudinal relaxation rate of folate-receptor-positive cells by 109%, an increase that, if observed with folate receptor-positive tumors in vivo, is sufficient for diagnostic use. The method used to synthesize the Gd-dendrimer-folate conjugate was one that produced two isomers via amide bond formation at the α- and γ-carboxylates of folate. As discussed above, modification of folate at the α-carboxylate generates agents that may not be recognized by the high-affinity folate receptor and so the encouraging results obtained with this mixture might be significantly improved upon by generation of a single isomer through modification of the γ-carboxylate of folate.

A method has been developed for the selective modification of the γ-carboxylate of folate (Reddy and Low 1998) but this method has not been employed for conjugation of MR contrast agents. The method involves inactivation of the α-carboxylate as a salt and activation of the γ-carboxylate as a methyl ester to generate a key intermediate for coupling (Figure 2, separate page). The intermediate can be coupled with ethylenediamine to yield a folate derivative with a pendant amine functionality for coupling with an activated carboxylate of a contrast agent (Figure 3).

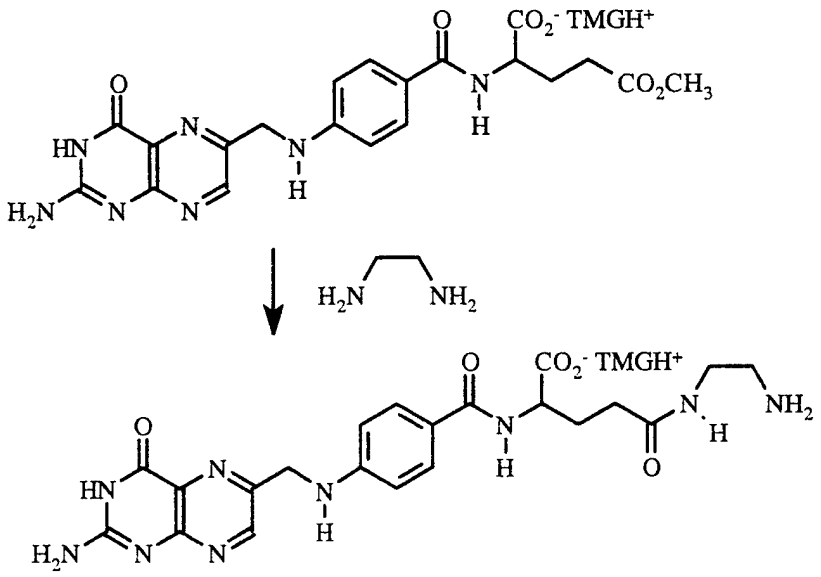

Figure 3

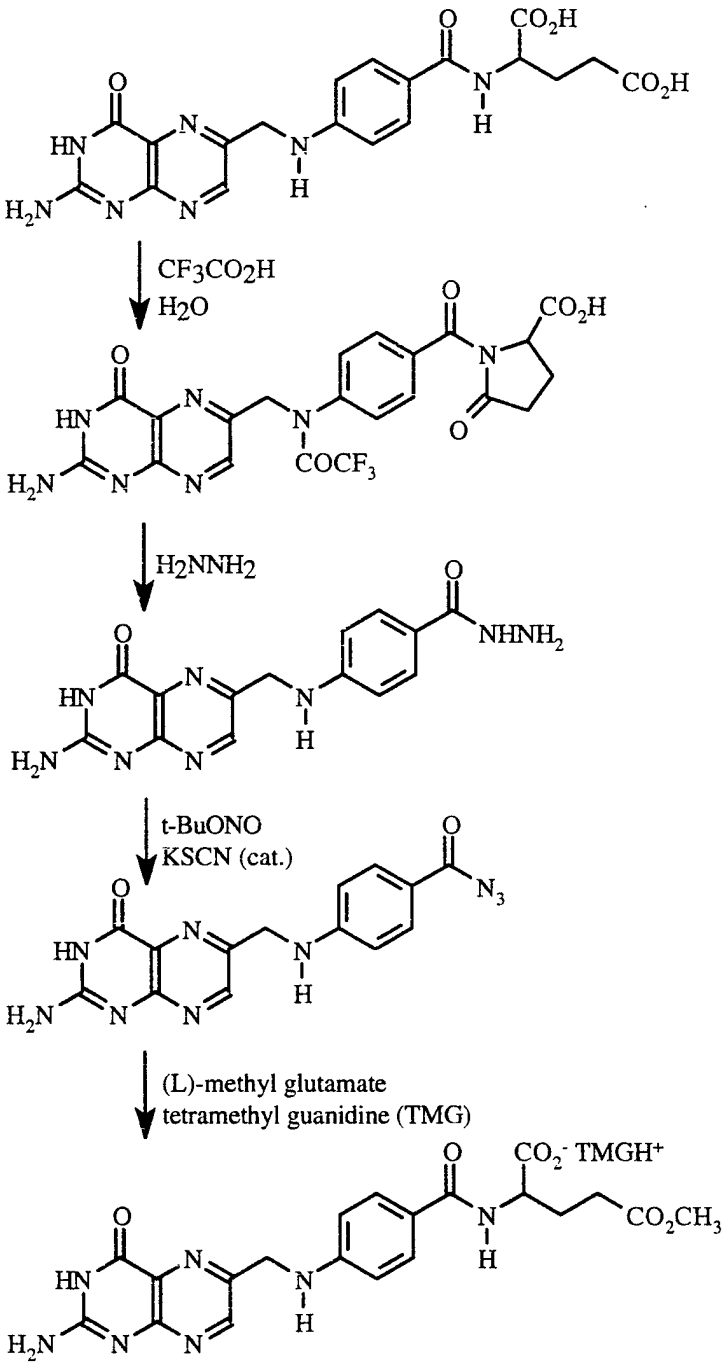

Figure 2

We proposed to explore the synthesis of MR contrast agents conjugated to folate through modification of the γ-carboxylate, exploiting the chemistry shown in Figures 2 and 3. The coupling of folate to a hydroxypyridinone ligand, suitable for complexation with iron(III) as a contrast agent, is one example of such a target Similar coupling reactions with the anhydride of diethylenetriaminepentaacetic acid (DTPA) will yield folate-conjugated ligands for

gadolinium(III). Folate-ligand conjugates will be synthesized, characterized, and employed in the complexation of iron(III) and gadolinium(III).

Similar coupling chemistry is to be developed with methotrexate (Figure 4), an anticancer drug that targets the high-affinity folate receptor (Gruner and Weitman 1999).

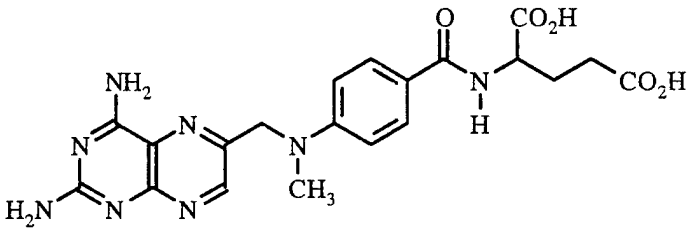

Figure 4

Body:

The synthesis of the folate-conjugated MR contrast agents encountered an unexpected difficulty as the proposed methods, which were based upon literature reports, were found to generate coupled products that contained an unacceptably high level of impurities which resisted all attempts at separation. These samples were unsuitable for uptake studies with cell lines. For this reason, we requested and were granted a no-cost extension to this award so that we can attempt to address the purification issue. We have begun to explore an alternative synthesis that may lead to less difficult purification problems (Leamon et al., 1993) and are currently at the final stages of the synthesis and the purification steps are in progress. In the interim, while this work is underway, Chinese hamster ovary (CHO) cells that have been modified to overexpress FR-β have been cloned and grown in the laboratory of DR. Manohar Ratnam at the Medical College of Ohio. Western blot analysis has been performed to confirm the overexpression of FR-β. The cells are easily visualized microscopically which will allow for qualitative analysis of the uptake of the dark red folate-hydroxypyridinone complexes of iron(III). Flow cytometry will be performed in order to measure the uptake of the gadolinium(III) complex of folate-DTPA.

Key Research Accomplishments:

Research is in progress and currently hinges upon solving a difficult purification problem that has prevented key uptake studies from being performed.

Reportable outcomes:

There are no reportable outcomes at this time.

Conclusions:

We are unable to draw conclusions based upon the experiments that we have performed to date. We have requested, and were granted, a no-cost extension to this award so that we can attempt to address key sample purification issues that have prevented us from performing the key cell uptake experiemnets that will be necessary before conclusions can be drawn.

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