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Discovery and Development of Folic-Acid-Based Receptor Targeting for Imaging and Therapy of Cancer and Inflammatory Diseases

PHILIP S. LOW,* WALTER A. HENNE, AND
DEREK D. DOORNEWEERD

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

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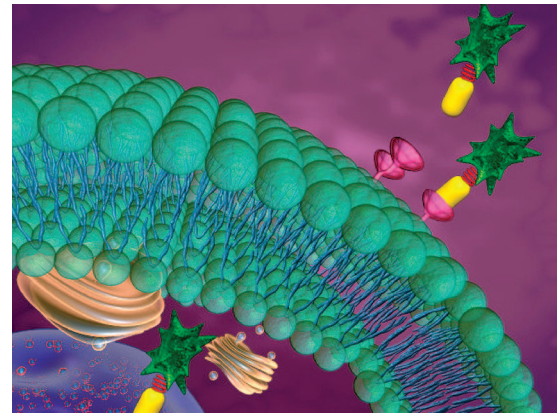
CONSPECTUS

In order to avoid the toxicities associated with prescription drug use today, we have explored novel methods for delivering drugs selectively to pathologic cells, thereby avoiding the collateral damage that accompanies their uptake by healthy cells. In this Account, we describe our quest for the ideal targeted therapeutic agent. This effort began with a search for ligands that would bind selectively to pathologic cells, displaying no affinity for healthy cells. After identification of an optimal targeting ligand, effort was focused on construction of linkers that would carry the attached drug to pathologic cells with receptors for the selected ligand. In the case of cancer, we exploited the well-characterized up-regulation of folate receptors on malignant cells to target folate-linked pharmaceuticals to cancer tissues *in vivo*. Drugs that have been linked to folic acid for tumor-selective drug delivery to date include (i) protein toxins, (ii) chemotherapeutic agents, (iii) gene therapy vectors, (iv) oligonucleotides (including small interfering RNA (siRNA)), (v) radioimaging agents, (vi) magnetic resonance imaging (MRI) contrast agents, (vii) liposomes with entrapped drugs, (viii) radiotherapeutic agents, (ix) immunotherapeutic agents, and (x) enzyme constructs for prodrug therapy. Current clinical trials of four folate-linked drugs demonstrate that folate receptor-targeting holds great promise for increasing the potency while reducing toxicity of many cancer therapies.



In the course of developing folate-conjugated drugs for cancer, we discovered that folate receptors are also overexpressed on activated (but not resting or quiescent) macrophages. Recognizing that activated macrophages either cause or contribute to such diseases as rheumatoid arthritis, Crohn's disease, atherosclerosis, lupus, inflammatory osteoarthritis, diabetes, ischemia reperfusion injury, glomerulonephritis, sarcoidosis, psoriasis, Sjogren's disease, and vasculitis, we initiated studies aimed at developing folate-conjugated imaging and therapeutic agents for the diagnosis and treatment of such diseases. In very brief time, significant progress has been made towards identification of clinical candidates for targeted treatment of several inflammatory and autoimmune diseases. This Account summarizes the discovery and development of a variety of folate-targeted drugs for the diagnosis and therapy of cancers and inflammatory/autoimmune diseases.

Introduction

Folic acid has emerged as an optimal targeting ligand for selective delivery of attached imaging and therapeutic agents to cancer tissues and sites of inflammation. The utility of folic acid in these applications has arisen primarily from (1) its ease of conjugation to both therapeutic and diagnostic agents, (2) its high affinity for the folate receptor ($K_d = 10^{-10}$ M), even after conjugation to its therapeutic/diagnostic cargo, and (3) the limited distribution of its receptor (FR) in normal tissues, despite its upregulation on both cancer cells (primarily FR-α isoform) and activated macrophages (FR-β isoform) (for a thorough review of FR iso-

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forms, see ref 1). Cancers found to overexpress FR include cancers of the ovary, lung, breast, kidney, brain, endometrium, colon, and hematopoietic cells of myelogenous origin.[2] Because activated macrophages are implicated in such pathologies as rheumatoid arthritis, psoriasis, Crohn's disease, systemic lupus erythematosus, atherosclerosis, diabetes, ulcerative colitis, osteoarthritis, glomerulonephritis, and sarcoidosis, applications for folate targeting now also include most inflammatory diseases.[2] While FR-directed antifolates have proven useful in the treatment of some of the above diseases,[3–7] this Account will focus on the discovery and development of folate receptor-targeted drugs for the diagnosis and therapy of these pathologies.

The Discovery

Discovery of vitamin-mediated drug targeting was totally fortuitous. A former graduate student, Mark Horn, was assigned the task of demonstrating that receptor-mediated endocytosis could occur in the plant kingdom, despite current dogma claiming the opposite. Not wanting to use radioactivity to quantitate internalization of his elicitors (molecules known to bind plant cells and elicit multiple disease resistance pathways), Mark linked biotin to his elicitors and followed their endocytosis with fluorescent streptavidin. Although the data revealed that the biotinylated elicitors indeed entered plant cells in a saturable, temperature- and energy-dependent process (i.e., receptor-mediated endocytosis), control studies with unrelated molecules did not behave as anticipated.[8,9] Thus, when biotin was linked to animal proteins (e.g., serum albumin, insulin, ribonuclease, and nonspecific IgG), the biotinylated proteins were also seen to enter plant cells, even though their nonbiotinylated counterparts remained completely extracellular. Although it was not our intention, this observation led to the fortuitous discovery that biotin could ferry attached proteins into live plant cells.[9]

The question then arose whether vitamins might also be exploited to mediate the delivery of otherwise impermeable macromolecules into animal cells. Evaluation of biotin–bovine serum albumin (BSA) uptake into several available animal cell types demonstrated that biotin-mediated internalization did indeed occur in some (e.g., bovine sperm, PC12 cells, etc.) but not all cells tested. Further studies revealed that riboflavin could also transport attached proteins into a limited selection of animal cells.[10] Most importantly, Christopher Leamon, a new graduate student in the lab, observed that folic acid could deliver tethered proteins into a variety of cultured animal cells.[11] Although we did not know at the time that FR expression was largely limited to malignant cells and activated macrophages, this observation marked the birth of folate-receptor-mediated drug-targeting research.

Initial Characterization of Folate-Receptor-Mediated Targeting

Stimulated by papers from Kamen and Antony and their colleagues showing upregulation of FR on certain mammalian cell lines,[12,13] cultured cells that overexpressed FR were obtained and shown to internalize folate conjugates of BSA, ribonuclease, horseradish peroxidase, IgG, and ferritin.[11] Ignorant of the selective expression of FR on cancer cells, we concluded erroneously that folate might be exploited to deliver otherwise impermeable macromolecules into many cells for both scientific and clinical applications.

Additional studies that immediately followed focused on the characterization of the folate conjugate uptake pathway. The folate receptor was shown to be responsible for conjugate internalization based on elimination of internalization by (1) competition with excess free folate, (2) cleavage of cells with phosphatidylinositol-specific phospholipase C (an enzyme known to release FR, a glycosylphosphatidylinositol-anchored protein, from cell surfaces), and (3) antibodies raised against FR.[14] Folate conjugate binding was also found to be saturable ($\sim 10^7$ molecules bound/HeLa cell) and of high affinity ($K_d \sim 24$ nM for folate–ribonuclease).[14] Folate-derivatized colloidal gold particles were found to enter KB cells in uncoated regions of membrane invagination, then traffic to multivesicular bodies, and eventually move into the cytoplasm or lysosomal-like compartments.[15] Other studies showed that folate-tethered proteins were not digested following internalization, suggesting that most trafficking was not directed to a degradative compartment.[16] This conclusion has been more recently confirmed and elaborated.[17]

Upregulation of Folate Receptors on Cancer Cells and Early Drug-Delivery Studies

Knowledge that FR was significantly upregulated on cancer cells was not obtained until late 1991 and early 1992 when a series of papers from different labs demonstrated that several monoclonal antibodies used to identify cancer cells in tissue biopsies actually recognized FR.[18–20] Learning of this observation, we realized that the distribution of FR was largely restricted to malignant tissues, a realization that changed the entire focus of our research. No longer was FR viewed as a tool for universal delivery of macromolecules into all mammalian cells but was now considered a receptor for mediat-

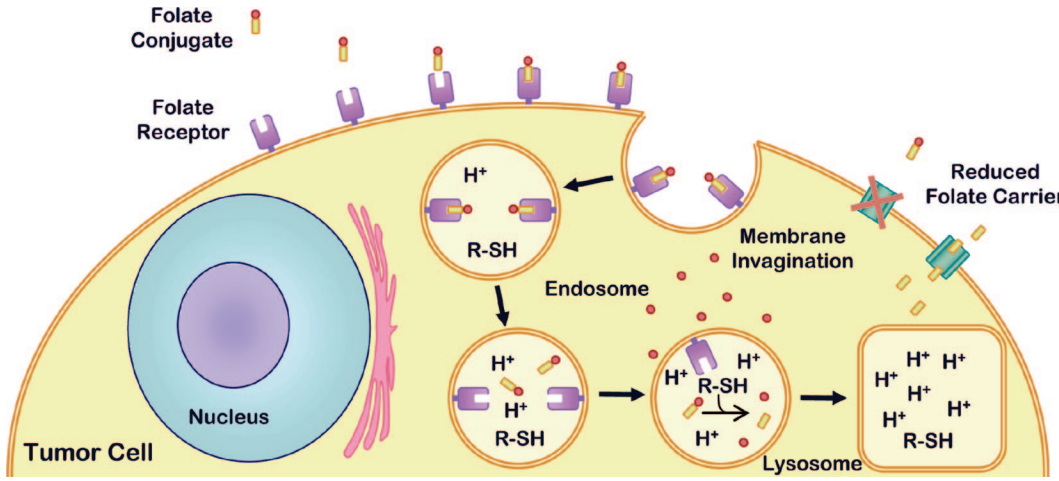

FIGURE 1. FR-mediated endocytosis of a folic acid drug conjugate. Folate conjugates bind FR with high affinity and are subsequently internalized into endosomes that can reduce disulfide bonds. Within the endosome, a folate–disulfide–drug conjugate is released from the FR and the prodrug is reduced to liberate the parent drug cargo. Because the pH of FR-containing endosomes is only mildly acidic, acid-labile linkers do not release the attached drug as efficiently.

ing selective targeting of drugs/imaging agents to cancer cells (Figure 1). Motivated by this new information, new research was directed at developing folate-linked cytotoxic agents.

Because of our biochemical background, initial efforts aimed at building folate-linked cytotoxics focused on folate-tethered protein toxins. Folate–momordin was shown to kill FR+ cancer cells with an IC_{50} of $\sim 10^{-9}$ M,[21] and a folate–pseudomonas exotoxin (PE38) conjugate was found to display an IC_{50} of $\sim 10^{-11}$ M.[16] More importantly, studies of the linkage between folic acid and PE38 demonstrated that the potency of the conjugate was intricately connected to the type of bond tethering the vitamin to the ribosome-inactivating protein. Thus, when the two components were linked via a reducible disulfide bond, full killing potency was observed ($IC_{50} \sim 10^{-11}$ M). However, when the components were bridged by a thioether bond (i.e., replacement of a sulfur with a carbon atom in the bridge), its potency decreased by over 4 orders of magnitude.[16] These results demonstrated for the first time the importance of building a cleavable linker into the folate-targeted cytotoxic agent, an imperative component in future folate conjugate drug designs.

Folate Liposomal Carriers for the Delivery of Chemotherapeutic Cargo

The desire to target larger quantities of drugs to cancer cells led rapidly to efforts aimed at delivering drug-loaded liposomes into malignant tissues. Initial studies with fluorescent liposomes demonstrated that liposomes with folate directly attached to the lipid headgroups did not efficiently bind FR+ cancer cells.[22] In contrast, liposomes tethered to folate via a polyethylene glycol (PEG) spacer were found to enter cancer cells in numbers of >200 000/cell.[22] With the possibility of loading >30 000 drug molecules into each liposome, the payload potential of these folate-targeted liposomes seemed enormous. Unfortunately, our unmodified liposomal formulations suffered from short circulation times *in vivo* because of nonspecific uptake by the reticuloendothelial system. This limitation was, however, solved by incorporating ~4% PEGylated lipids into the liposomes, with ~0.1% of the total lipids (folate–PEG–distearoylphosphatidylethanolamine) attached to folic acid.[23] Later studies would reveal that better tumor-specific delivery could be achieved if (1) the free (underivatized) PEG chains were shorter (PEG 2000) in length than the folate-derivatized chains (PEG 3350), (2) the liposome size was restricted to <100 nm in diameter, (3) cholesterol and saturated phospholipids were used as the primary building blocks of the liposomes, and (4) a pH-sensitive release or unloading mechanism was built into the liposomal structure.[22–26] Strategies to achieve the latter objective included incorporation of amphipathic peptides into the liposomes that would become fusogenic only at the low pH values found in intracellular endosomes,[25] inclusion of phospholipids in the bilayer that would hydrolyze to detergents at low intraendosomal pH values, and preparation of liposomes with caged phospholipids that became fusogenic only upon exposure to low endosomal pH values.[27,28]

In our first exploration of folate–PEG–liposome activity, liposomes were loaded with the common chemotherapeutic agent, doxorubicin, and tested for toxicity against FR+ tumor cells. Tissue cultures incubated with folate-tethered liposomes displayed a 45-fold higher uptake than their nontargeted con-

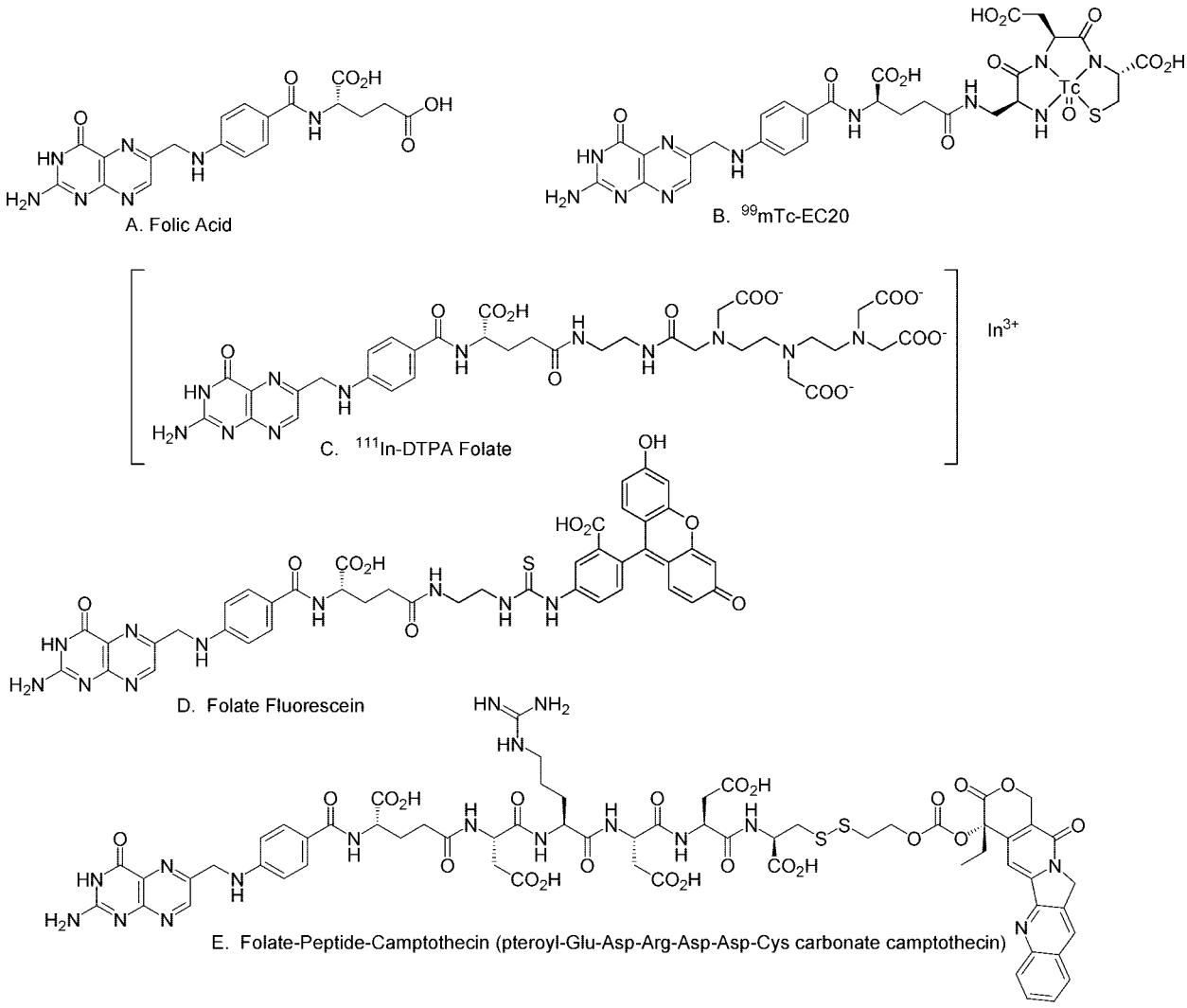

FIGURE 2. Structures of folic acid and several representative conjugates.

trols, and cell cytotoxicity of the targeted liposomes was found to be 85 times greater than similarly loaded controls.[23] Applied to antisense delivery, related folate-targeted liposomal formulations were observed to suppress the expression of the epidermal growth factor receptor >50-fold more potently than the same concentration of free antisense oligonucleotides.[29] Further studies of folate-targeted liposomal gene therapy vectors demonstrated that tumor-selective gene expression could be achieved if liposomal size, DNA compaction, particle charge, and nuclear delivery were optimized using commonly employed methods.[30]

Folic-Acid-Targeted Imaging Agents

With only limited data available on FR expression in normal tissues, there was significant concern that healthy tissues might be targeted with folate conjugates. The most efficient method for assessing whether normal cells might bind and internalize folate conjugates was to examine the biodistribution of radiolabeled folate-linked imaging agents (parts B and C of Figure 2). Folate–deferoxamine–^{67}Ga, folate–diethylenetriamine pentaacetic acid (DTPA)–^{111}In, folate–DTPA–^{99m}Tc, and folate–deferoxamine–^{66}Ga/^{68}Ga were then synthesized and tested in tumor-bearing animals in rapid succession.[31–36] Except for differences in imaging modalities, the biodistributions of several folate conjugates were quite similar. Thus, radioconjugates were taken up in large quantities by tumor and kidneys, to a lesser extent by the liver, and at very low levels by other tissues. As will be noted below, significant uptake would eventually also be seen at sites of inflammation because of the expression of FR on activated macrophages,[37] but in general, normal tissues, except the kidneys, did not appear to concentrate folate conjugates. These results were very encouraging, because they immediately implied that cytotoxic agents that did not damage the kidneys could be exploited for folate-targeted cancer therapies.

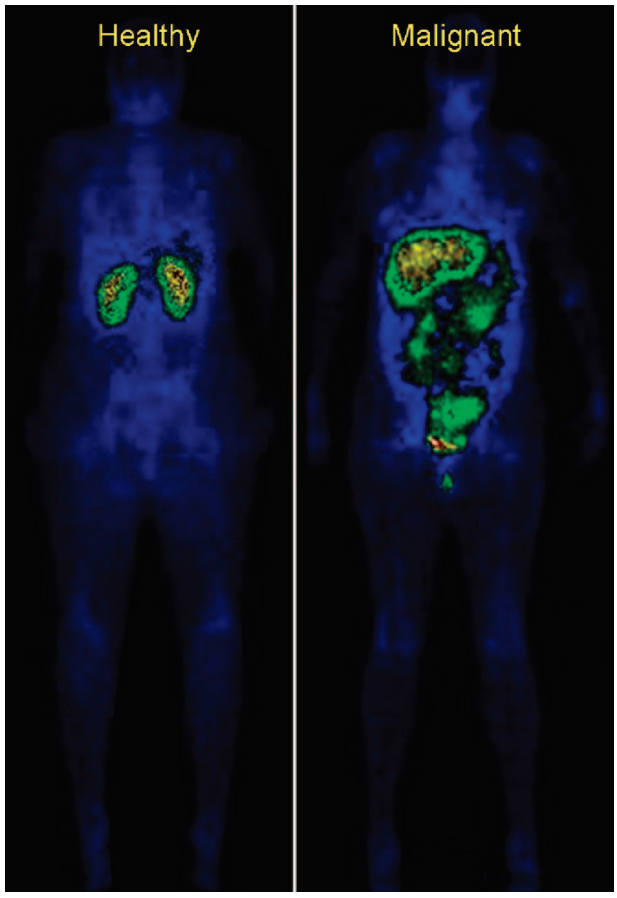

FIGURE 3. ^{111}In–DTPA–folate whole body scintigraphic images of a healthy volunteer and an ovarian cancer patient. Uptake in the cancer patient is seen in both the malignant tissue and kidneys, whereas only kidney uptake is observed in the healthy individual. Image reproduced with permission from Endocyte, Inc.

Concern about folate conjugate distribution in humans, however, would not be allayed until images of cancer patients were obtained. ^{111}In–DTPA–folate was moved rapidly into the clinic, and images similar to those shown in Figure 3 were collected. As seen, the absence of a significant uptake in liver, spleen, bone marrow, heart, lungs, brain, muscle, etc. argued that the tumor/kidney selectivity seen in animals was also realized in humans.[35] Also of importance was the observation that the ^{111}In–DTPA–folate would clear from FR-negative tissues in less than 1 h, suggesting that isotopes with shorter half-lives than ^{111}In could be developed for tumor imaging. Motivated by this realization, Endocyte, a company founded to develop folate-targeted imaging and therapeutic agents, designed EC20, a ^{99m}Tc-based folate-linked chelator. Because of its shorter half-life and the consequent lower radiation exposure, EC20 has proven to be the γ-emitter of choice for cancer and inflammation imaging.[38]

Folic-Acid-Targeted Low-Molecular-Weight Chemotherapeutic Agents

With tumor selectivity assured, the obvious next step was to exploit folate to target chemotherapeutic agents to cancer cells. However, initial studies using a folate-targeted taxol conjugate proved the construct to be less potent than anticipated,[39] most likely because of the poor water solubility or slow release of the drug from the conjugate. Poor water solubility was known to lead to nonspecific binding to nontargeted cells, and failure to release unmodified drug had been previously shown to lead to drug inactivity.[16] These concerns raised the need to understand more thoroughly the physical properties of folate–drug conjugates as well as the conditions in FR-mediated endocytic pathways that might be exploited to trigger drug release.

Two mechanisms had been exploited by other groups to promote the intracellular release of an active drug from its targeting ligand, but neither mechanism had been characterized in the FR endocytic pathway. Therefore, both would require validation before implementation in the design of folate conjugates. The first mechanism exploits the difference in reducing power between extra- and intracellular milieus to induce the selective release of a disulfide-linked drug inside its target cell. Evidence that this mechanism might be operative was provided by data showing the activity of a folate–disulfide conjugate of mitomycin C both *in vitro* and *in vivo*.[40,41] To visualize the rate and intracellular location of disulfide reduction, a folate-linked fluorescence resonance energy transfer (FRET) construct was prepared, which changed from red to green fluorescence upon reduction of an intramolecular disulfide bond.[17] Thorough analysis of the behavior of this construct both in cultured cancer cells and live tumor-bearing mice demonstrated that folate–disulfide–drug conjugate reduction (1) does not occur in circulation prior to conjugate capture by tumor cells, (2) occurs following endocytosis with a half-time of 6 h, (3) begins in endosomes and does not significantly depend upon the redox machinery located on the cell surface, within the lysosome, or the Golgi apparatus, (4) occurs independently of endocytic vesicle trafficking along microtubules, and (5) yields products that are subsequently sorted into distinct endosomes and trafficked in different directions.[17] On the basis of related disulfide linker chemistries, several folate–disulfide–drug conjugates have been prepared by scientists at Endocyte[40–43] and in our lab,[44] and two are now in clinical trials. The structure of a typical folate–disulfide–drug conjugate with a hydrophilic peptide spacer to increase water solubility is shown in Figure 2E.

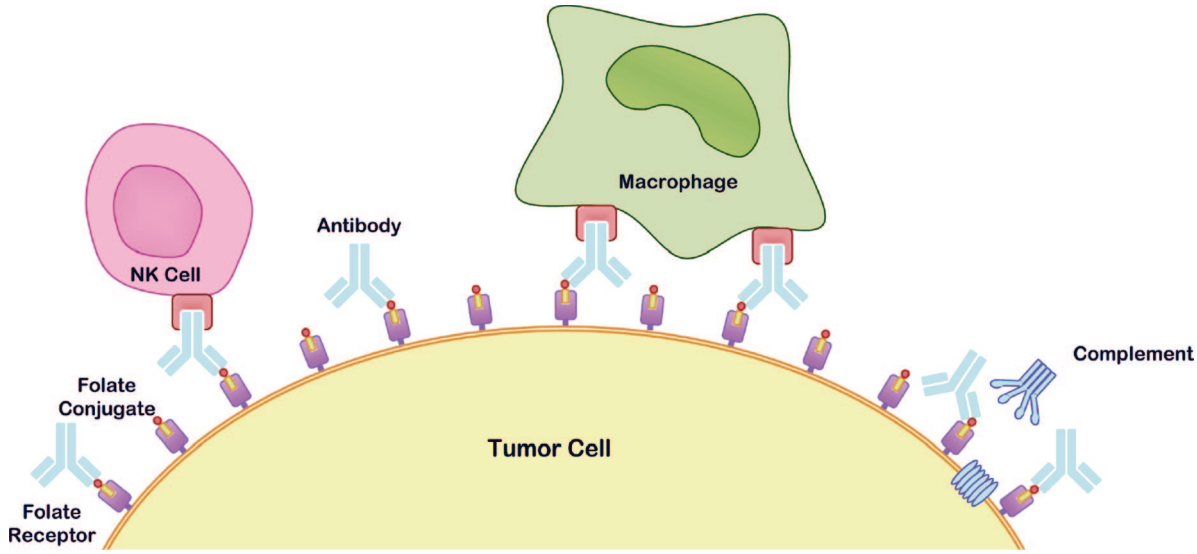

FIGURE 4. Illustration of folate–hapten-mediated immunotherapy. Mice previously immunized against the hapten (fluorescein) are treated with folate–fluorescein. Folate targeting decorates the cancer cell surface with large numbers of foreign haptens ($>10^6$), leading to antihapten (antifluorescein) antibody binding and destruction of the marked tumor cell by macrophages, natural killer cells, and complement.

The second mechanism envisioned to trigger the endosomal release of an active drug from its folate conjugate upon endocytosis involved the decrease in pH commonly observed in late endosomes and lysosomes.[45] To exploit this pH change for drug release, a variety of pH-sensitive linkers connecting folate to its therapeutic cargo were developed and tested both *in vitro* and *in vivo*.[39] Unfortunately, none of the conjugates demonstrated potencies similar to those of the disulfide-linked conjugates. To explore the molecular basis of this compromised efficacy, a second folate–FRET conjugate, in this case bridged by a pH-sensitive linker, was constructed and examined as described above for the disulfide-linked conjugate.[46] Surprisingly, little hydrolysis of the pH-sensitive linker was observed during endosomal trafficking. Subsequent studies with folate-linked pH-indicator dyes fortunately offered at least a partial explanation. These experiments revealed that the endosomal compartments visited by monovalent folate conjugates experience pH values only as low as 6.2,[46] whereas endosomal compartments visited by multivalent folate conjugates experience pH values as low as 5.0 and below.[26]

One additional aspect that is important for folic-acid-mediated drug delivery concerns the rate of FR recycling between the cell surface and its intracellular compartments. Net accumulation of folate conjugates in tumor tissues will depend upon not only the number and accessibility of FR on the malignant cell surfaces but also the time required for unoccupied receptors to recycle back to the cell surface for additional drug uptake. Using radioactive conjugates, we found empty FR+ to unload their cargo and return to the cell surface in $\sim$8–12 h.[47] Given that an average cancer cell will express anywhere from $\sim$0 to 10^7 FR/cell, this recycling time constraint suggested that only very potent chemotherapeutic agents could succeed as folate conjugates for the treatment of cancer. In our experience, agents possessing low nanomolar range IC_{50} values in their unconjugated forms have the highest potential for *in vivo* efficacy.

Folate-Targeted Immunotherapy

Given the complexities of designing linkers that would release free drug only after receptor-mediated endocytosis, we simultaneously explored therapies that required no drug release for therapeutic efficacy. One such strategy involved the use of folic acid to deliver a highly immunogenic hapten (low-molecular-weight immunogen) to the surfaces of cancer cells to render the malignant cells more "foreign" to the immune system (Figure 4). Recognizing that simple contact of skin cells with an oil (urushiol) from the poison ivy plant successfully marks skin cells as "foreign" or "non-self", leading to their rapid destruction by the immune system, we chose to use folic acid to similarly mark cancer cell surfaces with a related immunogenic hapten, fluorescein.[48] For this purpose, cancer-bearing animals were first vaccinated against fluorescein, after which the immunized animals were treated with folate–fluorescein (Figure 2D). Upon injection, the folate conjugate was found to rapidly localize to FR-expressing cells,[49] decorate their surfaces with $>10^6$ haptens/cell (Figure 5), and lead to their elimination by the immune system. In contrast, normal cells, because of their lack of FR, were largely spared any

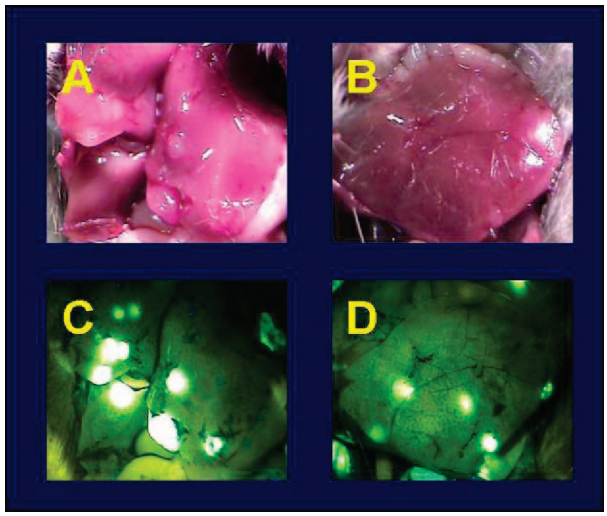

FIGURE 5. Fluorescence imaging of FR+ L1210A tumors present in two separate mouse livers. Mice were injected intravenously with 10 nmol of folate fluorescein and imaged after 2 h. A and B show the normal white light images of the mouse livers, while C and D display the fluorescent images of the same tissues. The data were reprinted with permission from the *Journal of Biomedical Optics*. Copyright 2003 SPIE.[49]

immune attack. Most importantly, the folate–hapten therapy (which is now in phase 2 clinical trials) was found to confer long-term immunity against the cancer, such that rechallenge with fresh tumor cells invariably led to the rejection of the implanted cells without the need for further therapy.[48] Given the potential for many cancers to recur, this induction of immune memory was viewed as a major strength of the targeted hapten approach.

Development of Folate-Targeted Therapeutic and Imaging Agents for Inflammatory Diseases

During the course of imaging patients for FR+ cancers, significant uptake of a radiolabeled folate conjugate was occasionally seen in the knee of a patient (Figure 6). Although we had no access to patient medical histories, upon consultation with the referring physician, it was learned that each of these patients was suffering from an inflammatory condition (presumably arthritis) in the imaged joint. Concurrently, a former graduate student, Mary Jo Turk, had observed that folate-targeted liposomes were taken up by macrophages as well as cancer cells in ascites fluid.[50] Upon further investigation, it was determined that uptake in macrophages was FR-mediated and that this FR was present only on activated but not resting/quiescent macrophages.[37,50–52]

To verify that inflamed joints are indeed sites of folate conjugate uptake, imaging studies were performed on nine dogs

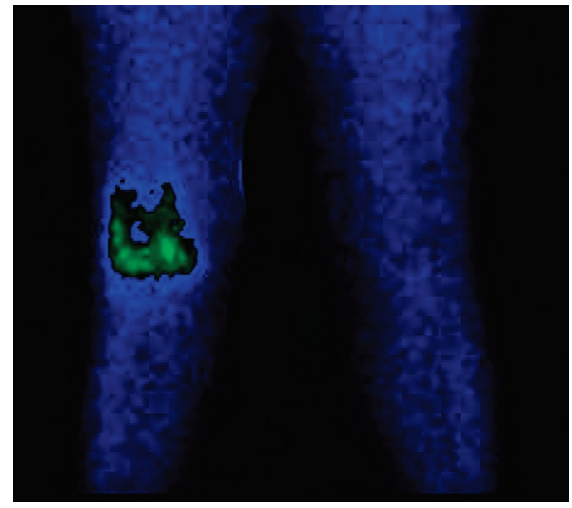

FIGURE 6. ^{111}In–DTPA–folate scintigraphic image of a patient with an inflamed right knee (presumably osteoarthritis). This image was reproduced with permission from *Advanced Drug Delivery Reviews*. Copyright 2004 Elsevier.[51]

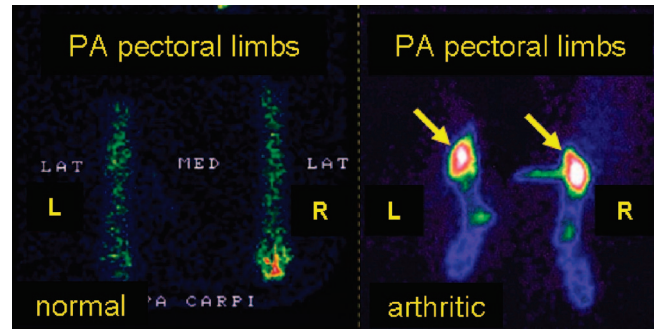

FIGURE 7. γ-Scintigraphic images of the front legs of a healthy dog (left) and a dog suffering from rheumatoid arthritis (right) 2 h postadministration of a ^{99m}TC EC20 imaging agent. Arrows indicate the areas of uptake in the arthritic joints. This image was reproduced with permission from *Advanced Drug Delivery Reviews*. Copyright 2004 Elsevier.[51]

suffering from rheumatoid arthritis along with five normal dogs to serve as controls. All nine arthritic dogs displayed dramatic uptake of the folate radiotracer at the expected sites of inflammation (Figure 7), while all five normal dogs displayed only background levels of radioactivity.[51] Subsequent studies of dogs, horses, rats, mice, and humans with both rheumatoid and osteoarthritis have demonstrated that essentially all joints experiencing active inflammation concentrate folate-linked imaging agents. These data obviously demonstrate that folate can target sites of activated macrophage enrichment *in vivo*; however, whether quantities of the drug sufficient to achieve effective therapy could be delivered remained uncertain.

Motivated by the potential to treat rheumatoid arthritis with folate-linked drugs, studies were undertaken to explore the impact of the previously described folate–hapten therapy on

rodent models of rheumatoid arthritis. The hypothesis was entertained that decorating an activated macrophage with large numbers of foreign haptens would lead to its immune-mediated elimination, much like the related marking of malignant cells with haptens was seen to promote their removal. For this purpose, rats with adjuvant-induced arthritis and mice with collagen-induced arthritis were first immunized against fluorescein and then treated with folate–fluorescein. A dramatic decline in arthritis symptoms was rapidly seen in both disease models, as manifested by decreases in paw swelling, spleen size, systemic inflammation, arthritis score, and bone erosion.[53] Furthermore, the anticipated decline in macrophage content of arthritic joints was accompanied by a concurrent decrease in CD4+ and CD8+ T cells, suggesting that elimination of activated macrophages can promote the disappearance of other inflammatory immune cells as well.

Recognizing that activated macrophages could be eliminated by treatment with folate-targeted therapy, the question naturally arose whether other diseases might be similarly benefited by elimination of their activated macrophages. To address this question, a literature search was undertaken to identify other diseases that are caused or worsened by activated macrophages. Search results revealed that psoriasis, Crohn's disease, systemic lupus erythematosus, atherosclerosis, diabetes, ulcerative colitis, osteoarthritis, and glomerulonephritis all have significant activated macrophage involvement.[2] Further research suggested that the symptoms of most inflammatory conditions are mediated in large part by products of activated macrophages, including TNF-α, IL-6, IL-1, prostaglandins, reactive oxygen species, collagenases, cathepsins, leukotrienes, and other potent cytokines.[54,55] Not surprisingly, elimination of the same proinflammatory products with drugs, such as Remicade or Enbrel (for TNF-α), Kineret (for IL-1), or Celebrex (for prostaglandins), etc., has emerged as a major approach for controlling inflammatory diseases.[56–59] Whether elimination of activated macrophages with folate-targeted therapies can suppress symptoms of these other inflammatory diseases will obviously require further investigation. However, to begin to assess the potential of such therapies, imaging studies have been recently conducted in animal models of the same pathologies. In all models tested to date, folate-linked imaging agents have been shown to concentrate in the inflamed tissues of diseased animals.

What About Folate Conjugate Toxicity?

The underpinning of all targeted therapies is the promise of reduced systemic toxicity because of selective drug targeting to diseased cells. While imaging studies revealed significant folate conjugate uptake in pathologic cells, enrichment of folate conjugates was also invariably seen in the kidneys. Using folate–Texas Red as a surrogate for folate–drug conjugates, conjugate binding and intracellular trafficking were monitored in real time in the kidneys of live rats using multiphoton intravital microscopy.[60] Folate–Texas Red was seen to bind to the apical surface of proximal tubule cells within seconds of tail vein injection. By 30 s postinjection, endocytosis of folate–Texas Red could be seen at the apical surface, followed by transcytosis of the conjugate in small vesicles across the kidney cell to the basal surface. Upon docking at the basal membrane, vesicle fusion led to the release of vesicle contents into the blood stream. Thus, most of the folate conjugate was not retained by the kidneys but rather returned to the circulatory system within minutes of its capture from the filtrate. Not surprisingly, no kidney toxicity has been observed for any folate conjugate tested in either preclinical animal studies or human clinical studies. Further, if kidney toxicity were to emerge in the future, recent data suggest that predosing with antifolates can significantly reduce kidney (but not tumor) uptake of the folate conjugate.[61]

Prospects for the Future

Because of its overexpression in cancer cells and activated macrophages, the folate receptor shows considerable promise as a therapeutic target for a large number of important human pathologies. Development of optimal therapies may, however, require "thinking outside of the box", because many limitations that have compromised traditional therapies (e.g., toxicity to normal tissues, intracellular delivery, export from the pathologic cell, etc.) may not hamper the efficacy of folate conjugates, whereas issues not commonly confronted with current therapies (e.g., oral bioavailability and folate receptor delivery capacity) may constitute challenges for folate-targeted conjugates. Nevertheless, with the exponential growth in folate-linked imaging and therapeutic agents, creative strategies to circumvent such potential obstacles should be rapidly forthcoming. Because of its small size, low cost, ease of conjugation to therapeutic and imaging agents, compatibility in both organic and aqueous solvents, lack of immunogenicity, and specificity for pathologic cells, we anticipate that folic acid will remain an attractive candidate for receptor-targeted therapeutics for the foreseeable future. Results from ongoing clinical trials will help reveal the full potential of this targeting ligand.

We thank our former graduate students, generous colleagues at Endocyte, Inc., and collaborators worldwide for their ongoing assistance.

BIOGRAPHICAL INFORMATION

Philip S. Low was born in Ames, Iowa, and raised in West Lafayette, Indiana. After graduating with a B.S. degree in chemistry from Brigham Young University (1971), Dr. Low obtained his Ph.D. degree from the University of California, San Diego (1975) and completed postdoctoral training at the University of Massachusetts (1976). He joined the faculty of Purdue University in 1976, where he is currently the Ralph C. Corley Distinguished Professor of Chemistry. Dr. Low has published over 250 articles on various topics, including the structure and function of the human erythrocyte membrane and the development of receptor-targeted imaging and therapeutic agents. The latter work has led to 30 patents and 2 targeted drugs that are currently undergoing clinical trials for the treatment of kidney, ovarian, breast, lung, brain, and endometrial cancers. Dr. Low is the founder of Endocyte, Inc., a company based on these technologies.

Walter A. Henne received his B.S. degree in biology and M.S. degree in analytical chemistry from Governors State University, University Park, Illinois, and his Ph.D. degree in chemistry from Purdue University, West Lafayette, Indiana. He is currently a postdoctoral researcher in the laboratory of Dr. Philip S. Low. His research interests include diagnostic and targeted drug-delivery systems.

Derek D. Doorneweerd received his B.S. degree in professional chemistry from Northern Illinois University, DeKalb, Illinois. He is currently a Ph.D. candidate in the department of chemistry, Purdue University, West Lafayette Indiana, under the direction of Dr. Philip S. Low. Presently, he studies low-molecular-weight-targeting ligands for use in therapeutics and diagnostic applications.

FOOTNOTES

*To whom correspondence should be addressed. E-mail: plow@purdue.edu. Telephone: (765) 494-5273. Fax: (765)-494-5272.

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