Chemotherapeutic Applications of Folate Prodrugs: A Review

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Abstract
Several techniques to assisting in the drug design and discovery stages have been developed during the last several decades. The bulk of these techniques aimed to find novel chemical entities that had the greatest significant interaction with the targeted receptors or enzymes while providing the least degree of risk of unwanted interactions. This approach, on the other hand, is time-consuming and expensive, as it requires the screening of thousands of molecules for biological activity, with only one making it to market. The prodrug strategy, in which the active drug molecule is disguised by a promoiety to change its undesirable characteristics, is one of the most appealing and promising methods. The folate receptor (FR)-targeted systems may also open the path for more advanced drug conjugates, especially because this receptor is now being targeted by a variety of technological innovations, including nanoparticles, small molecules, and protein-based technologies, resulting in a wealth of experience in the discipline.

Key Words: Folate Receptor, Chemotherapy, Nanotubes, Light-aided Drug Release, Nanotechnology, ⁹⁹mTc-Etarfolatide Imaging.


Introduction
A drug's biological and physicochemical characteristics have been used to describe it. Some of the medicines utilized have unfavorable characteristics, resulting in ineffective delivery and unpleasant side effects. To boost their utility and usage in clinical practice, these medicines' physicochemical, biological, and organoleptic characteristics should be enhanced (Karaman et al., 2013).

Numerous approaches have been presented to help in the drug design and discovery stages during the last several decades. The bulk of these approaches were committed to discovering novel chemical entities that have the most substantial interaction with the specific receptors or enzymes while offering the fewest risks of adverse effects. On the other side, this approach is time demanding, expensive, and requires the testing of hundreds of molecules for bioactivity, with just one reaching it to trade. Among the most intriguing and viable strategies is the prodrug approach, wherein the active drug component is masked by a promoiety to modify its unwanted properties (Jana et al., 2010). Albert coined the word prodrug, also known as proagent, to describe a pharmacologically inert moiety that is transformed to an active form within the body. This phrase has been effectively utilized to change a drug's physicochemical and pharmacokinetic characteristics (absorption, distribution, excretion, and metabolism) as well as its related toxicity (Stella et al., 2007). Prior to exerting therapeutic action, a prodrug must undergo regulated or predictable chemical and/or enzymatic biotransformation. The word prodrug refers to a covalent bond formed between an active drug and a promoiety (Figure 1).

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 10 June 2021 Accepted: 19 July 2021
Figure 1. Prodrug strategy represented as schematic steps

This technique uses a chemical approach rather than a formulation method to remove obstacles. In general, the immediate objective of using prodrugs is to create novel entities with higher effectiveness, selectivity, and low toxicity (Jana et al., 2010). In the biological system, an ideal prodrug should undergo fast biotransformation to its active form and a non-toxic moiety via chemical or enzymatic processes (Chipade et al., 2012). In addition, depending on the purpose for which the prodrug was developed, the active drug and the promoiety must be released prior to, during, or after absorption, or in a specified target tissue or organ. The prodrug method, which employs target cell- or tissue-specific endogenous enzymes and transporters, is now widely regarded as one of the most promising site-selective drug delivery methods. Acetanilide and phenacetin are two examples of chemicals that meet the traditional requirements of a prodrug and show their effects after being metabolized in the body. Acetanilide has been used as an antipyretic since 1886. It is converted to paracetamol by metabolism (aromatic hydroxylation). This is analogous to phenacetin, which undergoes O-dealkylation to generate paracetamol (Figure 2).

Figure 2. Metabolic transformation of phenacetin and acetanilide
Folates

Folates are natural pteroylpolyglutamates with at least two to seven glutamates linked to the γ-carboxyl of glutamate via amide connections. Pteroylpentaglutamates are the most common intracellular folates, while pteroylmethionylglutamates are the most common extracellular folates. There are natural pteroylpolyglutamates with up to 11 glutamic acid residues.

Folate Receptors

The folate receptors (FRs) are cell surface glycoproteins with molecular weights ranging from 35 to 40 kDa. It is split into three distinct isoforms: FR-α, FR-β, and FR-γ. The α and β variations are linked to the cell membrane by GPI anchors, whereas the FR-γ is only found in hematopoietic cells and lacks the GPI component, rendering it freely soluble. On normal granulocytes, FR-β, which shares 70% sequence homology with FR-α, is most commonly found in a non-folate-binding isoform, presumably due to an alternate posttranslational modification.

Upregulation of Folate Receptor in Malignancy

FR-β is increased in inflammatory and autoimmune disorders by activated myeloid cells (mainly monocytes and macrophages). Many malignancies, including those of the blood, lung, skin, kidney, liver, and soft tissue, have been shown to have the FR-β isof orm in tumor-associated macrophages. By suppressing CD8+ T cells and secreting proangiogenic molecules, these macrophages can enter solid tumors and encourage their spread and development. Retinoic receptors control FR-β expression, which can be boosted by all-trans retinoic acid, especially when used in conjunction with histone deacetylase inhibitors. Consequently, the FR-β isoform may afford an interesting applicant for the selective targeting of chemotherapeutic agents.

Despite the fact that FR-β is expressed on some cancers, the FR-α isoform has the greatest potential for targeted cancer therapy because it is the most widely expressed of all FR isoforms and is upregulated in various epithelial cancers, such as lung, kidney, and ovarian cancers, as well as breast (Patel et al., 2016).

Overexpression of FR-α is seen in cancers, such as renal cell carcinomas, mesotheliomas, cervix, endometrial, testicular choriocarcinoma, ovarian, colorectal, pediatric ependymomas, and lung. In
these carcinomas, FR-α overexpression is roughly 100–300 times greater than in healthy cells, with 1–10 million receptor copies per cell. The expression of FR-α on the apical surface of most normal cells has likewise been discovered to be low. Because of this differential in expression, FR-α is a promising therapeutic target for new anticancer drugs with low toxicity in normal tissues.

Small Molecule–drug Conjugates (SMDCs)

The ability to connect chemical agents to receptors that hunt for FR-α-expressing malignancies gives the structure extraordinary specificity while maintaining treatment effectiveness, and this method has resulted to the development of many folic acid–based small molecule–drug conjugates (FA-SDMCs).

Vintafolide

Vintafolide is the most successful FA-SDMC (formerly EC145). This medical agent is a hydrophilic molecule that affords the therapeutic agent named desacetyl vinblastine monohydrazine (DVABLH) to cancers that upregulated FR-α in a selective fashion. Vintafolide binds to FR-α with high affinity in preclinical tests, indicating that it has extremely selective and strong action against FR-α positive tumor xenografts compared to the untargeted DVABLH. Vintafolide is made up of four different modules: folate acid moiety that targets FR-α, self-immolative disulfide linker, hydrophilic peptide spacer, and microtubule-destabilizing medication, DVABLH (Figure 4).

Figure 4. Chemical backbone of the folic acid–dependent SMDC vintafolide. The grey is a self-immolative disulfide linker, green is a peptide spacer, blue is a folate targeting ligand, and red is a potent cytotoxic drug DVABLH.

Because folic acid is hydrophobic, the spacer helps to improve the drug conjugate’s overall water solubility, preventing non-specific diffusion across cell membranes and ensuring cell internalization via receptor-mediated endocytosis (RME). Polysaccharides, peptides, and polyethylene glycol (PEG) chains are examples of typical spacers used in FA-SDMCs (Srinivasarao et al.). The spacer likewise acts as a physical barrier between both the drug carrier and the intended receptor, preventing steric disruption and preserving the ligand’s binding site specificity (Srinivasarao et al.). Long, flexible spacers, on the other hand, might lead the drug unit to coil around rather than engage with the intended receptor, jeopardizing the receptor’s affinity (Srinivasarao et al., 2015). For better FA-SDMC tumor penetration and fast systemic clearance, small size (usually less than 2000 Da) is important. Vintafolide, with a molecular mass of 1917 Da and a diffusion interval of 6 minutes, satisfies these requirements (Bailly, 2014). This rapid absorption of the medication component by FR-positive cancer cells is a favorable characteristic since it reduces circulation time, limiting premature release of the drug. This FA-SDMC is also swiftly removed from the body via the kidneys and liver (half-life of elimination: 26 minutes).

Folate–taxoid Conjugates

Seitz et al. developed a highly potent succeeding folate–taxoid that would cure both drug-sensitive and –resistant malignant cells .(2015, Seitz et al.) Taxoid SB-T-1214, an analogue of the Taxol, and folic acid moiety are integrated in this folate–taxoid combination termed 2. This SMDC has a hydrophilic PEGylated dipeptide spacer, similar to that of vintafolide, and self-immolative disulfide linker, as shown in Figure 5 (Seitz et al., 2015).
Figure 5. Chemical backbone of the folate-taxoid conjugate 2 as reported via Seitz et al.

In culture, the efficacy of the free SB-T-1214 and taxoid compound 2 was compared in FR-α-positive and negative cells. As expected, the SB-T-1214 was very potent against both the cell lines tested. In contrast, taxoid compound 2 demonstrated substantial lethality against FR-α-positive cells, with IC₅₀ values that were more over three times lower than those found in FR-α-negative ones. The folate-taxoid 2 was taken up by RME, an internalisation pathway unaltered by the folic acid normally located on the cell culture media, suggesting that folic acid essential for cell development is predominantly carried into cells via folate transporters instead of RME. Furthermore, as compared to the free drug, taxoid conjugate 2 had a 1000-fold lower toxicity towards healthy cells. The cytotoxic action of folate-taxoid 2 is derived from an intracellular GluSH-triggered reduction of the disulfide linker, which releases the free cytotoxic drug, similar to the vintafolide and SB-T-1214 (Seitz et al., 2015).

As with conjugate 2, the drug should be released in its purest form for maximum biological activity, lending credence to the theory that the inefficiency of vintafoline analogs may be contributed to the free of the altered agent (Khalil and Mustafa, 2020; Mohammed and Mustafa, 2020; Mustafa, Bashir, et al., 2020; Mustafa, Mohammed, et al., 2020; Oglah and Mustafa, 2020a, 2020b). Furthermore, the effective release of chemical agents is dependent on the presence of GluSH in the intracellular environment (A.M. Nejres et al., 2020; Aws Maseer Nejres et al., 2020; Moath Kahtan Bashir et al., 2020; Mustafa, Khalil, et al., 2020; Mustafa, Oglah, et al., 2020; Oglah and Mustafa, 2020b; Oglah, Mustafa, et al., 2020). When specifying the cancerous cells for targeting via SMDCs, it is critical to take this difference into account (Mustafa, 2019; Aldewachi et al., 2020; Moath Khtan Bashir et al., 2020; Mustafa and Abdulaziz, 2020; Oglah, Bashir, et al., 2020). FA-SDMCs have been developed in which the degradation of unbound medication is not regulated by cellular GluSH in part because of this possible complication/limitation with specific cancer cells and serum stability concerns (Mahmood et al., 2014; Mustafa, 2018; Mustafa et al., 2018, 2021).

The aforementioned examples are brief but typical samples of FA-SDMCs from a large field of cytotoxic drug release conjugates that use a disulfide linker. Also, many additional medicines, such as mitomycins, tubulysins, and camptothecins, have been conjugated to folate through a disulfide linker.

**Dendritic β-galactosidase Sensitive Folate Monomethyl Auristatin E Conjugates**

The disulfide link in FA-SDMCs is vulnerable to breakage in circulation by a number of free thiol-containing substances, which might result in unwanted premature drug release. As a result, FA-SDMCs without disulfide linkers have been created, a structural feature that would ideally limit off-target drug release in the circulation. Alsarraf et al. created the galactosidase-sensitive conjugate numbered 3, which delivers the chemotherapeutic compound named monomethylauristatin E (MMAE) to tumor cells (Alsarraf et al., 2015). This SMDC has a phenolic or aniline self-immolative linker, galactoside trigger, folic acid targeted ligand, and two MMAE molecules, allowing these two drug molecules to be released via a single internalization and activation route. Incubation of folate-conjugate 3 at 37°C and at pH 7.2 with galactosidase was used to investigate the this release process. The fragmentation mechanism starts with the
hydrolysis of SMDC 3’s glycosidic bond by enzymes, yielding a phenolic compound 4 that undergoes the 1,6-elimination reaction followed by decarboxylation yielding quinone 5 as well as the aniline related product numbered 6. Two MMAE molecules are released as a result of subsequent 1,6- and 1,4-elimination operations (Figure 6). Another FA-SDMC that does not contain a disulfide bridge and is broken via an enzymatic is a folate–camptothecin conjugate that’s also degraded by the cathepsin-B protease.

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Apart from FA-SDMCs, that are digested by enzymes found in the tumor microenvironment, folate–enzyme conjugates have been developed to transport an enzyme to the folate binding site of the cancer cells anterior to the administration of a model drug that is converted to the activated state by this catalyst. This therapy uses penicillin-V amidase as well as a doxorubicin precursor as an instance.
Boronic Acids-Schiff Base Linker

In addition to the commonly utilized disulfide and carbon-based connectors, the covalent binding of boronic acids with Schiff bases to create boronate complexes is being used as a framework to specifically transport lethal medications to cancerous cells. Santos et al. created a compound numbered 10 containing the cytotoxic medication bortezomib, PEG chains, and two folate targeted backbones (Figure 7). The bivalent folate targeted unit was selected to simulate the bivalent Fab regions detected on the immunoglobulin Gs (IgGs), which provide a high affinity and selectivity of the antibodies for detected antigen epitopes (Santos et al., 2017).

Figure 7. Chemical backbone of boric acid complex 10 as depicted by Santos et al. The red is a cytotoxic agent bortezomib, blue is a folic acid moiety, black is a PEG chains

Compound 10 experiences low IC_{50} of 62 nM over MDA-MB-231 malignant cells than pure bortezomib, even though it had greater efficacy for these 1nd cells. Because GluSH is present in submicromolar levels in the cell, Santos et al. investigated the GluSH-mediated breakage pathway (Figure 9) by creating compound 11, a less sterically hindered equivalent of compound 10. The drug release process, as established by HPLC, is likely to include the attachment of GluSH to the iminium carbon, breakdown the five-membered ring, and finally the hydrolysis enhancing the drug numbered 15 release (Santos et al., 2017).
Light-activated Releasing of the Drug

Photodynamic therapy (PDT), for example, is a method of inducing cytotoxicity by light that has received a lot of interest for cancer treatment. This technique involves light-assisted photosensitizer stimulation in the atmospheric oxygen, following by the oxidative reactions that neutralize photosensitizer-affected cells. Furthermore, the benefits of the light-stimulated methods include the non-invasive motivation and increased specificity because of the simplicity with which this medium may be manipulated spatially and temporally (Dcona et al., 2017).

Boron dipyrromethene (BODIPY) derivatives, for example, are a potential family of photosensitizers with appealing optical and photo-physical characteristics as well as good aqueous medium stability. Ke et al. created two di-iododistyryl folate-conjugated BODIPY-dependent photosensitizers (16a and 16b), each with a different glycol linker length (Figure 9) (Dcona et al., 2017).

Figure 8. Suggested release mechanism of bortezomib (15) from complex 11
The in vitro photosensitizing potentials of 16a and 16b were investigated by incubating them with KB humans oropharyngeal carcinomas, which express high levels of FR-α, and MCF-7 breast cancer cells, which express low levels of FR-α. There was no evidence of damage in the form of light, however activity was seen once IR light phenotype was applied. Conjugate 16a, which lacking the triethylene glycol connector, had a 3-fold higher lethality (IC\textsubscript{50} of 60 nM) versus 16b (IC\textsubscript{50} of 180 nM) (Dcona et al., 2017).

The fact that 16b aggregates more in RPMI culture media than 16a can be explained by the fact that the former's triethylene glycol linker induces dipole–dipole interactions in adjacent oligoethylene glycol chains. As a result, conjugate 16a with the shorter linker appears to be a promising alternative for usage as a photosensitizer in PDT (Dcona et al., 2017).

FA-SDMCs, as previously stated, are a diverse family of conjugates for targeted drug delivery. While many of these systems have been designed for FR-α overexpression applications, because folic acid binds to both of these receptors, they can easily be used to FR-β overexpression cases (an emerging field). Antifolate antibodies that address FR-α or -β with precision and discrimination (since they don't comprise an arbitrary folic acid binding group) offer an alternative treatment option for FR-positive malignancies (Dcona et al., 2017).

**Monoclonal Antibodies Targeting FR**

In complement to very well therapeutic antibodies like even the aforesaid farletuzumab, drug–antibody conjugates (DACs) are still being utilized as carriers for the specific targeting of the potent pharmaceuticals to tumors. This algorithm includes antibodies' high binding specificity with the toxicological properties of a chemical compound, whose cell-killing capacity varies from antibody-dependent apoptosis, while also minimizing off-site harm (Chudasama et al., 2016). As a result, medicines that would normally be too toxic to use in traditional chemotherapy regimens can now be used. Additionally, the lethal agent's connection boosts the antibody's effectiveness and allows it to counteract the unconjugated antibodies' rare therapeutic impact.

In contrast to SMDCs, which have a short circulation half-life, antibodies have a significantly longer half-life in the bloodstream, which increases the proportion of the given dosage accessing the tumor. IMGN853 (Figure 10) is an example of a FR-α-targeting ADC, and it consists of three...
components, include the DM4, an antimitotic agent that blocks tubulin polymerization, anti-FR-α antibody that specifies FR-α expressing cancerous cells as well as microtubule assembly, and disulfide-dependant linker that links the chemotherapeutic agent with selected antibody (Vergote and Leamon, 2015).

IMGN853 attaches to FR-α, is imported by RME, and the DM4 drug is released as a result of enzymatic breakdown of the antibody and linker. This causes cell-cycle arrest and apoptosis by affecting microtubule activity. IMGN853 has shown anti-tumor efficacy and is now being tested in phase II studies for patients with FR-positive platinum-resistant ovarian tumor as a part of several combination regimens and as a single agent. This ADC is a prototype of its kind, so there's lots of potential for improving its chemistry if the clinical trials fail.

Nanotechnology-aided FR Targeting
As previously stated, conventional chemotherapy is restricted by its weak as well as poor selectivity, and the undesirable toxicity induced by platinum (Pt)-dependent regimen's non-specific cellular absorption can be particularly problematic. Despite this, platinum-based treatment is still utilized as a primary chemotherapy in virtually all stages of ovarian cancer due to its high responsiveness (Moore et al., 2014). However, the justification for continued support of this treatment option is dwindling. For example, frequent Pt-dependent therapy cycles can lead to acquired drug resistance, which can be caused by reduced cellular Pt uptake, which restricts the production of harmful Pt-DNA complexes. Furthermore, intracellular GluSH promotes Pt detoxification and leads to Pt inactivation via the production of cisplatin–thiol conjugates, avoiding cell death caused by the development of fatal Pt-DNA complexes.

As a result, there is a pressing need to change the Pt treatment choices that are now accessible. Patel et al. have described the production of NMI-350 Pt-theranostic nano-emulsions (NEs) (Figure 11) to this purpose. Natural polyunsaturated fatty acid rich omega-3 and -6 fatty acid oils, as well as gadolinium tagged multi-compartmental NEs, make up the NMI-350 family. The cytotoxic and hydrophobic di-fatty acid platins and C6-ceramide can be encapsulated in their oily core, and the NE surface can be used to attach imaging agents and folate ligands for targeting (Patel et al., 2016).

Because of the aforementioned design, these NEs allow for the controlled delivery of adjuvant chemotherapy and then also extend the circulation half-life of Pt to optimize uptake of nano-drug adducts in malignant cells over a prolonged period of time. In addition, the production of the di-fatty acid platinum design has been significantly improved; Patel et al. have developed a production that requires only 24 hours, rather than the well-known 21 days. Using this more efficient approach, di-fatty acids of various chain lengths were synthesized, and folate was bonded to the NE surface through a PEG3400-DSPE spacer (Figure 12). The particle size of the completely functionalized NEs was in the range of 120–150 nm.
The NEs’ FR-binding efficiency was subsequently assessed by flow cytometry on two FR-α-rich cell lines, KBCR-1000 (Pt-resistant) and KB-WT (Pt-sensitive). FA-targeted rhodamine labeled NEs and Non-targeted rhodamine labeled NEs were used to treat both lines, with the former being functionalized with 100, 300, 1200, and 3600 FA molecules. Higher amounts of FA conjugation enhanced cellular absorption in both lines, as predicted (Patel et al., 2016).

Due to being the most stable and cost effective relative to the other FA-targeted rhodamine tagged NEs, the FA-targeted rhodamine marked NEs tagged with 300 FA monomers were chosen for a lethal test. In a lethal experiment utilizing a certain Pt-sensitive and Pt-resistant cell cultures, this FA-targeted rhodamine tagged NE was matched to cisplatin, but this NE generated an approx. 30-fold enhancement in effectiveness when compared to unconjugated cisplatin. The simultaneous impact of the Pt and the exogenously introduced C6-ceramide can be attributed to this increased cytotoxicity, which has the ability to overcome Pt-resistance (Patel et al., 2016).

**Nanotubes-aided FR Targeting**

Wang et al. created the first Ni-folate with an inner diameter of 5–8 nm, which include hydrazine as a linker, cisplatin as a cytotoxic agent, Ni as a connector, and FA as a targeted ligand. The suitably large cavity of these nanotubes allows for a substantial drug loading, overcoming the low deliverable payload dosage associated with previous folate conjugates. Furthermore, unlike the smaller folate–drug conjugates, these nanotubes do not accumulate in the kidneys (Wang et al., 2015). The initial stage of nanotube synthesis includes the formation of a tape-like structure because the pteroic acid subunit of FA may form hydrogen bonds with the pteroic acid moiety of other FA molecules. The glutamic acid in FA may then coordinate to Ni
+2 without breaking hydrogen bonds, while hydrazine functions as a bridging mediator between two Ni ions, producing a nano-sheet. The high temperature of this reaction aggravates the nanosheets' relative intermolecular mobility, causing curling to occur for reducing the required energy. Extreme heat can promote the formation of nanotubes by breaking some initial linkages and establishing new ones, using hydrazine functioning as a structural comment stream to link the nano-sheets into nanotubes, as illustrated in Figure 13 (Wang et al., 2015).
Figure 13. Transformation of nanosheets into nanotubes

**99mTc-etarfolatide Imaging**

The evaluation of FR-α regulation may serve as a diagnostic protocol, in which, the FR-α status can be addressed during therapy, and many routes for FR-α detection have been studied. Despite these techniques' excellent specificity and sensitivity, clinical use frequently necessitates invasive cellular samples that are commonly gathered from a one lesion (Maurer et al., 2014). Furthermore, because of the diverse nature of FR-α expression on tumors and the changing features of tumors over time, constructing an accurate depiction of a patient's FR-α status is challenging, resulting in an incomplete picture. This restriction can be solved by using whole-body imaging with folate radio-conjugates to provide real-time and non-invasive FR-α assessment for various lesions at several time periods.

Etarfolatide (EC20) is a folate-targeted radio-imaging agent made up of 99m technetium (Tc) linked to a folic acid unit through a non-cleavable peptide linker (Figure 14). The 99mTc release isn't required for radio-folate imaging, therefore the EC20 linker is non-degradable.

Figure 14. Chemical backbone of the 99m technetium-etarfolatide.
99mTc is a commonly used radio-graphy tracer with a half-life of 6 hours and gamma emission as its primary mode of radioactive decay. Furthermore, 99mTc-etagolafolate has a significant binding affinity for FR-α, and tumors that overexpress this receptor isoform generally internalize a large proportion of the 99mTc-etagolafolate given (17 percent). Fast buildup at cancerous cells and renal elimination are further advantages of this probe combination. As a result, the non-specific tumor absorption of 99mTc-etagolafolate is reduced, allowing for faster picture creation.

Tc's optimum emission computed-tomography of single-photon (SPECT) imaging properties, namely a photon energy of 140 keV and t1/2 equals to 6 hours, are used in 99mTc-etagolafolate. As a result, this probe agent has been tested in a number of clinical studies, including those utilizing vintafolide in combination with 99mTc-etagolafolate as an imaging agent of high quality. Although no safety problems have been discovered with this course of treatment, unwanted side symptoms such as nausea, lower abdomen discomfort, and vomiting are linked to 99mTc-etagolafolate (Maurer et al., 2014).

While numerous phase II trials have shown that this imaging agent may utilize to identify individuals who are most likely to respond to vintafolide therapy, more research is needed. Physiological variables can affect imaging findings and interpretation, most notably the fact that 99mTc-etagolafolate is uptaken into spleen, bladder, kidneys, as well as with a small amount into bone marrow. As a result, tiny amount of FA can be administrated prior to 99mTc-etagolafolate intake to partially satisfy the FR-α, which may interfere with the receptor regulation in lesions near the affected tissues (Maurer et al., 2014).

Another drawback of this probe combination is that active macrophages (which express FR-β) also absorb 99mTc-etagolafolate, which can lead to FR-α-positive tumor tissue being mistaken for inflammation or infection (Maurer et al., 2014).

The requirement for independent SPECT and computational radiography (CT) imaging hampered initial 99mTc-etagolafolate scanning research, but contemporary SPECT/CT fused imaging has greatly enhanced spatial localisation and can determine whether lesions are FR-α-positive or -negative. Patients who are willing to respond to treatments that target the FR have been identified using 99mTc-etagolafolate (Maurer et al., 2014).

**Conclusion**

Over the years, the prodrug method has been refined to address a number of unwanted drug properties. Several forms of Vitamin B9 and their derivatives are referred to as "folate." Folates are required for physiological functions such as nucleic acid synthesis and red blood cell formation. Synthetic folic acid metabolism is highly individualized, thus natural folates are recommended. Besides, natural folates have less side effects and are generated by the body. Naturally occurring folates can be found in foods and in metabolically active forms in the human body. The FR-targeted innovations could open the door for more advanced drug conjugates, particularly as this receptor has been addressed by a variety of associated technologies, including tiny molecules, nanomaterials, and peptide-dependent techniques, resulting in a large amount of data.

**References**


Khalil RR, Mustafa YF. Phytochemical, antioxidant and antitumor studies of coumarins extracted from Granny Smith apple seeds by different methods. Systematic Reviews in Pharmacy 2020; 11(2): 57–63.


Mohammed ET, Mustafa YF. Coumarins from Red Delicious apple seeds: Extraction, phytochemical analysis, and evaluation as antimicrobial agents. Systematic Reviews in Pharmacy 2020; 11(2): 64–70.