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Review

Stability of folic acid under several parameters

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ABSTRACT

Folic acid is a small molecule, also known as vitamin B_9. It is an essential compound involved in important biochemical processes. It is widely used as a vector for targeted treatment and diagnosis especially in cancer therapeutics. Nevertheless, not many authors address the problem of folic acid degradation. Several researchers reported their observations concerning its denaturation, but they generally only took into account one parameter (pH, temperature, light or O_2 *etc.*). In this review, we will focus on five main parameters (assessed individually or in conjunction with one or several others) that have to be taken into account to avoid the degradation of folic acid: light, temperature, concentration, oxygen and pH, which are the most cited in the literature. Scrupulous bibliographic research enabled us to determine two additional degradation factors that are the influence of singlet oxygen and electron beam on folic acid stability, which are not considered as among the prime factors. Although these two factors are not commonly present as compared to the others, singlet oxygen and electron beams intervene in new therapeutic technologies and must be taken in consideration for further applications such photodynamic or X-rays therapies.

Contents

Abbreviations: AHMP, 6-(hydroxymethyl)pterin; DNA, deoxyribonucleic acid; DSC, differential scanning calorimetry; EOC, epithelial ovarian cancer; FA, folic acid; FPT, 6-formylpterin; FR, folate receptor; GC/MS, gas chromatography/mass spectrometry; IR, infra-red; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PABA, *p*-aminobenzoic acid; PCA, pterin-6-carboxylic acid; PDT, photodynamic therapy; PGA, *p*-aminobenzoyl-L-glutamic acid; pNBGA, *N*-(4-nitrobenzyl)-L-glutamic acid; PS, photosensitizer; ROS, reactive oxygen species; RP-HPLC, reverse phase high performance liquid chromatography; UV–Vis, ultraviolet-visible; XA, xanthopterin.

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1. Introduction

Folic acid is a small molecule, also known as vitamin B_9. It is an essential compound involved in many important biochemical processes in humans, mainly in its ionic form. It is involved in cell multiplication, regulation of gene activity, red and white cell production, renewal of skin and the intestine lining, as well as in the synthesis of chemicals that modulate brain function. Folic acid is available both naturally and synthetically. Folate is the anionic form of folic acid. At physiological pH the acid-base form is folate. Very often, the term "folic acid" refers to the fully oxidized synthetic compound used in dietary supplements, whereas "folate" refers to the various tetrahydrofolate derivatives naturally present in foods. Nevertheless, there is no difference in "*natural*" or "*synthetic*" folic acid. It is the same molecule (Marchetti et al., 2014).

The discovery of folic acid was first reported in the 1930s, when Wills reported the presence of a substance from yeast called the *Wills factor*, which could prevent megaloblastic anemia, a potentially fatal condition (Wills, 1931). In 1941, the term "folic acid" was coined from the Latin word *folium*, which means leaf, since folic acid is present in abundance in green leaves including the grass (Mitchell et al., 1941). Subsequent research done over the next few decades had helped to form the body of data on the components and metabolism of folic acid, as well as the inter-relationship between folic acid, vitamin B_{12} and methionine metabolism, and the role of folic acid in the synthesis of pyrimidine and purine (Herrmann and Obeid, 2011).

Folic acid's molecular structure can be divided into three parts: a glutamic acid (Glu) moiety, a *p*-aminobenzoic acid (PABA) moiety and a pterin moiety. The pterin moiety is linked to PABA by a methylene bridge and in turn PABA is connected by a peptidic bond to Glu to form folic acid (Fig. 1) (Vora et al., 2002b).

Folic acid has a low solubility in water and at 25 °C, it is practically insoluble (0.01 mg/mL). Its solubility is improved in alkaline or acidic medium, although it has been described that it is more stable in alkaline medium (Araújo et al., 2011).

Folic acid is crucial to ensure normal functions of the human body in various metabolic and biochemical processes. It plays an important role in the synthesis of purines and pyrimidines, in the metabolism of homocysteine and in the replication and methylation of DNA. It is also needed for normal development of tissues, particularly those associated with rapid cell division such as in embryology (Borradale et al., 2014; Vorobei and Vorobei, 2011). In the case of folic acid deficiency, a number of important health complications arise, including male infertility, neural tube defects in the developing foetus and other pregnancy complications, which are damaging to human evolution (Off et al., 2005). However, statistics and clinical studies (Steindal et al., 2007) concerning the seasonal influence of solar UV on the development of certain cancers suggest that the relative risk can be lower in summer and autumn compared to diagnostics established in winter and spring. This means that UV light can play a role of an antifolate derivative, as metotrexate does (Kamen, 1997).

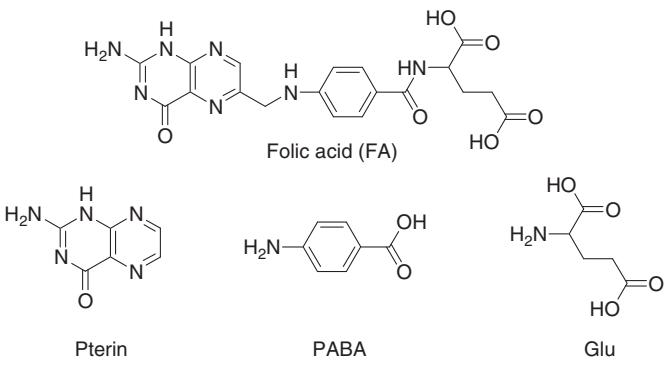

Fig. 1. Molecular structure of folic acid and the different moieties; pterin, para-aminobenzoic acid (PABA) and glutamic acid (Glu) that form a folic acid molecule.

Folic acid is also widely used as a vector for targeted treatment and diagnosis (Müller, 2013; Schieferstein and Ross, 2013) and numerous clinical applications have been carried out regarding folic acid targeted drugs, especially in the field of anticancer research. In actively dividing cancer cells, folic acid receptors (FR_α, FR_β and FR_δ) are highly expressed to meet the folate demand (Assaraf et al., 2014; Lu and Low, 2002, 2012) compared to normal cells. This phenomenon has thus become a good opportunity to exploit by attaching molecules to folic acid, which will then act as the targeting agent to deliver therapeutics to cancer cells (Azaïs et al., 2014; Chen et al., 2013; Gravier et al., 2008; Schneider et al., 2005).

FR is a promising anti-tumour target as it is over-expressed in 40% of solid tumours and negligible in the majority of healthy tissues (Parker et al., 2005). FR is highly over-expressed at the surface of a spectrum of solid tumour cells, including ovarian, kidney, lung, brain, endometrial, colorectal, pancreatic, gastric, prostate, testicular, bladder, head and neck, breast, and non-small cell lung cancer (Assaraf et al., 2014; Ledermann et al., 2015). In tumour tissues, FRs are known to be accessible to intravenously administered folate-targeted drugs (Elnakat and Ratnam, 2004). Therefore, these drugs could represent a target that minimizes toxicity and maximizes anti-tumour efficacy (Marchetti et al., 2014).

Targeted therapies directed towards folate receptors have been studied in several types of cancer, including lung cancer (Christoph et al., 2014; Ledermann et al., 2015; O'Shannessy et al., 2012). However, at present epithelial ovarian cancer (EOC) is the most studied indicator to develop this kind of treatment and is the one that is involved in clinical research trials. Among FR-positive tumours, 72–100% of EOC overexpress this receptor (Kalli et al., 2008; Markert et al., 2008; Parker et al., 2005), in particular serous carcinomas. The important role that the FR plays in EOC development and progression has led to increased FR-targeted therapeutic approaches (Azaïs et al.; Ledermann et al., 2015; van Dam et al., 2011; Vergote and Leamon, 2015).

In the field of photodynamic therapy (PDT), more and more researchers have been focussing their work on the development of photosensitizers-folic acid complex or nanoparticles, in which folic acid is acting as a targeting agent (Stallivieri et al., 2015). PDT could be part of innovative management of EOC peritoneal metastasis and in a preclinical study. We recently assessed the high specificity of a folate-targeted photosensitizer. This could enable intraperitoneal PDT for peritoneal carcinomatosis of ovarian origin, to decrease peritoneal cancer recurrence rate cytoreductive surgery and even for prophylactic intent on an apparently normal peritoneum in early-stage ovarian cancer (Azaïs et al., 2016a, 2016b). Nevertheless, it is very rare that authors are concerned with folic acid stability. This review clearly shows that folic acid is not stable in many conditions and moreover, singlet oxygen