Trakia Journal of Sciences, Vol. 3, No. 3, pp 55-59, 2005

Available online at:
http://www.uni-sz.bg

ISSN 1312-1723

Hypothesis

THEORY OF MUTAGENESIS, CANCEROGENESIS, AND PREVENTION OF CANCER

Z. Raikov, E. Raikova, V. Ivanov*

Department of Medical Chemistry and Biochemistry, Thracian University, Faculty of Medicine; 11 Armeiska Street, 6000 Stara Zagora, Bulgaria

ABSTRACT

The important role of folic acid in normal cell metabolism and development of some pathological processes has been demonstrated. In 1987 we published a hypothesis for the role of folic acid in cancer prevention. Many research data on the involvement of folates in carcinogenesis have since accumulated to give support to our hypothesis. We link folate depletion with the strong nucleophilic properties of hydrogenated folates, especially tetrahydrofolate, that determines it as one of the first targets of electrophilic attack of agents with carcinogenic potential, including reactive oxygen species.

Key words: tetrahydrofolate, nucleophiles, electrophiles, folate deficiency, carcinogenesis

INTRODUCTION

It is clear now that initiators of carcinogenesis fall into three broad groups: radiant energy, chemical compounds and viruses. In general these act by causing mutation or by introducing novel genes into cells (e.g. by viruses)

Apart from direct damaging effects on DNA the ionising radiation causes free radicals to form in tissues. The resulting reactive oxygen species (ROS) – superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$) and other radicals can interact with DNA and other cell macromolecules leading to their damage [1]. The oxidative DNA damage may lead either to activation of proto-oncogenes or to inactivation of tumour suppressor genes and in this way – to malignant cell transformation [2].

DNA is the main target molecule for the action of the ionising radiation, ROS and chemical carcinogens that appear electrophilic agents. Apart from the free nucleotides, other low molecular weight nucleophiles (LMN) in the cell are pteridines – folic acid (FA), dihydrofolic acid (DHFA) and tetrahydrofolic acid (THFA). The last plays an important role in the synthesis of purine and pyrimidine nucleotides and, in this way, in DNA synthesis and repair.

In the present article we promote our previous concept on the important role of LMN as the first targets of electrophilic attack in carcinogenesis, mutagenesis, and, also, in prevention and treatment of cancer [3].

MATERIALS AND METHODS

The hydrazine mustard spin-label 3-[N,N-bis(2-chloroethyl)carbohydrazide]-2,2,5,5-tetramethylpyrolidine-1-oxyl (HMSL) was synthesised in our laboratory by a method described elsewhere [4].

RESULTS AND DISCUSSION

Before the representation of a discussion on our and other authors results, confirming our view on the reasons for folic acid deficiency, we follow briefly the biological functions of folic acid and the established molecular effect of folate deficiency.

[1]***Correspondence to:*** *Veselin A. Ivanov, Department of Medical Chemistry and Biochemistry, Thracian University, Faculty of Medicine; 11 Armeiska Street, 6000 Stara Zagora, Bulgaria; E-mail: veskoasenov@abv.bg*

Biological functions of the folic acid

It is well known that the tetrahydrofolic acid (THFA), which is produced in human cells by reduction of the vitamin folic acid (FA), transfers one-carbon groups (methyl, methylene, methenyl and formyl) from one compound to another.

The nucleotide pool, filled in with folic acid participation, provides not only for the DNA replication, but for DNA repair. S-adenosylmethionine (SAM) is the principal methyl donor in methylation of nucleotides, especially cytosine in DNA. Approximately 4% of cytosine residues are modified post synthetically to 5-methyl cytosine (5 mC) [5]. Genes that are methylated at specific locations in the DNA molecule are either not transcribed or are transcribed at reduced rate. In this way site-specific DNA methylation controls gene expression.

Folic acid deficiency and cancer

There are accumulated data implicating folic acid deficiency in the development of cancer, notably of the cervix, lung, breast, brain, colorectum, etc.

There appear to be two principal mechanisms through which low folate status may increase the risk of malignancy. FA deficiency, by reducing intracellular SAM, can alter cytosine methylation in DNA, leading to inappropriate activation of proto-oncogenes and induction of malignant transformation. Folate deficiency may cause an imbalance in DNA precursors, uracil misincorporation into DNA and chromosome breakage [6].

A brief description of the evidence from cellular, animal and human studies that folic acid can modulate DNA by such mechanisms is present below.

a)Folic acid deficiency and DNA methylation

Folate depletion has been shown to induce hypomethylation on the coding region of the p53 tumour suppressor gene [7].

The findings of a relationship between folate status and methylation patterns in individuals already diagnosed with cancer and in healthy normal individuals provide credible evidence for a mechanism through which folate may modify DNA methylation and alter cancer risk (see **Figure 1**).

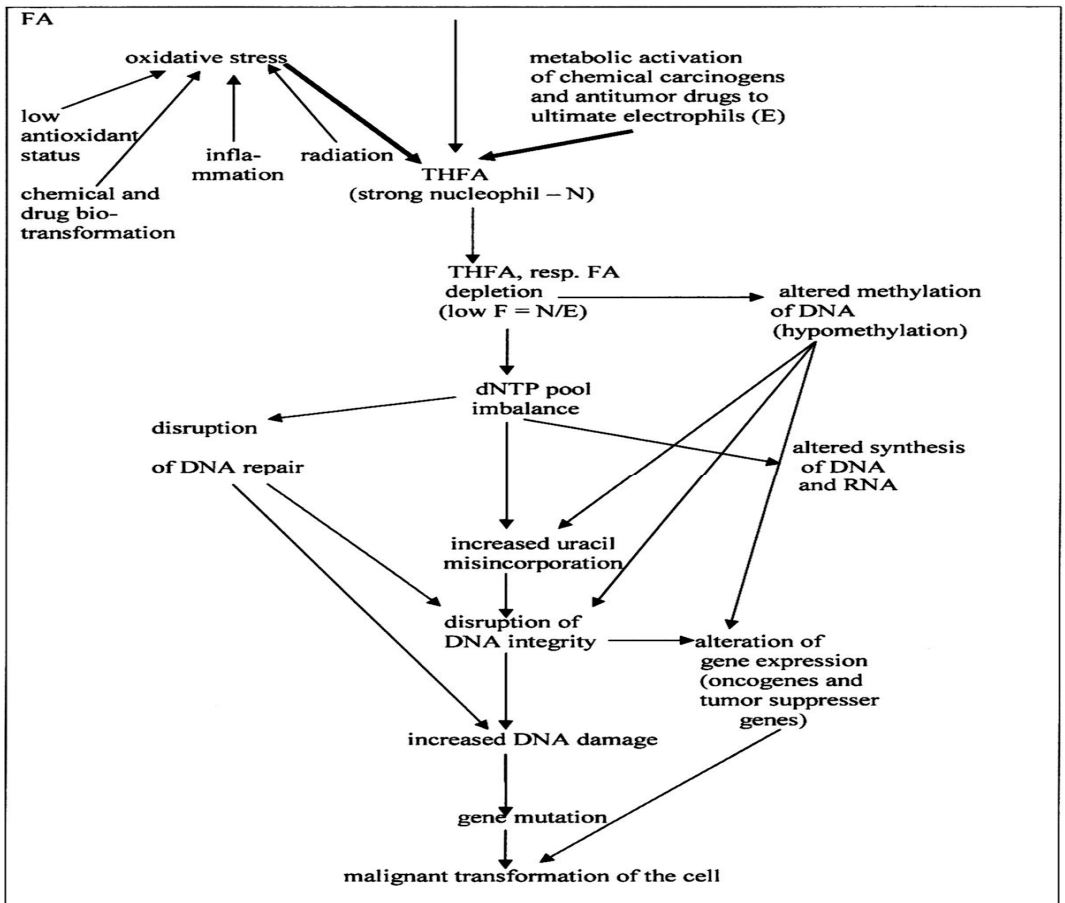

Fig. 1 Reasons for THFA, respectively FA deficiency and its molecular effects leading to cancer risk.

b)Folic acid deficiency, DNA integrity and repair

It has been shown that folate deficiency induces breaks in chromosomes and that such breaks are associated with an increased risk of cancer in humans. A mechanism by which folate deficiency might create such breaks is the misincorporation of uracil into DNA. Folate deficiency, by blocking the methylation of dUMP to TMP, can disrupt the balance of DNA precursors leading to accumulation of excess dUMP in the nucleotide pool. This may result in dUMP being misincorporated into DNA in place of thymidine as DNA polymerases cannot distinguish between dUMP and dTTP. Uracil DNA glycosylase removes any misincorporated uracil from DNA molecule and in the process a transient single-strand break develops in the DNA [8]. Simultaneous removal and repair of two adjacent uracil residues on opposite strands can result in a double-strand DNA breaks. If thymidine is continually limited under condition of folate deficiency, uracil misincorporation and repair may occur repeatedly in what is termed a "catastrophic'' repair cycle [9]. Strand breaks, as intermediates in excision repair, may destabilise the DNA molecule, leading to chromosome aberrations and malignant transformation. In those instances where cancers are enhanced by particular viruses, the phenomena of hypomethylation and strand breaks may have additional significance.

Ultimate electrophiles, obtained during biotransfomation of chemical carcinogens and ROS, which increased production (oxidative stress) might be due to radiation, inflammation, chemical carcinogens and drug biotransformation; low antioxidant status, etc. are the main reasons for THFA depletion. As one of the strongest nucleophiles in the cell, THFA combines readily with electrophilic agents and gets inactive.

Folate deficiency disrupts DNA repair. As was mentioned above, folate deficiency induced dNTP pool imbalance and uracil misincorporation into DNA. Although much of this uracil can be removed by DNA repair enzymes, the lack of available dTTP blocks the step of DNA repair catalysed by DNA polymerase; thus, base-excision repair, one of DNA repair mechanisms, is disrupted [10]. The results are DNA strand breaks and blockage of normal DNA replication. Site-specific DNA hypomethylation, induced by folate deficiency, might affect the methyl-directed mismatch repair- the other major cellular DNA repair system [11] (see **Figure 1**).

Alteration in DNA methylation (hypomethylation), disruption of DNA integrity, caused by increased uracil misincorporation, and DNA strand breaks, and disruption of DNA repair are related phemonena that can each be induced by folate depletion and are believed to enhance carcinogenesis by altering the expression of critical genes. Moreover, folate supplementation can reduce DNA instability in folate-deficient subjects. In this connection, it is important to reveal the causes for folate depletion.

Reasons for folic acid deficiency

Quantum biochemical studies of Pulman and Pulman on the purine and pyrimidine bases, FA, DHFA and THFA showed that the most powerful nucleophiles among them are guanine and hydrogenated folates – DHFA and THFA [12] (**Table 1**).

Table 1: *Quantum biochemical characteristics of pyrimidines, purines and pteridines according to Pulman and Pulman*

Pyrimidines, Purines, Pteridines	***Energy of the highest occupied molecular orbit***	***Energy of the lowest free molecular orbut***
Cytidine	0.60	- 0.80
Thymidine	0.51	- 0.96
Adenine	0.49	- 0.87
Guanine	0.31	- 1.05
Folic acid	0.49	- 0.65
7,8-Dihydrofolic acid	0.29	-0.75
Tetrahydrofolic acid	0.05	-1.07

Recently we studied the effect of LMNs – FA and THFA, and four deoxyribonucleoside-monophosphates (dGMP, dAMP, dCMP and TMP) on the alkylation of DNA with hydrazine mustard spin-label (HMSL), a bifunctional alkylating cytostatic (**see Materials and Methods**). According to their competitive effect on DNA alkylation the LMNs can be ordered in the following way: THFA>dGMP>FA>dAMP>dCMP>TMP (**Table2**).

Table 2. *Competitive effect of LMNs on the alkylatio of DNA with HMSL*

LNMs	***Area under the spectrum of the hydrolysed spin-labelled DNA (arbitrary units)***
THFA	2.8
dGMP	4.6
FA	11.8
dAMF	12.3
dCMP	23.3
TMP	57.3

CONCLUSION

An expanding body of epidemiological animal and human studies suggests that folate status modulates the risk of developing cancers in several tissues - folate depletion appears to enhance carcinogenesis while folate supplementation conveys a protective effect. There are two principal mechanisms whereby folate is thought to modulate DNA stability and cancer incidence. According to first mechanism the folate deficiency causes DNA hypomethylation and proto-oncogene activation. The second mechanism is that folate deficiency induces continuous uracil misincorporation during DNA synthesis leading to a catastrophic DNA repair cycle, DNA stand breakage and chromosome damage.

Considering that we know the reason for THFA depletion and FA deficiency, we could recommend the application of folic acid in pharmacological doses during and after antitumour therapy of cancer patients, for cancer prevention of individuals from the high-risk groups and for treatment of all disorders arising from oxidative stress.

REFERENCES

1. Wiseman, H. and Halliwell B., Damage of DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer. *Biochem J.*, 313, 17-28 (1996).
2. Allen, R.G. and Tresini M., Oxidative stress and gene regulation. *Free Radic. Biol. Med.*, 28, 463 – 499 (2000).
3. Raikov, Z.D., Hristova-Georgieva, N.I and Raikova, E.T., Nucleophilic targets in carcinogenesis, mutagenesis and chemotherapy of cancer. *Med. Hypotheses*, 22, 15 – 22 (1987).
4. Raikova, E., Inanov, I., Kaffalieva, D., Demirov, G. and Raikov, Z., Spin-labelling of DNA with hydrazine mustard spin-label (HMSL). *Int. J. Biochem*., 14, 41 – 46 (1982).
5. Schmute, C. and Jones, P.A., Involvement of DNA methylation in human carcinogenesis *J.Biol. Chem*., 379, 377 – 388 (1998).
6. Dutie S.J., Folic acid deficiency and cancer: mechanism of DNA instability. *British Med. Bull.*, 55, 578 – 592 (1999).
7. Kim, Y.I., Pogribny, I.P., Basnakian, A.G., Miller, J.W., Selhub, J.,Jame, S.J. and Mason, J.B., Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. *Am. J. Clin. Nutr*., 65, 46-52 (1997).
8. Blount, B.C., Mak, M.M., Wehr, C.M., Macgregor, J.T., Hiatt, R.A., Wang, G., Wickramasinghe, S.N., Everson, R.B. and Ames, B.N., Folate deficiency couses uracil misincorporation into human DNA and chromosomal breakage: implications for cancer and neuronal damage. *Proc. Nat. Acad. Sci. USA*, 94, 3290-3295 (1997).
9. Reidy, J.A., Role of deoxyuridine incorporation and DNA repair in the expression of human chromosomal fragile sites. *Mutat. Res*., 211, 215-220 (1988).

10. Choi, S.W., Kim, Y.I., Weitzel, J.N. and Mason, J.B., Folate depletion impairs DNA excision repair in the colon of rats. *Gut*, 43, 93-99 (1998).
11. Modrich, P., Mismatch repair, genetic stability and cancer. *Science*, 266, 1959-1960 (1994).
12. Pulman, B. and Pulman, A., Quantum Biochemistry. *Intersci. Publ., John Wiley, New York* – London, (1963).