J. Radiat. Res., 45, 527–533 (2004)

Regular Paper

Modulation of Enzymes Involved in Folate Dependent One-carbon Metabolism by γ-radiation Stress in Mice

Vipen BATRA, Vellappan KESAVAN* and Kaushal P. MISHRA

One-carbon matabolism/Folate pool/DNA damage/Radiation.

The role of various enzymes in folate dependent one-carbon metabolism, which are involved in mobilizing the folate pool for DNA synthesis and the DNA methylation reaction, was investigated. Male Swiss mice (6 weeks old) were subjected to 2, 5 and 7 Gy total body γ-irradiation. The animals were killed at intervals of 24, 48, 72, 96, 120 and 192 h and the livers were removed. Using a 12000 × g supernatant of 10% tissue homogenate, the activities of dihydrofolate reductase, thymidylate synthase and methylenetetrahydrofolate reductase were determined. The profiles of these folate enzymes were correlated to DNA damage by monitoring p53 protein profile and by comet tail moment analysis. A significant increase in activity of dihydrofolate reductase and thymidylate synthase was observed up to 96 h post-irradiation and the activity subsided thereafter, reaching control value after 192 h. A sharp decline in methylenetetrahydrofolate reductase activity was observed until 192 h after irradiation. Total folates declined by 54% after 96 h following irradiation, and p53 protein concentration in nuclei increased after irradiation, proportionate to radiation dose, and subsided slowly. Thus results indicate a significant drop in total folate levels and rise in p53 protein concentration in the liver after total body γ-irradiation. It may appear that, under radiation stress conditions, levels of enzymes involved in one-carbon metabolism for DNA repair, are modulated up to a certain time interval, in a dose specific manner. It may also appear that the requirements of folate for nucleotide base synthesis seem to be met at the expense of other one-carbon transfer reactions.

INTRODUCTION

Folate coenzymes act as donors and acceptors of one-carbon units in a variety of reactions involved in one-carbon metabolism. Folate dependent one-carbon metabolism is essential for DNA synthesis[1)], DNA repair[2)], DNA methylation[3)] and metabolism of certain amino acids.[4)] The *de novo* synthesis of thymidylate from deoxyuridylate and 5,10-methylenetetrahydrofolate is catalysed by thymidylate synthase (TS) and results in formation of dihydrofolate that in turn is reduced to tetrahydrofolic acid (THFA) by dihydrofolate reductase (DHFR).[5)] On the other hand the mobilization of the folate pool towards DNA methylation reactions occurs through methylenetetrahydrofolate reductase (MTHFR) catalysed synthesis of methyltetrahydrofolate from methylenetetrahydrofolate.[6)] Methyltetrahydrofolate is utilized for synthesis of S-adenosyl methionine (SAM) which is ultimate methyl donor for DNA methylation reactions.[7,8)] It has been reported that under conditions of folate deficiency dUMP accumulates and, as a result, uracil is incorporated into DNA instead of thymine[9,10)] resulting in strand breaks, chromosome breakage and micronucleus formation.[11)]

Gamma radiation is well known to induce damages in the DNA.[12)] To counteract this damage, a variety of DNA repair pathways have evolved protecting cells against the genotoxic effects of gamma radiation.[13)] These repair pathways require regular supply of DNA bases, which in turn depends on sufficient pools of folate and folate dependent enzymes.[14)] It is therefore clear that gamma radiation stress induces a state of folate pool deficiency by consuming existing folate pool of the cell. Further, irradiation produces oxygen radicals and thereby induces oxidative damage in biomolecules. Recently γ-radiation has been shown to cause dose dependent oxidative damage, splitting the folate molecule into pterin and p-aminobenzoylglutamic acid.[15)] Intestinal absorption of folates was also shown to be impaired after irradiation.[16,17)] Thus there is progressive depletion in folate levels in the animals after irradiation mainly due to increased requirements, impaired intestinal absorption and increased loss due to oxidative cleavage of folate molecule. Any functional or radia-

*Corresponding author: Phone: +91-22-25590411,
Fax: +91-22-25560750,
E-mail: vkesavan@rediffmail.com
Radiation Biology and Health Sciences Division Bhabha Atomic Research Centre, Mumbai - 400 085 India.

tion mediated folate deficiency may adversely affect nucleotide synthesis and DNA methylation reactions which may further lead to cell death or carcinogenesis.[17,18)]

Although many of the enzymes of one-carbon metabolism are known and their specificities for folate substrates have suggested potential regulatory features, the *in vivo* regulation of the interconnected cycles of one-carbon metabolism is still poorly understood.[19)] The radiation mediated *in vitro* perturbation in enzymes involved in one carbon metabolism,[20)] and interdependence of p53 and folate enzymes profile has been suggested by few studies. These studies showed that in cells over expressing TS, the level of p53 protein gets reduced.[21)] The effect of different doses of γ-radiation on *in vivo* levels of folate dependent enzymes and corresponding p53 protein concentration is unknown. The present studies were planned to explore the radiation stress modulated profile of various enzymes involved in mobilization of cellular folate pools for DNA methylation and DNA synthesis reactions.

MATERIALS AND METHODS

Irradiation

Male Swiss mice (5 weeks old), maintained on a stock laboratory diet (consisting of 70%

broken wheat, 20% Bengal gram, 4% yeast powder, 5% fish meal, 0.25% shark liver oil, and 0.75% sesame oil) were obtained from the Departmental Animal House facility. They were housed three per cage in a room with a constant temperature of 23 ± 1°C and a 12 h light-dark cycle. The average initial body weight of the mice were 22.1 ± 1.6 g. The animals were subjected to total body γ-irradiation at a rate of 79 cGy/min by using a Co-60 Theratron Junior Teletherapy unit (Atomic Energy of Canada Ltd., Ottawa, Canada). The area of exposure was kept constant, and the total dose given was 2, 5, or 7 Gy. Unirradiated animals acted as controls. The mice were given free access to food and water throughout the study. The average food consumed estimated per mouse per day for each group is as follows: control, 4.4 ± 0.22 g; 1 h postirradiated: 4.4 ± 0.1 g; 24 h postirradiated: 4.0 ± 0.12 g; 72 h postirradiated: 3.8 ± 0.12 g; and 120 h postirradiated: 3.7 ± 0.05 g. The difference in food consumption between different dose groups was however found to be statistically insignificant.

The animals were killed by cervical dislocation at intervals of 24, 48, 72, 96, 120 and 192 h after irradiation; the livers were removed and homogenized with 3–4 strokes in ice–cold 0.1 M phosphate buffer pH 6.0 containing 1% ascorbate and made to 10% w/v suspension. An aliquot of the homogenate was immediately heated in a water bath (100°C) for 5 min to inactivate endogenous folyl conjugase. The folates were then extracted for 10 min at 80°C, clarified by centrifugation and made to the original volume in the same buffer containing 1% ascorbate and preserved for assays. A 12000 × g supernatant was isolated from the remaining aliquot of the liver homogenate and was used for enzyme assays.

Preparation of dihydrofolate

Dihydrofolate (DHF) was prepared by reducing purified pteroylglutamate with sodium dithionite.[22)] The dihydrofolate prepared in a crystalline form was stored in dark in a dessicator over phosphorus pentaoxide and potassium hydroxide and was used within 2 days for DHFR assay.

DHFR assay

DHFR assay system[23)] contained in total volume of 1 ml, Tris HCl buffer, pH 7.4, 100 mmole; KCl 150 μmole; NADPH 0.5 μmole; Dihydrofolate 0.5 μmole; 2-mercaptoethanol 10 μmole and 5 μg enzyme protein. The assay is based upon marked change in absorption at 340 nm due to oxidation of NADPH as the DHF is reduced to THF. DHFR activity is defined as equivalent to the amount of enzyme required to transform 1 μmole of DHFA to THFA per minute under condition of assay.

TS assay

Thymidylate Synthase assay system contained Tris HCL buffer, pH 7.4, 60 mM; 2-Mercaptoethanol, 100 mM; Magnesium chloride, 40 mM; Formaldehyde, 20 mM; 5 μg enzyme protein, dUMP, 0.1 μM and Tetrahydrofolate, 1.2 mM in 1 ml of reaction mixture.[24)] The assay is based on marked spectral change due to increase of absorbance at 340 nm, which occurs when tetrahydrofolate is converted to dihydrofolate. One international unit of activity is defined as equivalent to the amount of enzyme required to transform 1 μmole of dUMP to dTMP per minute under condition of assay.

MTHFR assay

MTHFR assay mixture contained potassium phosphate buffer, 100 mμmole, pH 6.3; FAD, 5 mμmole; menadione bisulphate, 2 μmole; ascorbic acid, 5 μmoles; ^{14}C-methyl terahydrofolate (110 disintegration per minute per mμmole), 320 mμmoles and ethylenediamine tetra acetic acid, 1mmole in the total reaction mixture of volume 0.6 ml. After the incubation period of 1 h at 30°C, 0.3 ml of dimedone solution (3 mg per 1 M acetate buffer, pH 4.5) was added, the mixture heated for 5 minutes at 95°C and cooled in ice.[25)] The assay is basically the reverse reaction and the 5, 10-methylene tetrahydrofolate formed dissociates easily to yield labeled formaldehyde, which in turn is extracted as formaldehyde dimedone condensation product and is counted for radioactivity. The MTHFR activity is expressed in terms of nmole formaldehyde formed per gram liver per hour.

Folate assay

Folate activity was determined by microbiological assay,

using *Lactobacillus casei* ATCC 7469 with 5-formyltetrahydrofolate (Sigma Chemical Co., Missouri, USA), as standard as described earlier.[26] The total folate was assayed after prior digestion of the sample with chicken liver folyl conjugase.

Liver nuclei isolation and p53 protein assay

The liver was homogenized in ice-cold nuclear isolation buffer (low salt) containing 50 mM Tris-HCl pH 7.5, 0.25 M sucrose, 5 mM MgCl, 1 mM DTT and protease inhibitor cocktail (Sigma Chemical Co., Missouri, USA). The homogenate (10% w/v) was centrifuged at 5000 × g (Sorvall SS-34 centrifuge rotor) for 10 minutes at 4°C to obtain nuclear pellet and supernatant fractions. The nuclear pellet was resuspended in ice cold 0.8 ml of nuclear lysis buffer (high salt) containing 50 mM Tris-HCl pH 7.5, 5 mM MgCl, 100 mM KCl, 1 mM DTT and protease inhibitor cocktail and centrifuged at 12000 × g for 15 minutes to obtain nuclear extract. Nuclear p53 protein was estimated by quantitative sandwich ELISA principle using p53 pan ELISA kit (Roche Diagnostics Gmbh., Mannheim, Germany). It involved single step incubation of p53 containing sample with capture antibody and peroxidase labeled detection antibody to form a stable immunocomplex. The peroxidase bound to the complex is developed by tetramethylbenzidine as a substrate. The photometrically determined colour is proportional to p53 protein concentration.

Comet assay

The alkaline gel comet assay was done to assess the DNA damage by method as described earlier.[27] The DNA damage in Lymphocyte nuclei after various time intervals at different doses of irradiation was assessed by tail moment analysis.[28]

RESULTS

DHFR profile after g-radiation

Dihydrofolate reductase was monitored in mouse liver at various periods after irradiation. No significant change in dihydrofolate reductase activity was seen in animals exposed to 2 Gy. The DHFR activity after 5 Gy exposure increased progressively. The increase was found to be 7.71% over control value after 24 h post-irradiation and 78.35% after 96 h post-irradiation. The increase in activity started subsiding after 96 h and enzyme levels were only 5.81% over and above control after 192 h of irradiation (see Table 1). On the other hand animals exposed to 7 Gy showed an increase in the enzyme activity (39.19%) over the control after 24 h of irradiation and an upward modulation of the enzyme activity was seen till 96 h where the activity showed an increase of 143.5% over the control. The increased levels of enzyme at 7 Gy subsided and approached closer to control after 192h (Table 1).

Modulation of Thymidylate synthase by γ-radiation

Thymidylate synthase activity increased progressively from 8.9 to 21.70% over control during 24–192 h after irradiation at 2 Gy (see Table 2). The increase in enzyme activity was more pronounced after 5 Gy where the activity increased from 32.41 IU per gram liver (after 24 h post-irradiation) to 154 IU per gram liver (after 96 h post-irradiation). The enzyme activity declined thereafter, approaching control values at 192 h post-irradiation. There was a similar increase in TS activity after 7 Gy at 72 h (154%), which was maintained until 96 h (128.9%). The enzyme activity thereafter subsided and reached closer to control values after 192 h (Table 2).

Modulation of Methylene-tetrahydrofolate reductase by γ-radiation

The methylenetetrahyderofolate reductase is involved in catalyzing the transformation of methylenetetrahydrofolate to methyltetrahydrofolate. MTHFR activity in mice liver was largely unchanged after 2 Gy. However, a progressive decrease in MTHFR activity was seen after various doses of radiation. The decline in enzyme activity was prominent at 5 and 7 Gy radiation dose (see Table 3). The MTHFR activ-

Table 1. Effect of various doses of γ-radiation on dihydrofolate reductase activity[#] after different Time intervals.

Radiation dose	24 h	48 h	72 h	96 h	120 h	192 h
Control	30.08 ± 1.59	30.08 ± 1.59	30.08 ± 1.59	30.08 ± 1.59	30.08 ± 1.59	30.08 ± 1.59
2 Gy	30.35 ± 1.23	30.89 ± 1.14	30.35 ± 1.23	30.62 ± 1.75	30.89 ± 1.45	31.22 ± 1.60
	(Nil)	(Nil)	(Nil)	(Nil)	(Nil)	(3.78)
5 Gy	32.40 ± 1.36	38.95 ± 2.01*	45.70 ± 2.16**	53.65 ± 4.65**	32.90 ± 1.75	31.83 ± 1.68
	(7.71)	(29.48)	(51.9)	(78.35)	(9.37)	(5.81)
7 Gy	41.87 ± 1.94*	45.67 ± 2.86*	49.08 ± 3.05**	73.25 ± 5.80**	38.46 ± 2.11*	29.94 ± 1.29
	(39.19)	(51.82)	(63.16)	(143.51)	(27.85)	(Nil)

[#] DHFR activity is expressed in international units (IU) per gram liver. One IU equivalent to the amount of enzyme required to transform 1 μmole of DHFA to THFA per minute. The values are mean ± standard error of samples (in triplicate) from 4 animals in each group. The values given in parenthesis are % increase in DHFR activity over control. (*$P < 0.05$, **$P < 0.01$).

Table 2. Effect of various doses of γ-radiation on thymidylate synthase activity[#] after different time Intervals.

Radiation dose	24 h	48 h	72 h	96 h	120 h	192 h
Control	2.35 ± 0.18	2.35 ± 0.18	2.35 ± 0.18	2.35 ± 0.18	2.35 ± 0.18	2.35 ± 0.18
2 Gy	2.56 ± 0.21	2.84 ± 0.22	2.90 ± 0.26	2.74 ± 0.21	2.95 ± 0.28	2.86 ± 0.19
	(8.9)	(17)	(23.40)	(16.5)	(25.53)	(21.70)
5 Gy	3.11 ± 0.23*	5.26 ± 0.47**	5.40 ± 0.69**	5.98 ± 0.74**	2.55 ± 0.19	2.38 ± 0.16
	(32.41)	(123.8)	(129.78)	(154)	(8.51)	(1.27)
7 Gy	3.15 ± 0.22*	5.76 ± 0.71**	6.59 ± 0.71**	5.38 ± 0.71**	2.38 ± 0.16	2.33 ± 0.17
	(34.04)	(145)	(154)	(128.9)	(1.27)	(Nil)

[#] TS activity is expressed in terms of international units (IU) per gram liver. One IU is equivalent to the amount of enzyme required to transform 1 μmole of DHFA to THFA per minute. The values are mean ± standard error of samples (in triplicates) from 4 animals in each group. The values given in parenthesis are % increase in TS activity over control. (*$P < 0.05$, **$P < 0.01$).

Table 3. Effect of various doses of γ-radiation on methylene-tetrahydrofolate reductase activity[#] after different time intervals.

Radiation dose	24 h	48 h	72 h	96 h	120 h	192 h
Control	56.40 ± 2.7	56.40 ± 2.7	56.40 ± 2.7	56.40 ± 2.7	56.40 ± 2.7	56.40 ± 2.7
2 Gy	56.79 ± 2.67	55.65 ± 2.59	55.43 ± 2.49	54.68 ± 2.35	56.41 ± 2.49	56.46 ± 2.67
	(Nil)	(1.32)	(1.71)	(3)	(Nil)	(Nil)
5 Gy	48.17 ± 1.86*	46.21 ± 1.74*	40.34 ± 1.65**	37.67 ± 1.52**	39.24 ± 1.37**	27.51 ± 1.26**
	(14)	(18.06)	(28.47)	(33.2)	(30.42)	(51.22)
7 Gy	36.21 ± 1.78**	36.15 ± 1.66**	33.53 ± 1.43**	30.89 ± 1.35**	29.11 ± 1.16**	23.94 ± 1.01**
	(35.79)	(35.90)	(40.54)	(45.23)	(48.38)	(57.55)

[#] MTHFR activity is expressed in terms of nmole formaldehyde formed/ g liver/ hour (see materials and methods for definition). The values are mean ± standard error of samples (in triplicates) from 4 animals in each group. The values given in parenthesis are % decrease in MTHFR activity over control. (*$P < 0.05$, **$P < 0.01$).

ity declined continuously from 14 to 51% after 24 to 192 h at 5 Gy. The decline in activity was equally significant at 7 Gy where in enzyme activity was 57.5% impaired after 192 h (Table 3).

Liver total folate profile

The total liver folate profiles in mice irradiated with 7 Gy over a period of up to 192 h are shown in Fig. 1. The folate exists inside the cell in various polyglutamyl forms. The total folates that were determined after prior digestion of the sample with chicken liver folyl conjugase were steadily dropped following irradiation and the trend continued up 192 h studied (see materials and methods). The folate levels showed only 295 ng per g liver compared to 673 ng per g liver folate for the control indicating 56% drop in total folate status after 192 h.

p53 profile of liver nuclei

The levels of p53 in nuclei were found to increase after various doses of gamma irradiation (Table 4). The levels of p53 in nuclei were. in co-relation with increase in radiation

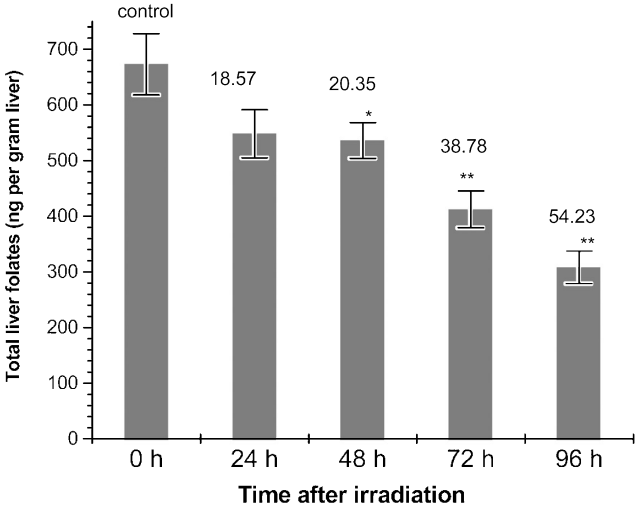

Fig. 1. Mice liver folate levels at various periods after irradiation at 7 Gy. The values are mean ± standard error for four mice in each group. The values given above bar chart are % decrease in folate levels per ng liver. (*$P < 0.05$, **$P < 0.01$).

Table 4. Effect of various doses of γ-radiation on amount of nuclear p53 protein[#] after different time intervals.

Radiation dose	24 h	48 h	72 h	96 h	120 h	192 h
Control	62.0 ± 2.1	62.0 ± 2.1	62.0 ± 2.1	62.0 ± 2.1	62.0 ± 2.1	62.0 ± 2.1
2 Gy	89.2 ± 2.7**	81.0 ± 2.7**	85.0 ± 2.6**	75.5 ± 2.4*	70.2 ± 2.4*	65.4 ± 2.0
5 Gy	125.0 ± 3.7**	120.4 ± 3.7**	104.6 ± 3.3**	95.8 ± 3.0**	95.0 ± 3.0**	85.6 ± 2.5**
7 Gy	140.0 ± 4.1**	125.8 ± 3.9**	122.0 ± 3.8**	112.6 ± 3.6**	100.0 ± 2.9**	95.0 ± 2.7**

[#] The values are expressed as p53 protein concentration in terms of picogram p53 protein per ml of liver homogenate (10% w/v). The values are mean ± standard error of samples (in triplicates) from 4 animals in each group. (*$P < 0.05$, **$P < 0.01$).

dose and were observed to subside slowly. p53 levels increased by 43.8–125% after 24 h of irradiation at 2–7 Gy. The p53 levels progressively declined to control levels after 192 h at 2 Gy while showing slow declination at 5 and 7 Gy. The p53 levels continued to be higher than control at 5 Gy (38.0%) and 7 Gy (53.2%) dose after 192 h.

DISCUSSION

Radiation stress mediated DNA damage induces several metabolic processes some of which involve facilitation of folate-mediated reactions.[29] Folate stores of liver, the central organ involved in one-carbon metabolism, can be mobilized to meet the requirements of different tissue systems in the body.[30] Total body irradiation may adversely affect supply of this essential vitamin to all the tissues, especially those containing rapidly dividing cells. Rapidly dividing cells are critically dependent on an abundant supply of fully reduced folates, which are essential for formation of nucleotides required for DNA synthesis and repair[31,32] Present study indicated the presence of sufficient pool of folate dependent enzymes in liver. The fact that there is almost no increase in levels of DHFR and TS at 2 Gy probably indicates that existing pools of DHFR and TS in liver are sufficient to meet the synthesis of increased requirements of reduced folate coenzymes at this dose. Quantitatively the pool size of DHFR was found to be larger than TS. It is possibly due to the role of DHFR in regenerating THF, a reaction essential for DNA methylation reactions (via methyl-THF) as well as purine and pyrimidine synthesis reactions (via methylene-THF), compared to TS which is required only for thymidylate synthesis.[33] Thymidylate synthase, which is involved in catalysis of deoxyuridylate to deoxythymidylate, acts as the rate-limiting step in DNA synthesis.

The increase in activity of DHFR and TS at doses as high as 5 and 7 Gy occurs only up to 96 h post-irradiation (Table 1 and 2). The increase in levels of these enzymes may play a pivotal role in regulating the synthesis of tetrahydrofolate cofactors required to meet the increased demand for DNA repair pathways like base excision repair, nucleotide excision repair[34] and recombination repair. These studies on various folate dependent enzymes indicate significant enzyme mediated mobilization of folate pool for DNA repair reaction only up to a certain time interval in a dose specific manner. At higher doses of gamma exposure the initial upsurge in DHFR activity followed by its fall might be reflecting the threshold levels beyond which DNA repairing events are incomplete and the cells start dying shortly thereafter.[35]

MTHFR is involved in catalyzing the transformation of methylenetetrahydrofolate to methyltetrahydrofolate, which enters the methylation pool through SAM. SAM acts as an ultimate methyl donor to DNA through the enzyme DNA methyltransferase.[36] The levels of MTHFR were found to be unaffected at 2 Gy. However a sharp decline in enzyme activity was observed at 5 and 7 Gy, which might lead to methyl-tetrahydrofolate deficiency (Fig. 1). This may adversely affect overall DNA methylation and also methylation status of various genes. A change in methylation status of tumor suppressor gene can disturb its functions or it may activate an oncogene, setting a path for the cell leading towards carcinogenesis. p53 is one such tumor suppressor gene that is highly methylated by methyl donors such as folate.[37] A change in folate and MTHFR dependent methylation status may have adverse effect on regulation of cell proliferation. The levels of various folate dependent enzymes may therefore become rate limiting for DNA repair and DNA methylation reactions under gamma radiation stress condition.[38]

A significant decline in total liver folate levels (Fig. 1) may cause a state of functional folate deficiency in the animal. Folate deficiency can mimic ionizing radiation in damaging DNA by causing strand breaks and telomere instability.[39,40] The decline in folate levels increases dUMP to dTMP ratio which causes huge incorporation of uridylate into DNA in place of thymidylate, as the DNA polymerase can not effectively differentiate between uridylate and thymidylate.[41] So a radiation mediated folate deficiency may initiate a catastrophic repair cycle, which may lead to double strand break, chromosomal damages and cancer. Chromosome breaks have been demonstrated *in vivo* in humans from folate deficiency.[42]

In the present studies, DNA damage was assessed directly by comet tail moment analysis (Table 5) and indirectly by monitoring the amount of p53 protein (Table 4). Tail

Table 5. Effect of various doses of γ-radiation on nuclear DNA damage after different time intervals assessed by comet tail moment[#].

Radiation dose	24 h	72 h	120 h
Control	3.2 ± 0.4	3.2 ± 0.4	3.2 ± 0.4
2 Gy	4.2 ± 0.6	4.4 ± 1.1	5.3 ± 1. 2*
5 Gy	33.6 ± 2.9**	32.8 ± 2.5**	22.3 ± 1.8**
7 Gy	38.4 ± 3.6**	34.6 ± 3.4**	34.1 ± 3.1**

[#] The values are mean ± standard error of samples from 4 animals in each experimental group. Tail moment is the product of the tail length and the fraction of total DNA in the tail of the comet (*$P < 0.05$, **$P < 0.01$).

moment is the product of the tail length and the fraction of total DNA in the tail of the comet. Tail consists of only damaged DNA and so tail moment incorporates both size of migrating DNA and the number of fragments. Tail moment analysis clearly indicated that recovery from DNA damage occurs maximally up to 2 Gy and damage was much more significant at 5 and 7 Gy compared to 2 Gy (Table 5).

P53 levels were also found to reach close to control level after 96–120 h of irradiation at 2 Gy, thus indicating possibility of significant recovery of damaged DNA. However the p53 levels continued to be higher than control at 5 and 7 Gy radiation after 192 h, possibly indicating that at higher doses, cells may run short of nucleotides whose synthesis in turn requires some crucial biomolecules (e.g. deoxyribose and ATP) or catalytic coenzymes such as folate.

This study showed the dose dependent effect of radiation-mediated damage of DNA and its recovery after various time intervals. Significant dose dependent changes in folate metabolism and folate enzymes in liver were observed which might hinder DNA synthesis and repair processes. The whole body irradiation may mobilize folate pool of liver for DNA repair processes in other organs. Therefore the changes observed in folate metabolism in liver may partly be attributed to indirect effect of radiation on other organs, which due to increased folate requirements, may influence the course of biochemical events in liver after whole body irradiation.

In summary, the enzymes involved in folate dependent one-carbon metabolism are significantly modulated in dose dependent manner. The increased mobilization of folate pool towards DNA synthesis and repair reactions is hindered by the decline in total folate levels of liver possibly due to increased utilization and increased oxidative catabolism.

REFERENCES

1. Koury, M. J., Horne, D. W., Brown, Z. A., Pietenpol, J. A, Blount, B. C., Ames, B. N., Hard, R., Koury, S. T. (1997) Apoptosis of late-stage erythroblasts in megaloblastic anemia: association with DNA damage and macrocyte production. Blood, **89**: 4617–4623.
2. Duthie, S. J., Hawdon. A. (1998) DNA instability (strand breakage, uracil misincorporation, and defective repair) is increased by folic acid depletion in human lymphocytes *in vitro*. The FASEB J. **12**: 1491–1497.
3. Rampersaud, G. C., Kauwell G. P., Hutson A. D., Cerda J. J., Bailey L. B. (2000) Genomic DNA methylation decreases in response to moderate folate depletion in elderly women. Am. J. Clin. Nutr. **72**: 998–1003.
4. Niculescu, M. D., and Zeisel S. H. (2002) Diet, methyl donors and DNA methylation : Interactions between dietary folate, methionine and choline J. Nutr. **132**: 2333S–2335S.
5. Kim, Y. I., (1999) Folate and carcinogenesis: Evidence, mechanisms, and implications. J. Nutr. Biochem. **10**: 66–88.
6. Anguera, M.C., Suh, J. R., Ghandour, H., Nasrallah, I. M., Selhub, J., Stover, P. J. (2003) Methenyl tetrahydrofolate synthetase regulates folate turnover and accumulation. J. Biol. Chem. **278**: 29856–29862.
7. Caudill, M. A., Wang, J. C., Melnyk, S., Pogribny, I, P., Jernigan, S., Collins, M. D., Santos-Guzman, J., Swendseid, M. E., Cogger, E. A., James, S. J. (2001) Intracellular S-adenosylhomocysteine concentrations predict global DNA hypomethylation in tissues of methyl-deficient cystathionine beta-synthase heterozygous mice. J. Nutr. **11**: 2811–2818.
8. Sibani, S., Melnyk, S., Pogribny, I. P., Wang, W., Hiou-Tim, F., Deng, L., Trasler, J., James, S. J., Rozen, R. (2002) Studies of methionine cycle intermediates (SAM, SAH), DNA methylation and the impact of folate deficiency on tumor numbers in Min mice. Carcinogenesis. **23**: 61–65.
9. Wagner, C. (1995) Biochemical role of folate in cellular metabolism. In Folates in health and disease , Eds. L. B. Baily, pp 23–45 Marcel Decker : New York.
10. Fenech, M. and Crott, J. W. (2002) Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes- evidence for breakage-fusion- bridge cycles in the cytokinesis block micronuclus assay. Mutat. Research. **504**: 131–136.
11. Wang, X. U., and Fenech, M. (2003) A comparison of folic acid and 5-methyltetrahydrofolate for prevention of DNA damage and cell death in human lymphocytes. Mutagenesis, **18**: 81–86.
12. Pogozelski, W. K., Xapsos, M. A., Blakely, W. F. (1999) Quantitative assessment of the contribution of clustered damage to DNA double-strand breaks induced by 60Co gamma rays and fission neutrons. Radiat. Research, **151**: 442–448.
13. Teoule, R. (1987) Radiation-induced DNA damage and its repair. Int. J. Radiat. Biol. **51**: 573–589.
14. Steinberg. S. E., Fonda, S., Campbell C. L., Hillman, R. S. (1983) Cellular abnormalities in folate deficiency. Brit. J. Haemat. **54**: 605–612.
15. Kesavan, V., Pote, M. S., Batra, V., and Viswanathan, G. (2003) Increased folate catabolism following total body γ-irradiation in mice. J. Radiat. Res. **44**: 141–144.
16. Kesavan, V., Noronha, J. M. (1971) Effect of X-radiation on the absorption of naturally occurring folates. Int. J. Radiat. Biol. **3**: 205–214
17. Kesavan, V., and Noronha J. M. (1978) An ATPase dependent

radiosensitive acidic microclimate essential for intestinal folate transport J. Physiol. (London) **280**: 1–7.
18. Calvert, H. (1999) An overview of folate metabolism: features relevant to the action and toxicities of antifolate anticancer agents. Seminars in oncology, **26**: No 2, Suppl 6, 3–10.
19. Choi, S. W., Kim Y. I., Weitzel, J N, Mason J. B. (1998) Folate depletion impairs excision repair in the colon of the rat. Gut, **43**: 93–99.
20. Shane, B. (1989) Vita. Hormone. **45**: 263–335.
21. Jingfang J., Pedersen-Lane, J., Maley, F., and Chu, E. (1999) Regulation of p53 expression by thymidylate synthase. Proc. Natl. Acad. Sci. USA. **96**: 3769–3774.
22. Blakley, R. L. (1960) Crystalline dihydropteroylglutamic acid. Nature, **188**: 231–232.
23. Kawai, M., Hillcoat, B. L. (1974) Interaction of thymidylate synthetase and dihydrofolate reductase enzymes *in vitro and in vivo*. Can. Research. **34**: 1619–1626.
24. Wabha, A. J., Friedkin, M. J. (1961) Direct spectrophotometric evidence for the oxidation of tetrahydrofolate during the enzymatic synthesis of thymidylate. J. Biol. Chem. **236**: PC11.
25. Kutzbach, C., Stakstad ELR. (1971) Mammalian methylene tetrahydrofolate reductase : Partial purification, properties and inhibition by S-adenosylmethionine. Bioche. Biophy. Acta, **250**: 459–477.
26. Pote, M. S.,Viswanathan, G., Keasvan, V. (1998) Increased intestinal folate absorption in experimental diabetes. J. Clin. Biochem.Nutr. **25**: 25–30.
27. Duez, P., Dehon, G., Kumps, A., Dubois, J. (2003) Statistics of the Comet assay: a key to discriminate between genotoxic effects. Mutagenesis, **18**: 159–166.
28. Krown, K. A., Page, M. T., Nguyen, C., Zechner, D., Gutierrez, V., Comstock, K. L., Glembotski, C. C., Quintana, P. J. E., Sabbadini, R. A. (1996) Tumor Necrosis Factor Alpha-induced Apoptosis in Cardiac Myocytes. J. Clin. Invest. **98**: 2854–2865.
29. Mason, J. B., Choi, S. W. (2000) Folate and carcinogenesis: developing a unifying hypothesis, Adv. Enz. Regu. **40**: 127–141.
30. Baggott, J. E., and MacKenzie, R. E. (2003) 5,10-Methenyltetrahydrofolate cyclohydrolase, rat liver and chemically catalysed formation of 5-formyltetrahydrofolate. Biochem. J. **374**: 773–778.
31. Kim, Y. I., Salomon R. N., Graeme-cook. F. (1996) Dietary folate protects against the development of macroscopic colonic neoplasia in a dose responsive manner in rats. Gut. **39**: 732–740.
32. Yehuda, G., Rothem, A. L., Hooijberg, J. H., Stark, M., Ifergan, I., Kathmann, I., Dijkmans, B. A. C., Peters, G. J., Jansen, G. (2003) Loss of multidrug resistance protein 1 expression and folate efflux activity results in a highly concentrative folate transport in human leukemia cells. J. Biol. Chem. **278**: 6680–6686.
33. Sowers, R., Toguchida, J., Qin, J., Meyers, P, A., Healey, J. H., Huvos, A., Banerjee, D., Bertino, J. R., Gorlick, R. (2003) mRNA expression levels of E2F transcription factors correlate with dihydrofolate reductase, reduced folate Carrier, and thymidylate synthase mRNA expression in osteosarcoma. Mole. Cancer Therap. **2**: 535–541.
34. Duthie, S. J., Narayanan, S., Brand, G. M., Pirie, L., Grant, G. (2002) Impact of folate deficiency on DNA stability. J. Nutrition. **132**: 2444S–2449S.
35. Pasupathy, K., Nair C. K. K. (2003) Response of DNA repair enzymes in murine fibrosarcoma, lymphosarcome and ascites cells following gamma irradiation. J. Radiat. Res. **44**: 229–235.
36. Vanoni, M. A., Ballou D. P., Matthews, R. G. (1983) Methylene tetrahydrofolate reductase : Steady state and rapid reaction studieson the NADPH-methylene tetrahydrofolate, NADPH-menadione and methyltetrahydrofolate-menadione oxidoreductase activities of the enzyme. J. Biol. Chem. **10**: 11510–11514.
37. Ding, B. C., Johnathan R., Witt, T. L., Schuetz, J D., and Matherly L. H. (2001) Repression of Human Reduced Folate Carrier Gene Expression by Wild Type p53 J. Biol. Chem. **276**: 8713–8719.
38. James, S. J., Yin, L., Swendseid, M. E. (1989) DNA strand break accumulation, Thymidylate synthesis and NAD levels in lymphocytes from methyl donor-deficient rats. J. Nutrition. **119**: 661–664.
39. Branda, R. F., Blickensderfer, D. B. (1993) Folate deficiency increases genetic damage caused by alkylating agents and gamma radiation in Chinese hamster ovary cells. Cancer Res. **15**: 5401–5408.
40. Ojima, M., Hamano, H., Suzuki, M., Suzuki, K., Kodama, S., Watanbe, M. (2004) Delayed induction of telomere instability in normal human fibroblast cells by ionizing radiations. J. Radiat. Res, **45**: 105–110.
41. Blount, B. C., Mack, M. M., Wehr, C, M., Macgregor, J. T., Hiatt, R. A., Wang, G., Wickramasinghe, S. N., Everson, R. B., and Ames, B. N. (1997) Folate deficiency causes uracil misincorporation in to human DNA and chromosome breakage: Implications for cancer and neuronal damage. Proc. Natl. acad. Sci. USA. **94**: 3290–3295.
42. Ame, B. N. (2001) DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutation. Res. **475**: 7–20.

Received on May 14, 2004
1st Revision on August 9, 2004
Accepted on September 17, 2004