Queuine promotes antioxidant defence system by activating cellular antioxidant enzyme activities in cancer

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Synopsis

Constant generation of ROS (reactive oxygen species) during normal cellular metabolism of an organism is generally balanced by a similar rate of consumption by antioxidants. Imbalance between ROS production and antioxidant defence results in an increased level of ROS, causing oxidative stress, which leads to promotion of malignancy. Queuine is a hyper-modified base analogue of guanine, found at the first anticodon position of the Q-family of tRNAs. These tRNAs are completely modified with respect to queuosine in terminally differentiated somatic cells; however, hypo-modification of Q-tRNAs is closely associated with cell proliferation. Q-tRNA modification is essential for normal development, differentiation and cellular function. Queuine is a nutrient factor for eukaryotes. It is found to promote the cellular antioxidant defence system and inhibit tumorigenesis. The activities of antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase are found to be low in the DLAT (Dalton’s lymphoma ascites transplanted) mouse liver compared with normal mouse liver. However, exogenous administration of queuine to the DLAT cancerous mouse improves the activities of antioxidant enzymes. These results suggest that queuine promotes the antioxidant defence system by increasing antioxidant enzyme activities and in turn inhibits oxidative stress and tumorigenesis.

Key words: antioxidant, cancer, queuine, queuosine, Q-tRNA, reactive oxygen species (ROS)

INTRODUCTION

Antioxidants contribute to the body’s major defence system against free radicals. They react with free radicals and provide protection against the toxic effect of ROS (reactive oxygen species) by chelating or scavenging them. ROS, such as the superoxide anion (O$_2^−$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (HO•), are constantly generated in vivo during normal cellular processes and metabolism, which are essential for cell growth, proliferation and cell death. Low concentrations of ROS are indispensable in many biochemical processes of cell differentiation, cell progression and apoptosis [1], as well as providing defence against micro-organisms [2], modulating activities of redox-sensitive transcription factors [3] and regulating mitochondrial enzyme activities [4]. In contrast, high levels or inadequate removal of ROS results in oxidative stress, which may cause metabolic malfunctions and damage to macromolecules such as DNA, proteins and lipids [5]. The steady-state formation of free radicals (pro-oxidants) is normally balanced by a similar rate of consumption by antioxidants [6]. An imbalance between ROS production and antioxidant defence results in increased levels of ROS, causing oxidative stress, which leads to various pathological conditions, including metabolic dysfunction, cardiovascular diseases, neurodegenerative diseases, cancer and premature aging [7]. To maintain the redox balance in order to protect themselves, organisms have evolved an endogenous antioxidant defence mechanism to scavenge and prevent formation of ROS. Thus enzymatic antioxidants such as catalase, SOD (superoxide dismutase) and GPX (glutathione peroxidase), and non-enzymatic ones, including glutathione, ascorbic acid and flavonoids etc., protect against oxidative stress and prevent damage to cells [8]. SOD catalyses the spontaneous dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen, which is further detoxified by catalase and GPX [9]. GR (glutathione reductase) is essential for the glutathione redox cycle that
maintains an adequate level of cellular GSH [10]. These antioxidant enzymatic functions are necessary for life in all oxygen metabolizing cells. Free radicals and ROS are known to be involved in the initiation and promotion of carcinogenesis in vivo and in vitro. The activity of antioxidant enzymes is reported to be low in most animal and human cancers [11,12]. Therefore the activities of antioxidant enzymes are proposed to be important in the regulation of oxidative stress and tumorigenesis.

Queuine (7-[(4,5-cis-dihydroxy-2-cyclopentenyl-1-yl)-amino]-methyl]-7-deazaguanosine) is a hyper-modified base analogue of guanine [13]. In the mammalian system, it is present as a free base or as Q-tRNA in a bound state within the cell and plays an important role in cellular functions. It is found at the first anticodon position of four tRNAs: tRNA^{51y}, tRNA^{His}, tRNA^{Asp} and tRNA^{Asn}. These four tRNAs are known as Q-family tRNAs [14]. The original transcript of Q-family tRNAs contains guanine in the first anticodon position, which is post-transcriptionally modified with queuine during maturation [15]. Queuine is ubiquitously present throughout the living system from prokaryotes and eukaryotes, including plants, with the exception of archaebacteria, Mycoplasma and yeast [16]. Prokaryotes synthesize queuine de novo by a complex biosynthetic pathway; however, eukaryocytes are unable to synthesize either the precursor or queuine itself. They utilize the salvage system and acquire queuine as a nutrient factor from their diet or from intestinal microflora [17]. Queuine or Q-tRNA deficiency does not occur under normal physiological conditions. The tRNAs of the Q-family are completely modified in terminally differentiated cells. Queuosine deficiency of tRNAs is associated with lymphoma, leukaemia and various kinds of tumours [18–20]. Queuosine modification of tRNAs are often incomplete in cancer cells; however, exogenous queuine has been shown to improve Q-tRNA deficiency [21–23]. Alteration in the level of queuosine modification of tRNA is observed during differentiation and developmental stages in different species, i.e. Dicyostelium discoideum [24] and Dro sophila melanogaster [25], during development and aging of rats [26], embryonic tissues [27] and neoplastically transformed cells [28]. The physiological role of queuine remains ill-defined, but direct or indirect evidence suggests that queuine participates in many cellular functions. Earlier reports have suggested that hyper-modified nucleosides found in the tRNA anticodon contribute to the high fidelity of codon recognition and influence translation [29]. Apart from its classical role, queuine has other important roles in the regulation of cell proliferation [30,31], differentiation [18], control of aerobic/anaerobic metabolism [32,33], protection from oxidative stress and oxidative radicals [34], down-regulation in the expression of proto-oncogenes [35,36] and modulation of signal-transduction pathways [37,38].

Cancer cells are known to have a high energy metabolism and thus produce high levels of ROS. Accumulation of ROS promotes impairment of the antioxidant defence system, causing oxidative stress, which is one of the major causes of initiation and promotion of malignancy. Therefore the present study was focused to analyse the role of queuine in protecting the cellular antioxidant defence system during malignancy.

**MATERIALS AND METHODS**

**Materials**

All chemicals used were of analytical and molecular biology grade. PMSF, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide], GR, NADPH and haematoxylin and eosin stain were purchased from Sigma–Aldrich, Tris buffer, NAD, NBT (Nitro Blue Tetrazolium), NADH, PMS (phenzeline methosulfate), GSH, GSSH, DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)], riboflavin, ferric chloride and potassium ferrocyanide were purchased from Sisco Research Laboratories (Mumbai, India). EDTA, HCl, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, hydrogen peroxide, picric acid, formaldehyde, glacial acetic acid and Leishman’s stain were purchased from Merck.

**Animals**

Mice (AKR strain) were bred and maintained at 25 ± 2°C under a 12 h light/12 h dark schedule with ad libitum supply of standard mices feed and drinking water. AKR mice were used in the present study because of their susceptibility to lymphoma and short lifespan. Adult male mice (15–20 weeks old) were used in the experimental work. Animal experiments were performed with the approval of the Institutional Animal Ethics Committee, Jiwaji University, Gwalior, India.

**Induction of lymphoma into mouse**

Lymphoma was introduced into the adult (15–20 weeks old, weighing 30 ± 2 g) male mouse by serial transplantation of live DLAT (Dalton’s lymphoma ascites transplanted) cells. About 1 × 10⁶ ascites cells in PBS (pH 7.4) were introduced intraperitoneally to each mouse as described previously [31,38].

**Treatment of queuine to cancerous mouse**

DLAT cancerous mice were treated with exogenous administration of queuine. Queuine was dissolved in autoclaved 0.9% NaCl and used for treatment of DLAT cancerous mice. DLAT cancerous mice were divided in four groups. Three groups were treated with different doses of queuine: 12.5 μg (0.416 mg/kg), 25 μg (0.832 mg/kg) and 50 μg (1.66 mg/kg) at 24 h intervals to each mouse per day intraperitoneally for 18 days consecutively, and the control group received 0.9% NaCl in a similar manner. In addition, one group of normal mice (15–20 weeks old, 30 ± 2 g) was used without any treatment. The doses of queuine were selected on the basis of previous work [22].

All the groups of mice were killed on day 19 of DLA transplantation by cervical dislocation, because the DLAT cancerous mouse survives for 21 ± 2 days after induction of lymphoma. The liver was excised on ice immediately after killing the animal, washed in 0.9% NaCl and used for further study.

**Lymphocyte staining**

Lymphocytes of normal, DLAT cancerous and queuine-treated DLAT cancerous mice were stained with Leishman’s stain following the method of Haybae and Flemens [39]. A blood smear was prepared on a glass microscope slide, air-dried and stained.
with Leishman’s stain for 45 s. The slides were incubated in 50 mM potassium phosphate buffer (pH 7.0) for 5–7 min, rinsed with distilled water, air-dried and mounted with 1.3-diethyl-8-phenylxanthine.

**Histopathological analyses of liver**

Histopathological analyses were carried out according to the method of McManus and Mowrey [40]. Liver was fixed in freshly prepared Bouin’s fixative, dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. Tissue sections were cut with a thickness of 4 μm using an automated microtome (Leica). The sections were fixed on glass slides (76 mm × 26 mm) and incubated at 40°C overnight. The sections were deparaffinized in xylene and hydrated in decreasing concentrations of ethanol and stained with haematoxylin and eosin staining solution using an automated histopathological slide-staining machine (Bio-Rad).

**Assay of antioxidant enzymes**

Spectrophotometric and activity gel assays were used to measure the activity of the antioxidant enzymes catalase, SOD, GPX and GR. The enzyme activities of catalase, SOD, GPX and GR were determined in the livers of normal, DLAT cancerous and queuine-treated (12.5 μg, 25 μg and 50 μg) DLAT cancerous mice. Activity is expressed as units of enzyme/ml of extract and specific activities as units/mg of protein.

**Catalase**

The tissue was homogenized in 50 mM potassium phosphate buffer, pH 7.4, containing 1 mM PMSF in a cold-room maintained at 4 ± 2°C and centrifuged at 12,000 g for 30 min at 4°C. The supernatant was collected and used to determine catalase activity. Total protein was estimated using the method of Bradford [41]. The catalase activity was measured spectrophotometrically according to the method of Aebei [42] with minor modifications. The reaction was performed in a final volume of 1 ml containing total cellular protein (15 μg) and 20 mM hydrogen peroxide in 50 mM potassium phosphate buffer (pH 7.0). The rate of hydrogen peroxide decomposition was followed by monitoring the absorbance (A) at 240 nm. The activity of catalase was calculated by using a molar absorption coefficient of 43.6 M−1·cm−1 for hydrogen peroxide.

The activity assay of catalase was analyzed by non-denaturing PAGE, followed by the ferricyanide method as described previously [43]. Protein from each sample was equally loaded and separated by non-denaturing PAGE (7 % gels) at 4°C. The gel was stained in a staining solution containing 1 % ferric chloride and 1 % potassium ferrocyanide for 4–5 min at room temperature (25°C) in the dark. Catalase activity was shown by the appearance of transparent bands on the dark-blue background of the gel. A few drops of 1 M HCl were added after the appearance of bands to stop the reaction. The intensity of bands was analyzed by densitometric scanning using an Alpha Image Analyser System (Alpha Innotech, San Leandro, CA, U.S.A.).

**SOD**

SOD activity was determined in the same cytosolic fraction used to determine the activity of catalase. The enzycomic activity of SOD was measured spectrophotometrically according to the method of Winterbourn et al. [44] with minor modifications. The reaction mixture was prepared in a final volume of 1 ml containing 67 mM potassium phosphate buffer (pH 7.8), 100 mM EDTA, 300 mM sodium cyanide, 0.12 mM riboflavin, 1.5 mM NBT and different concentrations of protein (0.2–20 μg). The reaction mixture was incubated under fluorescent light for 10 min at room temperature and the absorbance (A) was measured at 560 nm. One unit of activity was defined as the amount of enzyme causing half-maximal inhibition of NBT reduction.

The activity gel assay of SOD was performed following the procedure of Beauchamp and Fridovich [45] with minor modifications. Equal amounts of protein were loaded from each sample and separated by non-denaturing PAGE (10 % gels) at 4°C. The gel was then soaked in 1.23 mM NBT solution for 20 min in the dark, washed with distilled water and incubated for 15–20 min in the dark in 100 mM potassium phosphate buffer (pH 7.0) containing 28 mM TEMED (N,N′,N′,N′-tetramethylethylenediamine) and 0.28 mM riboflavin. The gel was subsequently exposed to fluorescent light until achromatic bands were observed.

**GPX**

GPX activity was determined following the method of Paglia and Valentine [46] with minor modifications. The tissue was homogenized in homogenization buffer containing 10 mM Tris/HCl (pH 7.5), 0.25 M sucrose, 0.5 mM EDTA, 0.5 mM DTT (dithiothreitol) and 1 mM PMSF in a cold-room maintained at 4 ± 2°C. The homogenate was centrifuged at 12,000 g for 30 min at 4°C. The supernatant was collected and used to determine the enzyme activity of GPX by using an indirect-coupled test procedure. As GS(GSH is produced on reduction of an organic peroxide by GPX and is recycled to its reduced state with oxidation of NADPH by GPX and is recycled to its reduced state with oxidation of NADPH by GR, the oxidation of NADPH was therefore monitored at an absorbance (A) of 340 nm to determine GR activity. The reaction mixture was prepared in a final volume of 1 ml containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide, 0.5 mM NADPH, 0.2 mM GSH and 1 unit of GR. The protein sample (50 μg) and 0.1 mM hydrogen peroxide were added to the reaction mixture and the absorbance (A) was recorded at 340 nm. The activity of GPX was calculated by using a molar absorption coefficient of 0.00622 M−1·cm−1 for NADPH.

The activity assay of GPX was performed using the method of Lin et al. [47]. GPX activity was determined by non-denaturing PAGE (10 % gels) at 4°C. After completion of electrophoresis, the gel was submerged in 50 mM Tris/HCl buffer (pH 7.9) containing 13 mM GSH and 0.01 % hydrogen peroxide. The gel was then placed in a staining solution containing 1.2 mM MTT and 1.6 mM PMS at room temperature in the dark. The gel was subsequently exposed to light until GPX was detected (indicated by the appearance of a clear zone on the gel).
GR assay was carried out in the same cytosolic fraction used to determine the activity of GPX. GR activity was measured spectrophotometrically by monitoring the rate of oxidation of NADPH by GSSG according to the method of Horn [48] with minor modifications. The reaction mixture was prepared in a final volume of 1 ml containing 50 mM potassium phosphate buffer (pH 7.0), 12 mM NaHCO₃, 0.5 mM EDTA, 0.5 mM 2-mercaptoethanol, 1.5 mM GSSG and 0.2 mM NADPH. The protein sample (50 µg) was added to the reaction mixture and the absorbance (A) was recorded at 340 nm. The activity of GR was calculated by using the molar absorption coefficient for NADPH (see above).

The activity gel assay of GR was performed following the method of Hou et al. [49] with minor modifications. GR activity was determined by non-denaturing PAGE (7.5 % gels) at 4 °C. After completion of electrophoresis, the gel was submerged in 50 mM Tris/HCl buffer (pH 7.9) containing 4 mM GSSG, 2 mM NADPH and 2.5 mM DTNB for 30 min at room temperature. The gel was then subjected to a staining solution containing 1.2 mM MTT and 1.6 mM PMS for 10–15 min at room temperature in the dark. The gel was then exposed to light until the GR activity was detected (indicated by the appearance of a clear zone on the gel).

Statistical analyses
Statistical analyses were performed by ANOVA, followed by the Bonferroni t test to evaluate the significant difference between the different groups, and comparison was made with the DLAT control. Results are means ± S.E.M. (n = 3). P < 0.05 was considered to be statistically significant (*). The significant difference was determined by using statistical analysis software [Sigma Stat version 2.0 for Windows (Integrated Bio-system, Varanasi, India)].

RESULTS

Characterization of Dalton’s lymphoma
Dalton’s lymphoma is a T-cell lymphoma. The number of lymphocytes is found to be increased in the DLAT cancerous mouse compared with the normal mouse (Figure 1). Induction of lymphoma with serial transplantation of live ascites cells into an adult mouse leads to the development of lymphoma, with accumulation of ascites fluid in the peritoneum and angiogenesis is observed. The body mass of the mouse is increased, and the liver is found to be increased in size. The life-span of a DLAT cancerous mouse is found to be 21 ± 2 days after induction of lymphoma; however, the normal mouse lives for about 18 months. Histopathological details of the liver show that hepatocytes are closely packed and well arranged in the normal mouse. However, in the DLAT cancerous mouse liver, dividing cells are present and hepatocytes are impaired in shape and arrangement. The nucleus is enlarged, the number of sinusoids is increased and there is infiltration of a large number of blood cells (lymphocytes) into the liver (Figure 2). The effect of queuine on in vitro and in vivo cell proliferation has been reported previously by Pathak et al. [31].

Effect of queuine on activities of antioxidant enzymes
Spectrophotometric assays show that the activity and specific activity of catalase is decreased to approx. 33 % in the DLAT cancerous mouse liver compared with normal mouse liver. Treatment of the DLAT cancerous mouse with 12.5 µg, 25 µg and 50 µg doses of queuine leads to an elevation in catalase activity to approx. 1.2-, 1.7- and 2-fold respectively compared with the non-treated DLAT cancerous mouse (Figure 3a). The activity gel assay shows that catalase activity is low in the DLAT cancerous mouse liver compared with normal mouse liver; however, queuine treatment of the DLAT cancerous mouse is found to increase the catalase activity gradually (Figures 3b and 3c). SOD activity and specific activities in the DLAT cancerous mouse liver are found to be decreased to approx. 27 % compared with normal mouse liver. Queuine treatment of the DLAT cancerous mouse promotes the activity of SOD gradually to 1.1-, 1.9- and 2.5-fold compared

Figure 1 Lymphocytes from mouse blood stained by Leishman's stain
Mouse blood from (a) normal mouse and (b) DLAT cancerous mouse at ×40 magnification is shown. The arrows indicate the presence of lymphocytes.
Effect of queuine on antioxidant defence system

Figure 2 Transverse sections of mouse liver stained with haematoxylin and eosin
Liver sections from (a) normal mouse and (b) DLAT cancerous mouse at ×40 magnification are shown, with arrows indicating an enlarged nucleus (1), dividing cells (2) and infiltration of blood cells (3).

with the non-treated DLAT cancerous mouse (Figure 4a). The native gel assay shows low activity of both SOD isoenzymes, i.e. MnSOD and CuZnSOD, in the DLAT cancerous mouse liver compared with normal mouse liver. However, queuine treatment enhances MnSOD and CuZnSOD activities (Figures 4b and 4c). GPX activity is found to be decreased to approx. 45% in the DLAT cancerous mouse liver compared with normal mouse liver. Queuine treatments promote the activity and specific activity to approx. 1.2-, 1.5- and 1.7-fold respectively compared with the non-treated DLAT cancerous mouse (Figure 5a). The activity gel assay shows a decline in GPX activity in the DLAT cancerous mouse liver compared with normal mouse liver, and this activity increased with queuine treatment (Figures 5b and 5c). Similarly GR activity is low in the DLAT cancerous mouse liver, approx. 30% compared with normal mouse liver. Treatment with 12.5 µg, 25 µg and 50 µg queuine elevated GR activities to 1.2-, 1.7- and 2-fold respectively compared with the DLAT cancerous mouse (Figure 6a). The activity gel assay shows lower GR activity in the DLAT cancerous mouse liver compared with normal mouse liver. Treatment with 25 µg and 50 µg queuine promoted GR activity compared with the untreated DLAT cancerous mouse liver (Figures 6b and 6c).

As seen by the activity assays, the enzyme activities of catalase, SOD, GPX and GR are low in the DLAT cancerous mouse liver compared with normal mouse liver. These enzyme activities are found to be increased after queuine treatment of the DLAT cancerous mouse. In summary, queuine promotes the activity of antioxidant enzymes such as catalase, SOD, GPX and GR.

DISCUSSION

The generation of ROS in normal cells is under tight homeostatic control. The steady-state formation of free radicals (pro-oxidants)
Figure 4 SOD activity in the liver of normal, DLAT cancerous and queuine-treated DLAT cancerous mice
Livers from normal mice, DLAT cancerous mice and DLAT cancerous mice treated with queuine (Q) (12.5, 25 and 50 µg) were examined for SOD activity (see the Materials and methods section). (a) Determination of the activity and specific activity of SOD. U, units. (b) Non-denaturing PAGE (7.5% gels) stained by activity staining. (c) Densitometric scanning of MnSOD and CuZnSOD. Results are means ± S.E.M. *P < 0.05 compared with the non-treated DLAT cancerous liver sample.

Figure 5 GPX activity in the liver of normal, DLAT cancerous and queuine-treated DLAT cancerous mice
Livers from normal mice, DLAT cancerous mice and DLAT cancerous mice treated with queuine (Q) (12.5, 25 and 50 µg) were examined for GPX activity (see the Materials and methods section). (a) Determination of the activity and specific activity of GPX. U, units. (b) Non-denaturing PAGE (7.5% gels) stained by activity staining. (c) Densitometric scanning of isoenzymes 1 and 2 of GPX. Results are means ± S.E.M. *P < 0.05 compared with the non-treated DLAT cancerous liver sample.

is normally balanced by a similar rate of consumption by antioxidants. An imbalance between ROS production and antioxidant defence results in an increased level of ROS, causing oxidative stress, which leads to various pathological conditions, including metabolic dysfunction, cardiovascular diseases, neurodegenerative diseases, cancer and premature aging [6]. ROS are transient species as a result of their high chemical reactivities, which may lead to oxidative damage and promote tumorigenesis [5]. The level of ROS increases with promotion of malignancy. Accumulation of ROS leads to a decrease in the antioxidant enzyme activities. Previous reports have suggested that the activities of antioxidant enzymes are low in most animal and human cancers [11,12]. Therefore any agent leading to an improvement of the activities of antioxidant enzymes in malignancy would contribute to anti-carcinogenic effects, as antioxidants are one of the major regulating agents protecting the cell from oxidative damage and removal of ROS.

Queuine is a nutrient factor for eukaryotes and is present in mammalian systems as a free base or in a bound state with tRNA, and participates in various cellular functions. Previous work has suggested that it has an important role in the regulation of cell proliferation. Queuosine deficiency of tRNA is associated with the promotion of malignancy. Previous reports have suggested that the tRNA is found to be hypo-modified in cancer cells; however, exogenous administration of queuine improves modification of Q-tRNA [21–23]. Queuine is found to down-regulate phosphorylation of tyrosine phosphoproteins and participates in cell signalling [37,38]. Consequences of oxidative stress include modification of cellular proteins, lipids and DNA. When oxidative stress is severe, survival is dependent on the ability of the cell to adapt to or resist stress, and to repair or replace the damaged molecules. Ultimately cells may respond to the insult by undergoing cell death. Cell death or apoptosis is under control of genes of the Bcl-2 family. Queuine down-regulates the level of the anti-apoptotic protein Bcl-2, thereby stimulating apoptosis to inhibit cell proliferation [31]. Cancer cells are known to be active in energy metabolism. Thus they produce high levels of ROS, promoting oxidative stress and malignancy. Elevated levels of ROS leads to over-expression of c-fos, AP-1 (activator protein 1) and ras genes, which ultimately stimulates cell proliferation [50]. High levels of ROS suprpresses the intracellular antioxidant defence system by decreasing antioxidant enzyme activities. The activities of antioxidant enzymes such as catalase, SOD,
GPX and GR are found to be low in the DLAT cancerous mouse liver compared with normal mouse liver. Queuine improves the antioxidant defence system by promoting antioxidant enzyme activities. Therefore it is proposed that increased activity of antioxidant enzymes may reduce oxidative stress, which probably contributes to check over-expression of oncogenes such as c-fos, AP-1 and ras. We have analysed the level of proto-oncogenes and found that the levels are high in the DLAT cancerous mouse liver compared with normal mouse liver. The levels of c-Myc and c-Fos were found to decrease on treatment of the DLAT cancerous mouse with queuine [36]. Thus queuine has an important role in the regulation of various cellular functions. The present study focuses on the effect of queuine on regulation of the activities of antioxidant enzymes, such as catalase, SOD, GPX and GR, in cancer. Catalase is a highly conserved haem-containing antioxidant enzyme known for its catalytic ability to degrade hydrogen peroxide into water and oxygen as well as to oxidize low-molecular-mass alcohols to aldehyde and water. Kehrer [51] has suggested that the enzyme protects cells by removing hydrogen peroxide produced by flavin-containing oxidases in the peroxisomes, thereby preventing the accumulation of toxic levels of this reactive intermediate. Catalase activity is found to be low in the DLAT cancerous mouse liver compared with normal mouse liver. Queuine treatment of the DLAT cancerous mouse gradually improves catalase activity. The activity of catalase is reported to be low in Morris hepatomas, Lewis lung carcinomas and the tumorigenic hamster kidney, as well as in various tumour cells and human cancers [52,53]. In mammalian cells, the catalase monomer binds to one molecule of haem; the holoenzyme also binds to two molecules of NADPH and maintains catalase in an active state. NADPH may protect the enzyme from inactivation by hydrogen peroxide [54]. High oxidative stress in the DLAT cancerous mouse may decrease cellular NADPH levels, lifting the protection of catalase by hydrogen peroxide. In turn, catalase may be inactivated partially in the DLAT cancerous mouse. SOD catalyses the dismutation of the superoxide radical into hydrogen peroxide, which is further metabolized to water and oxygen by catalase and GPX. Therefore the variations in the activities of these two antioxidant enzymes are expected to be similar. The decreased activities found in the DLAT cancerous mouse may be contributing to the development of lymphoma. Previous studies demonstrated a lower activity of catalase and SOD in tumours as a result of accumulation of free radicals, inhibiting antioxidant activity [11,55]. MnSOD activity is reduced in a variety of tumour types. Increased susceptibility of MnSOD to oxidative stress is due to mitochondrial dysfunction, resulting in elevated ROS levels in tumours [56]. CuZnSOD activity is reported to be low in malignant cells compared with normal cells [57]. In the present study, the activities of both isoenzymes of SOD are low in the DLAT mouse liver compared with normal mouse liver. Queuine treatment of the DLAT cancerous mouse elevates SOD activity. Glutathione is one of the most abundant thiol-containing molecules in animal cells and plays an important role in the protection of tissues from the toxic effects of xenobiotics and endogenous electrophiles [58]. Glutathione and glutathione-metabolizing enzymes protect mammalian cells against oxidative and alkylating agents. GPX is a selenium-containing enzyme which catalyses the reduction of a variety of hydroperoxides and lipid peroxides using glutathione and other cofactors [59]. GPX protects haemoglobin in red blood cells from oxidative degradation [60]. Five different isoenzymes of GPX are found in the mammalian system. Their mRNA expression and protein level varies depending on the tissue type. The activity of glutathione peroxide activity is found to be low in the DLAT cancerous mouse liver compared with normal mouse liver. The enzyme activities are improved in the DLAT cancerous mouse liver with queuine treatment. Overexpression of GPX is reported to rescue the growth of tumour cells [61]. Similarly, GR is a ubiquitous enzyme which catalyses the reduction of GSSG to GSH. GR is essential for the glutathione redox cycle that maintains an adequate level of cellular GSH. Low levels of GSH triggers expression of oncogenes such as AP-1, JNK (c-Jun N-terminal kinase), MAPK (mitogen-activated protein kinase) and p38 [62]. GR is also sensitive to oxidative damage. Low activity of GR is found in DLAT cancerous mouse liver compared with...
normal mouse liver, which is enhanced with queuine treatment of the DLAT cancerous mouse. Improvement in the activities of antioxidant enzymes by queuine probably contributes to decreased oxidative stress which, in turn, may check oxidation-induced damage of macromolecules such as lipid peroxidation, protein carbonylation and DNA damage. Thus queuine may promote anti-carcinogenic action in the DLAT cancerous mouse.

The promotion of the antioxidant defence system by queuine administration may be brought about by queuine or Q-tRNA modification. However, the possibility of regulation of antioxidant enzyme activities by queuine as a base cannot be ruled out. Further study is required to understand the mechanism of regulation by queuine. On the basis of these results, the use of queuine as an antioxidant compound may be suggested to protect the antioxidant defence system during malignancy.

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