Temporal Changes in Tissue Repair Permit Survival of Diet-Restricted Rats from an Acute Lethal Dose of Thioacetamide

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Although, diet restriction (DR) has been shown to substantially increase longevity while reducing or delaying the onset of age-related diseases, little is known about the mechanisms underlying the beneficial effects of DR on acute toxic outcomes. An earlier study (S. K. Ramaiah et al., 1998, Toxicol. Appl. Pharmacol. 150, 12-21) revealed that a 35% DR compared to ad libitum (AL) feeding leads to a substantial increase in liver injury of thioacetamide (TA) at a low dose (50 mg/kg, ip). Higher liver injury was accompanied by enhanced survival. A prompt and enhanced tissue repair response in DR rats at the low dose (sixfold higher liver injury) occurred, whereas at equitoxic doses (50 mg/kg in DR and 600 mg/kg in AL rats) tissue repair in AL rats was substantially diminished and delayed. The extent of liver injury did not appear to be closely related to the extent of stimulated tissue repair response. The purpose of the present study was to investigate the time course (0-120 h) of liver injury and liver tissue repair at the high dose (600 mg TA/kg, ip, lethal in AL rats) in AL and DR rats. Male Sprague-Dawley rats (225-275 g) were 35% diet restricted compared to their AL cohorts for 21 days and on day 22 they received a single dose of TA (600 mg/kg, ip). Liver injury was assessed by plasma ALT and by histopathological examination of liver sections. Tissue repair was assessed by 3H-thymidine incorporation into hepatonuclear DNA and proliferating cell nuclear antigen (PCNA) immunohistochemistry during 0-120 h after TA injection. In AL-fed rats hepatic necrosis was evident at 12 h, peaked at 60 h, and persisted thereafter until mortality (3 to 6 days). Peak liver injury was approximately twofold higher in DR rats compared to that seen in AL rats. Hepatic necrosis was evident at 36 h, peaked at 48 h, persisted until 96 h, and returned to normal by 120 h. Light microscopy of liver sections revealed progression of hepatic injury in AL rats whereas injury regressed completely leading to recovery of DR rats by 120 h. Progression of injury led to 90% mortality in AL rats vs 30% mortality in DR group. In the surviving AL rats, S-phase DNA synthesis was evident at 60 h, peaked at 72 h, and declined to base level by 120 h, whereas in DR rats S-phase DNA synthesis was evident at 36 h and was consistently higher until 96 h reaching control levels by 120 h. PCNA studies showed a corresponding increase in cells in S and M phase in the AL and DR groups. DR resulted in abolition of the delay in tissue repair associated with the lethal dose of TA in ad libitum rats. Temporal changes and higher tissue repair response in DR rats (earlier and prolonged) are the conduits that allow a significant number of diet restricted rats to escape lethal consequence.

Diet is one of the most important components of our environment that impacts public health. Diet restriction (DR) alters a number of key biological processes in such a way as to promote good health, decrease disease, and increase longevity including cancer (Allaben et al., 1990; Masaro, 1988; Weindruch and Walford, 1992; Hass et al., 1996). A variety of mechanisms such as DNA repair, drug metabolism, immune function, hormonal regulation, and cytoprotection against free radical injury have been invoked for the beneficial effects of DR (Turturro et al., 1993; Hart et al., 1992, 1995). Increased incidence of apoptosis as a mechanism for protection from DNA damage-inducing agents and disease by eliminating mutated cells (James and Mushkhillishvili, 1994), increased fidelity of DNA replication (Srivastava et al., 1993), lowered oncogene expression and preserved inducible cellular responses (Himeno et al., 1992), and increased repair of various forms of DNA damage are examples of mechanisms invoked for the beneficial effects of DR (Busbee et al., 1995). Toxicity of chemotherapeutic agents such as ganciclovir (Berg et al., 1994) and isoproterenol (Duffy et al., 1995) has been shown to be decreased by diet restriction. In light of the potential exposure to hepatotoxins understanding the mechanisms and the impact of these changes induced by diet restriction are important to better extrapolate to man the results of toxicity tests in animals, thereby allowing better assessment of risk from exposure to environmental toxicants, especially under the conditions of human dietary ecology. However, the mechanisms by which restricted human diet may modulate the toxicity of

2 Abbreviations used: AL, ad libitum; ALT, alanine aminotransferase; DR, diet restriction; GSH, glutathione; 3H-T, 3H-thymidine; PCNA, proliferating cell nuclear antigen; TA, thioacetamide; EGFR, epidermal growth factor receptor; STAT, signal transducers and activators of transcription.

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environmental toxicants are not understood. While studies have shown that nutritional status is an important factor in determining susceptibility to toxic chemicals (Chanda and Mehendale, 1996a), it is also known that survival after hepatocellular injury and necrosis may depend on the ability of the remaining hepatocytes to divide and restore an adequate population of functioning cells (Chanda and Mehendale, 1994, 1996b). Several studies have established the critical role of liver cell division and compensatory tissue repair (Calabrese and Mehendale, 1996; Mehendale, 1991; Mehendale et al., 1994). Thioacetamide, originally used as a fungicide, is a potent model hepatotoxicant and exposure to environmental agents like this is known to cause liver injury leading to hepatitis and fulminating hepatic failure (Mehendale et al., 1997).

Our earlier studies showed that low-dose thioacetamide (50 mg/kg ip) results in an enormous increase in liver injury in DR rats (Ramaiah et al., 1998). However, there was a higher and a timely enhancement of tissue repair response in DR group. Equitoxic dose (50 mg TA/kg in DR and 600 mg TA/kg in AL rats) studies revealed that despite equal liver injury, DR rats were able to mount an adequate tissue repair response allowing them to escape death. The purpose of this study was to investigate if the mechanism of increased cell proliferation response seen at the low dose of TA was also operating at the lethal dose to account for increased survival in DR rats. Since the low dose of TA caused a 6-fold higher liver injury in DR rats, it was of interest to investigate the extent of liver injury at the lethal dose (which is 12-fold higher) in DR and AL rats and to investigate the accompanying tissue repair. We report here that enhanced responsiveness of the liver to regenerate and replace dead cells in DR rats allows animal survival despite substantially higher mechanism-driven infliction of liver injury. Since DR is considered as a lifestyle option to prolong disease-free life, these findings may substantially impact the outcome-based risk assessment of human exposure to environmental hepatotoxicants, under varied diet regimens such as voluntary diet restriction.

METHODS

Chemicals. Thioacetamide and [3H-methyl]thymidine (3H-T, sp act 1.7 Ci/mmol) were obtained from Sigma Chemical Company (St. Louis, MO). The scintillation fluid (Scintiverse SX 16-4) was purchased from Fisher Scientific Co (Pittsburgh, PA). All other biochemicals and chemicals were obtained from Sigma.

Animals and treatment. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc. Indianapolis, IN; 225–275 g, 8–9 weeks of age) were maintained in our central animal facility. They were housed individually over sawdust bedding known to be free of any chemical contamination. The ad libitum group had free access to water and normal rodent chow (Harlan Teklad Rat Chow No. 7001, Madison, WI; protein 25%, fat 4.25%, fiber 4.67%, vitamins and minerals supplemented, calories 3.94 kcal/g) at all times. The rats in the diet-restricted group were allowed 65% (17.7 g/day) of the ad libitum daily food consumption (27.2 ± 5.4 g/day) for a period of 21 days and maintained on the same regimen during and after TA treatment (Ramaiah et al., 1998).

Food consumption of the DR group was adjusted relative to that observed in AL group twice weekly. Forty rats were diet restricted individually in two batches of 20 in our central animal facility for time course studies (0–120 h, n = 4 for each time point). The first batch of 20 rats was used for 0-, 12-, 24-, 36-, and 48-h time course studies. Sixteen rats from the second batch of 20 rats were used for the remaining time points (60, 72, 96, and 120 h). On day 22 of the diet restriction regimen they were used for TA treatment. The AL (n = 32) and DR (n = 32) rats received a single ip injection of TA (600 mg/kg) dissolved in normal saline (0.9% NaCl). The respective controls received only the saline (1 ml/kg) vehicle. The plasma and liver tissue from these rats was used for further studies. Since administration of 600 mg TA/kg resulted in mortality in the AL group at 72 h and after, the surviving rats were used to obtain plasma and liver tissues for various parameter estimations.

Plasma enzymes. Blood was collected in heparinized tubes from the dorsal aorta of rats under diethyl ether anesthesia at 0, 12, 24, 36, 48, 60, 72, 96, and 120 h after TA or vehicle administration, and the plasma was separated by centrifugation to estimate alanine aminotransferase (ALT; EC 2.6.1.2.) as marker of liver injury using Sigma kit No. 59 UV (ALT, Sigma).

Histopathology. Livers were surgically removed from rats under diethyl ether anesthesia. Portions of liver from each rat collected at various periods after TA treatment were washed with ice-cold normal saline (0.9% NaCl), cut into small slices, and fixed in 10% phosphate-buffered formaldehyde fixative for 48 h. The tissues were then transferred to 70% ethyl alcohol, processed, and embedded in paraffin. Liver sections (5 μm thick) were stained with hematoxylin–eosin (H & E) for histological examination under a light microscope. Unstained liver sections were prepared for proliferating cell nuclear antigen immunohistochemistry on these slides.

In vivo incorporation of [3H]thymidine into rat liver nuclear DNA. Stimulation of S-phase synthesis was quantified by 3H-T incorporation into hepatonuclear DNA using the procedure of Chang and Looney (1965) and Chauveau et al., (1956). The DNA content was estimated according to the method by Burton (1956). Since 3H-T incorporation study may indicate DNA repair instead of DNA synthesis, proliferating cell nuclear antigen assay was used to confirm S-phase stimulation and cell cycle progression.

Proliferating cell nuclear antigen (PCNA) assay. The proliferating cell nuclear immunohistochemical analysis was conducted as described by Greenwell et al. (1991). Briefly, the liver sections mounted on glass slides were first blocked with casein and then reacted with monoclonal antibody to PCNA (Dako Corporation, Carpinteria, CA). The antibody was then linked with biotinylated goat anti-mouse IgG antibody (Boehringer/Mannheim, Indianapolis, IN), which was then labeled with streptavidin-conjugated peroxidase (Jackson Immunoresearch, West Grove, PA). Color was developed by exposing the peroxidase-labeled streptavidin to diaminobenzidine, which forms a brown reaction product. The sections were then counterstained with Gill’s hematoxylin. G0 cells were blue and did not take the PCNA stain, G1 cells were light brown in color, S-phase cell nuclei stained dark brown, G2 cells had cytoplasmic staining with or without a speckled nuclear appearance, and M cells were visualized as mitotic figures. PCNA studies were conducted in order to confirm stimulated cell division and cell cycle progression suggested by 3H-T incorporation studies. Each liver section was quantified for different cells in the cell cycle by counting 1000 cells in 10 high-power fields (Kulkarni et al., 1996; 1997; Rao et al., 1996).

Liver glycogen levels. Total hepatic glycogen was estimated as marker of hepatocellular injury using Sigma kit No. 59 UV (ALT, Sigma). The acceptable level of statistical significance was established at the criterion of P ≤ 0.05.

3 Doses that induce equivalent acute hepatic injury (enzyme elevations and relative degrees of necrosis).
RESULTS

Lethality study. In the AL group 90% mortality occurred whereas 30% mortality was recorded in the DR rats. Deaths occurred between 3 and 6 days (average time of death, 4.5 days) in AL-fed rats whereas they occurred between 5 and 6 days in DR rats (average time of death, 5.5 days; Ramaiah et al., 1998).

Plasma enzyme elevations. ALT was estimated as a marker of liver injury over a time course (0–120 h) after TA administration in the DR and AL group. Results are expressed as means ± SE (n = 4). *Values significantly different from the AL group at the corresponding time point. #Values significantly different from control AL group. †Values significantly different from control DR group.

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TABLE 1 Effects of Diet Restriction on Hepatic Injury of Thioacetamide (600 mg/kg)

**Note.** A single dose of thioacetamide was administered ip to male Sprague-Dawley rats (225–275 g) in saline (1 ml/kg).

* Lesions scored as multifocal necrosis and hepatitis. Scoring: 0, no necrosis; +1, minimal, defined as only occasional necrotic cells in any lobule; +2, defined as less than one-third of the lobular structure affected; +3, moderate, defined as between one-third and two-thirds of the lobular structure affected; +4, severe, defined as greater than two-thirds of the lobular structure affected; +5, more severe, defined as damage to most of the parenchyma of the liver.
FIG. 2. Representative liver histopathology during a time course after administration of thioacetamide (600 mg/kg, ip) in diet-restricted and ad libitum rats. (A) Photomicrographs of liver sections from ad libitum rats at 12, 36, 48, 60, 96, and 120 h after 600 mg/kg TA treatment. (B) Photomicrographs of liver sections from diet restricted rats at 12, 36, 48, 60, 96, and 120 h after 600 mg TA/kg treatment. c, central vein; p, pyknotic nuclei; v, vacuolization; n, areas of necrosis.

DNA synthesis observed in AL rats was abolished in DR rats. The deficiency of tissue repair response corresponds with the mortality pattern in ad libitum rats whereas prompt and consistent tissue repair in DR rats is concordant with substantial survival (70%) in DR rats.

Proliferating cell nuclear antigen assay. $^3$H-T incorporation studies (Fig. 3) were further corroborated by PCNA immunohistochemical staining procedure (Figs. 4 and 5). Normally most cells are in resting phase and a relatively small proportion of cells are in other phases of cell cycle with about 3–4% in the G₂ phase. Lethal dose of TA administration to DR rats resulted in a prompt and continuous cell cycle progression resulting in a large number of cells in G₁ at 24 h and proportional increase in S-phase cells starting from 36 h and thereafter until 96 h, finally resulting in mitosis as evidenced by mitotic figures at and beyond 72 h. An increased number of cells in S-phase was evident in AL rats at 60 h and peaked at 72 h. However, at later time points number of S-phase cells did not increase. The maximum number of cells in S-phase was seen at 72 h in both AL and DR rats by this method, in agreement with the wave of S-phase stimulation in $^3$H-T incorporation observed at 72 h, after TA administration in DR and AL rats. Most of the cells had progressed to the M phase by 120 h in DR rats while in AL rats this was minimal to none. This was evidenced by predominant number of G₀ cells in AL rats at 120 h. It should be noted that wave of S-phase stimulation was apparent at 36 h in the DR rats while there was a significant delay in the appearance of these cells in the AL rats until 60 h. Thus, diet restriction caused the abolition of delay in tissue repair response induced by lethal dose of TA in ad libitum rats, as evidenced by $^3$H-T incorporation and PCNA data.

Liver glycogen levels. Liver injury results in breakdown of glycogen causing a significant reduction in glycogen stores. As delayed and was evident at 60 h, peaked at 72 h, and returned to control levels by 120 h (two of four deaths at this time point) in the AL rats. Although the peak of S-phase DNA synthesis was higher than DR rats, the peak was delayed leading to progression of injury characteristic of fulminant hepatic failure. In contrast, DR rats showed an enhanced S-phase DNA synthesis which occurred at 36 h and persisted until 96 h before it returned to control levels by 120 h. The delay in S-phase DNA synthesis observed in AL rats was abolished in DR rats. The deficiency of tissue repair response corresponds with the mortality pattern in ad libitum rats whereas prompt and consistent tissue repair in DR rats is concordant with substantial survival (70%) in DR rats.

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FIG. 4. Results of the proliferating cell nuclear antigen (PCNA) study following treatment with 600 mg/kg TA in ad libitum and diet-restricted rats. (A) Representative photomicrographs of liver sections from ad libitum rats at 0 (control), 12, 36, 60, 72, and 120 h after thioacetamide treatment. (B) Representative photomicrographs of liver sections from diet-restricted rats at 0 (control), 12, 36, 60, 72, and 120 h after thioacetamide treatment. Details of cell types are described under Methods.

anticipated, DR alone resulted in significant reduction in glycogen stores (Fig. 6). TA administration is known to cause depletion of hepatic glycogen (Mehendale et al., 1997). Since tissue repair involves replacement of dead cells by newly divided cells, energy utilization occurs and thus glycogen depletion occurs. A significant reduction in glycogen levels was evident in AL rats at 12 h and the decrease continued until 72 h before glycogen levels were restored by 96 h in surviving

FIG. 5. Graphical representation of cell cycle progression as measured by proliferating cell nuclear antigen, immunohistochemical procedure. Percentage was calculated from a total of 1000 viewed cells in the centrilobular region of the liver for each animal. Each time point had four rats per group. Rats received a single dose of 600 mg TA/kg ip. Percentages of cells in different phases of cell cycle were then counted during a time course of 0–96 h. *Significantly different from control (p ≤ 0.05). Control rats received vehicle only (1 ml/kg, ip).
Highly stimulated compensatory tissue repair is a biological response that follows partial hepatectomy or chemical-induced injury (Michalopoulos, 1990; Chanda et al., 1995; Kulkarni et al., 1997). In chemical hepatectomy, the regeneration response is a dose-related event in which cell division and tissue repair occur in a dose-dependent fashion until a threshold is reached, beyond which injury results in lethality due to unrestrained progression of injury (Mangipudy et al., 1995a; Mehendale, 1994; Rao et al., 1997). These studies suggest that prompt and exacting tissue repair plays a critical role in the final outcome of injury and that this biological event is an opposing and parallel response to tissue injury.

Modulation of food intake can influence the development of chronic disease, cancer, and longevity (Hart et al., 1992). Diet restriction enhances liver regeneration in aged animals following partial hepatectomy (Chou et al., 1995), significantly decreases the cardiotoxicity of isoproterenol (Duffy et al., 1995), and decreases the mortality in mice treated with an antiviral drug, ganciclovir (Berg et al., 1994). A number of mechanisms such as increased apoptosis, increase in repair of DNA damage, and increased fidelity of DNA replication have been proposed as the benefits of diet restriction. However, the exact mechanism for the decreased mortality was not investigated (Berg et al., 1994).

In an earlier study we found diet restriction to enhance tissue repair response upon hepatotoxic injury with thioacetamide (Ramaiah et al., 1998). A lethal dose of thioacetamide administration resulted in 30 vs 90% mortality in diet-restricted vs ad libitum fed rats, respectively. Paradoxically, diet restriction substantially increased liver injury of thioacetamide. Tissue repair was also enhanced in the DR rats. Equitoxic dose study revealed that a dose of 600 mg TA/kg in AL rats yields liver injury similar to that observed in DR rats receiving 50 mg TA/kg. However, tissue repair response was quite dissimilar suggesting that higher tissue repair in DR rats was not merely due to higher liver injury. The objective of the present work was to investigate the dynamic relationship between liver injury and tissue repair in rats maintained on DR and AL regimens and challenged with 600 mg TA/kg dose.

Plasma enzyme elevations of ALT revealed an approximately twofold higher peak liver injury in DR compared to the AL rats. Injury progressed in the AL group while it regressed toward normal in the DR rats. Liver necrosis scoring and histomorphometric analysis of H & E-stained liver sections revealed liver necrosis concordant with ALT elevations. ALT levels and histopathology indicated progression of liver injury in AL rats as opposed to regression and recovery in DR rats. The time of mortality in AL rats was concordant with the magnitude of ALT elevation and liver histopathology.

Liver injury as evidenced by ALT and necrosis scoring of H & E-stained liver sections revealed approximately 2-fold higher liver injury in DR rats receiving 600 mg TA/kg. A 6-fold higher liver injury was noted in DR rats receiving a low dose of 50 mg TA/kg (Ramaiah et al., 1998). Interestingly, a 12-fold higher dose did not produce the corresponding increase in liver injury in DR rats. The present study revealed that the peak liver injury in both DR and AL rats was delayed by 12 to 24 h at the higher dose. The peak of liver injury in DR rats at the low dose (Ramaiah et al., 1998) was evident at 24 h while it appeared at 48 h after the high dose, whereas in AL rats it was evident at 36 h at the low dose while it was delayed until 60 h after the high dose. The mechanism underlying this delay in the causation of injury is worthy of experimental scrutiny. A plausible explanation based on substrate inhibition of bioactivation enzymes merits consideration (Scott et al., 1991; Schlosser et al., 1993; Mangipudy et al., 1995a). The mechanism of thioacetamide hepatotoxicity is by the formation of a sulfoxide which is metabolized further to thioacetamide sulfone by cytochrome P450 and flavine-containing monooxygenases (Chieli and Mavaldi, 1984; Hunter et al., 1977). It is possible that 600 mg TA/kg may exert inhibition of the metabolism of sulfoxide to reactive sulfone. Since the enzymes which catalyze the conversion of thioacetamide to sulfoxide also catalyze sulfoxide to sulfone, high thioacetamide concentration reached at 600 mg TA/kg dose may inhibit the formation of reactive species. Lower liver injury at earlier points was also observed in a dose–response study with TA, where liver injury at 600 mg/kg dose was significantly lower at 12 and 24 h compared to that observed after 50, 150, or 300 mg/kg doses (Mangipudy et al., 1995a). Kulkarni et al. (1996) reported similar delay in maximum liver injury in Sprague-Dawley and Fischer 344 rats treated with higher doses of 1,2-dichlorobenzene.
Liver tissue repair measured by S-phase DNA synthesis and cell cycle progression by proliferating cell nuclear antigen immunohistochemistry reveal substantial differences between DR and AL rats. Normally most cells are in the resting phase and a relatively small proportion of cells is in the other phases of cell cycle with about 3–4% in the G2 phase. Following high-dose TA administration, there was a peak of S-phase DNA synthesis at 72 h in the AL rats. However, stimulation of S-phase occurred significantly earlier in DR rats receiving the same dose: the S-phase started at 36 h and persisted until 96 h before declining to control levels by 120 h; PCNA studies revealed corresponding increases in mitotic cells in both groups. A substantial number of mitotic figures was present at 120 h in DR rats while in AL rats the mitotic figures had disappeared by 120 h. The most striking feature from S-phase DNA synthesis and PCNA study was the abolition of delay in tissue repair response in rats maintained on the DR regimen. Thus, recovery from liver injury results from the prompt and sustained liver regeneration response in DR rats. These findings are consistent with other reports of enhanced animal survival if tissue repair is stimulated (Chanda et al., 1995; Mehendale, 1991, 1994; Rao et al., 1997; Mangipudy et al., 1995a, b) and failure to survive even a nonlethal challenge if tissue repair is inhibited (Mangipudy et al., 1996; Rao et al., 1997). Thus, the lethal dose of TA in AL rats was converted to an infraletal dose not because of decreased liver injury but due to enhanced tissue repair response in DR rats. Other possible mechanisms of protection such as higher hepatic GSH may be considered. It is known that hepatotoxicity of TA is enhanced after fasting but not after pretreatment with diethyl maleate, an agent that causes GSH depletion with minimal toxicity (Reed and Fariss, 1984). Moreover, Chanda et al. (1995) have shown that following thioacetamide (50 mg/kg) treatment the levels of GSH and oxidized glutathione were not affected. Since 50 mg/kg TA in DR rats caused the same level of injury as 600 mg/kg in AL rats (equitoxic doses), it is reasonable to suggest that changes in GSH levels after the lethal dose are unlikely to explain the differences between the toxic outcomes in DR and AL rats. Therefore, it is unlikely that increased GSH may play a role in the ability of DR rats to escape mortality. Survival or death thus is a function of timely amplitude (efficiency) of repair response in these animals.

The mechanisms leading to the efficient tissue repair in DR rats are of investigational interest. The ability of the liver to regenerate is regulated by complex signaling and transducing events that depend on the metabolic activity of the liver and the endocrine, autocrine, paracrine, and neural regulatory circuits (Diehl and Rai, 1996; Steer, 1995). Cell cycle check points are recognized as positions of control that ensure the order of events in the cell cycle and that integrate DNA repair with cell cycle progression (Hartwell and Kastan, 1994). Although TA is not known to cause DNA damage, the presence of early signaling mechanisms following DR can be envisaged to enhance cell cycle transition. It is well established that the G0 to G1 transition begins almost immediately after partial hepatectomy (Steer, 1995) and chemical hepatectomy (Mehendale, 1991, 1994), triggered by growth factors already present in blood or liver. Growth factors regulate hepatic regeneration by providing both stimulatory and inhibitory signals for cell proliferation. TGF-α and EGF exert their effects on cells through binding to EGF-receptor (EGF-R), EGF-R dimerization, and autophosphorylation of tyrosine residues (Kumar et al., 1995). Mitogenic growth factors promote G1 phase cell cycle progression by stimulating the formation or activation of cyclin D and cyclin E (Assoian, 1997). Other cellular proteins that transduce cell proliferation signals are signal transducers and activators of transcription (STAT), nuclear factor-κB, and certain CCAAT/enhancer binding proteins which are under regulatory control of proinflammatory cytokines such as tumor necrosis factor-α, interleukin-1 (IL-1), and IL-6 (Diehl and Rai, 1996).

Studies have shown that administration of EGF to mice results in the phosphorylation of tyrosine residues of STAT1α and STAT1β which can be detected within 6 and 20 min after treatment (Zhong et al., 1994). Metallothionein (Waalkes and Goering, 1990; Moffatt et al., 1995) and heat shock proteins (Reddy et al., 1995) are other proteins implicated in cell growth and hepatic regeneration. The later events of cell proliferation like the expression of protooncogenes are also likely candidates for regulation. Early protooncogenes like c-fos and c-myc may be expressed early in DR rats and may be responsible for early and increased tissue repair response. Stimulation of TA-induced cell division by fatty acid supplement has shown to be associated with protooncogene expression (Chanda and Mehendale, 1994, 1996b). Dissecting these signal-transducing events responsible for transforming lethal dose to an infraletal dose in DR rats should help us understand the molecular players for increased tissue repair response in DR rats.

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