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Effects of S-allyl Cysteine on N-Nitrosodiethylamine Induced Hepatocarcinogenesis: Variations in Temporal Patterns of Circulatory Tumor Marker Enzymes

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Abstract: In this study, the protective effects of S-allyl cysteine on the temporal patterns of tumor marker enzymes in N-Nitrosodiethylamine (NDEA)-induced hepatocarcinogenesis were studied. Experimental animals (160-180 g) were divided into control, NDEA (single i.p. injection of 200 mg kg^{-1} body weight followed by weekly subcutaneous injections of 3 mL kg^{-1} body weight CCl_4) treated, NDEA+SAC (200 mg kg^{-1} body weight) treated and SAC treated groups. The characteristics of circadian rhythms (acrophase, amplitude and mesor) of tumor marker enzymes such as alkaline phosphatase (ALP), aspartate and alanine transaminases (ALT and AST) and γ-Glutamyl transpeptidase (GGT) were analysed. Variations in acrophase, mesor, amplitude and r and p-values were noted. The detectable circadian rhythms of tumor marker enzymes and their alterations during NDEA/SAC treatments, in the present study, deserve further investigation for the diagnosis, prognosis and for the therapeutic efficacy of cancer. As to conclude, this study indicates the necessity of more research to reveal the temporal interplay between the central biological clock (suprachaismatic nucleus), peripheral tissue (liver) based oscillators and cancer processes.

Key words: Circadian, experimental hepatocarcinogenesis, S-allyl cysteine, tumor marker enzymes

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumor, responsible for atleast 5% of all cancer deaths in the world[1]. N-Nitrosodiethylamine (NDEA) is one of the potent hepatocarcinogenic dialkyl nitrosamine agent present in tobacco smoke, waste water, cheddar cheese, cured and fried meals and in number of alcholic beverages[2]. Studies on NDEA induced hepatocarcinogenesis in number of animals including mice, rats, hamsters, guinea pigs, rabbits, dogs and monkeys have been reviewed[3].

Garlic has been reported to reduce the risk of cardiovascular disease and cancer, stimulate immune function, alter the blood glucose, offer radioprotection, has antimicrobial function and possesses potential anti-aging effects[4,5] due to the presence of organosulfur compounds such as S-allyl cysteine (SAC), S-allylmercapto cysteine (SAMC), diallyl sulphide (DAS), diallyl disulphide (DADS) etc. Interest in SAC[6] is highlighted by the fact that it lacks the unpleasant, pungent odor of the volatile lipid soluble constituents of garlic such as DAS, DADS etc and therefore could, more readily used as chemopreventive agent in human beings.

The circadian patterns of serum ALP[7], AST and ALT[8] were reported in our lab. Disturbances in the rhythms of GGT[9] were also studied in tumor bearing rats. Tumor marker enzymes such as γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), asparate transaminase (AST) and alamine transaminase (ALT) have been found to be useful in detection and monitoring the induction and progression of NDEA induced hepatocarcinogenesis[10]. AST and ALT activities in the blood serum are generally accepted as an index of liver damage and this tendency is also known to be distinct in rodents. ALT is recognized to be a highly liver-specific enzyme. On the other hand, AST might be a non-specific index because it was disturbed not only in liver but also in heart, skeletal muscle, kidney and brain[11]. GGT, a plasma membrane bound enzyme plays a main role in multistage hepatocarcinogenesis, therefore being regarded as a neoplastic marker[9].

The connection between circadian rhythms and cancer extends well beyond the chronotherapy. Day-Night cycle plays a vital role in both the initiation and promotion of cancer. When rats were treated with a chemical carcinogen (safrole) either during day or night, the quantities of damaged DNA in the liver were varied dramatically, thus during the day, the carcinogen induced

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significantly more molecular genomic destruction compared with its night time administration[12]. In addition to cancer initiation, exposure of continuous light (LL)[13] as well as dim light[14] exerted a promoting effect similar to that caused by phenobarbital in NDEA induced hepatocarcinogenesis. The disruption of the circadian pacemaker (SCN) in the cancer bearing rodents results in rapid growth of tumors[15] .

In our lab, disturbances in the temporal patterns of thiobarbituric acid reactive substances and antioxidants such as reduced glutathione, catalase, glutathione peroxidase and superoxide dismutase in normal and NDEA treated rats were studied previously[16]. Moreover, greater disturbances in circadian system of endocrine, metabolic and immunological parameters were reported in tumor tissue, tumor bearing animals and cancer patients. Serum enzymes such as γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) have been found to useful in detection and monitoring the induction and progression of NDEA induced hepatocarcinogenesis[10]. However present study aims to study the effect of SAC in the temporal patterns of these marker enzymes in NDEA induced hepatocarcinogenesis, which can be involved in early diagnosis and prognosis of cancer and its treatment.

MATERIALS AND METHODS

Animals: Adult male Wistar rats (160-180 g) obtained from Central Animal House, Faculty of Medicine, Annamalai University were used in the study. Commercial pellet diet (Hindustan Lever Ltd., Bangalore, India) and water were made available to animals *ad libitum*. Animals were maintained in a controlled environment under standard conditions of humidity and temperature with alternating 12:12 light:dark cycles[10,16].

Methods: The animals were randomized and grouped into experimental and control rats (n = 6 in each group). Group I rats served as controls. Group II (NDEA+CCl_4) rats received single intraperitoneal injection of NDEA (200 mg kg^{-1} body weight) followed by weekly injections of CCl_4 (3 mL kg^{-1} body weight) for 6 weeks[10,16]. Group III rats received NDEA+CCl_4 as in Group II. In addition, 1 mL of SAC (200 mg kg^{-1} body weight)[17] were orally administrated. Group IV rats received SAC alone as in Group III. After 20 weeks of experimental study, blood samples were collected after every 4 h from each group of experimental and control rats (00:00, 04:00, 08:00, 12:00, 16:00, 20:00 and 24:00 h) throughout the 24 h period continuously. Minimal amount of blood was collected from orbital sinus with great care using heparinized tubes[16]. The serum from all the group animals was separated by centrifugation (3000 rpm for 20 min) with out hemolysis.

Estimation of marker enzymes: The activity of ALP was assayed by the method of King and Armstrong[18]. The activities of aspartate and alanine transaminases (AST and ALT) were estimated by the method of Reitman and Frankel[19]. The activity of GGT was assayed by the method of Fiala *et al*.[20]. The values (mean±SD) obtained from each group were plotted versus the time of blood collection. The characteristics of circadian rhythms (acrophase, amplitude and mesor) were analysed by cosinor analysis (using 'cosinorwin' computer software program). Acrophase (time at which the activity of variable is highest over 24 h period) is expressed in h. Amplitude (half of the difference between maximum and minimum levels of the variable) and mesor (mean value of the variable for equidistant data covering 24 h period) were expressed in the same units of documented variables.

RESULTS AND DISCUSSION

The 24 h patterns of tumor markers revealed a detectable rhythmicity in Groups I, III and IV (Fig. 1 - 4). All the studied markers were found to be disturbed in Group II rats ($p<0.05$) (Fig. 1 - 4). Acrophase of ALP, ALT, AST and GGT were delayed in Group II rats compared to control and advanced in Groups III and IV compared to NDEA treated rats. Increased mesor in Groups II and decreased mesors in Groups III and IV were found in all the tumor marker enzymes. Variations in amplitude and r-values were also noted. Circadian rhythm alterations occur in tumor tissues, tumor bearing animals and cancer patients with greater seen in more advanced cases. Rhythm alterations include diminished amplitude, phase shifts, period changes and erratic peak and troughs in endocrine, metabolic, immunological and rest-activity cycles[21]. This lack of synchronization have been reported to alter circadian fluctuations in experimental tumor model including this study and patients.

Increased mesor values in Group II rats indicated that high serum enzyme activities were due to hepatocellular degeneration. The activities of these enzymes such as AST, ALT, ALP and GGT were established themselves as an index of liver function and recovery degree in liver transplants and in CCl_4 induced hepatocellular carcinoma[11].

Advanced acrophase, decreased mesor and increased r value indicates the therapeutic potential of SAC. Studies on pharmacokinetics of SAC in a number of animal

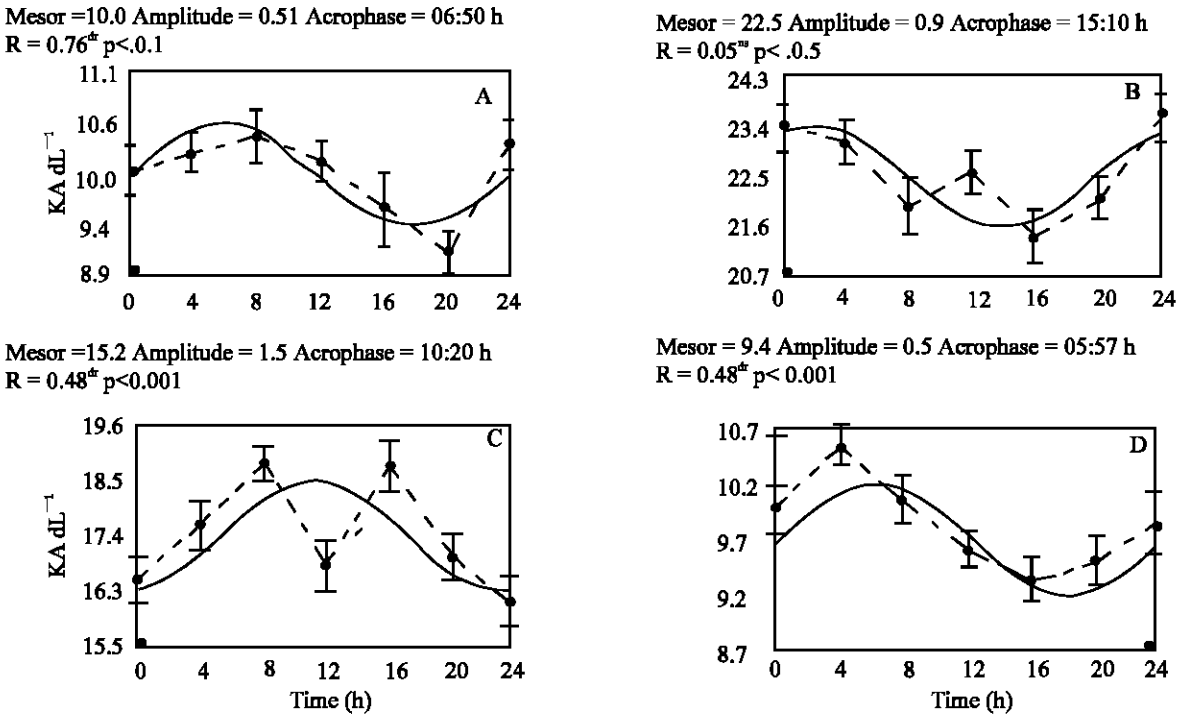

Fig. 1: Temporal patterns of ALP activity in Wistar rats. A-normal; B-NDEA treated; C-NDEA+ SAC treated; D-SAC treated. Dotted lines represent the raw data (with mean±SD) and smooth lines represent lines the best fitting cosinor curve (obtained using 'cosinorwin' computer software program)

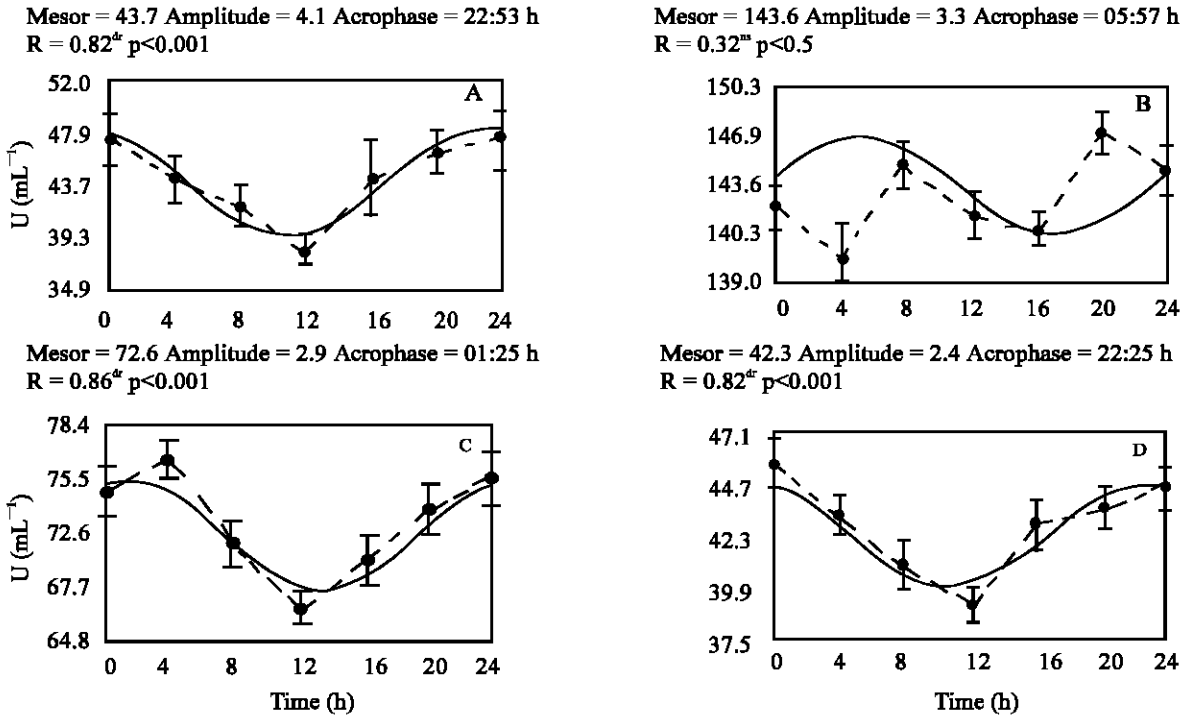

Fig. 2: 24 h patterns of ALT in Wistar rats. A-normal; B-NDEA treated; C-NDEA+ SAC treated; D-SAC treated. Other details as in Fig. 1

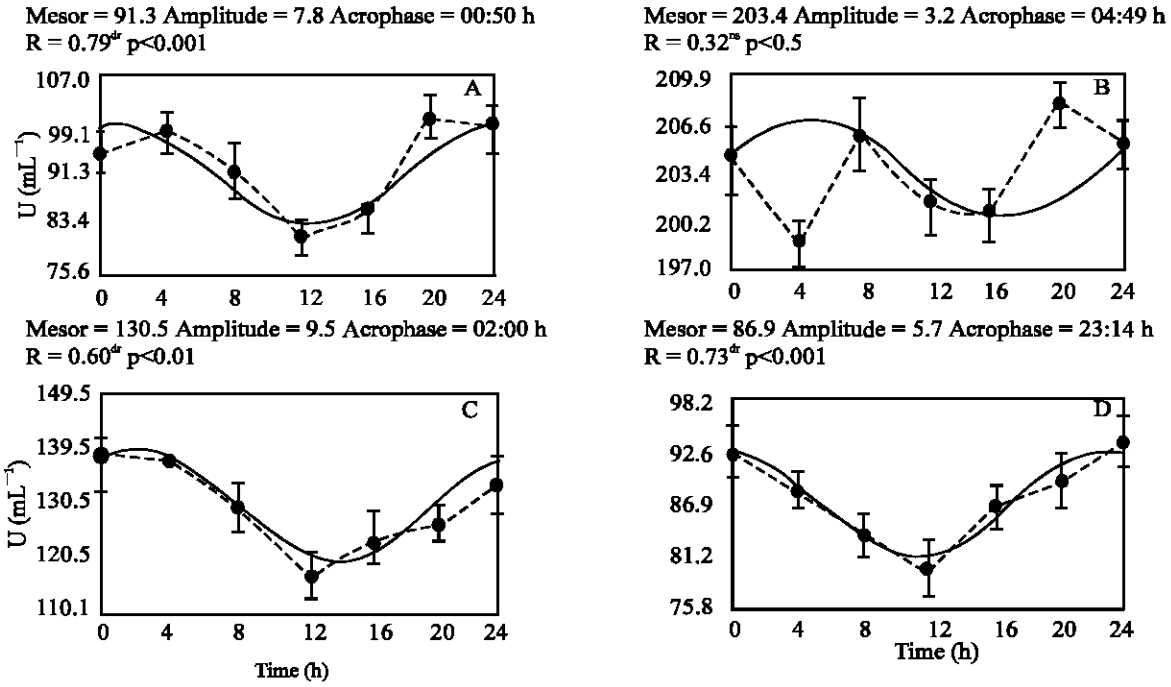

Fig. 3: Temporal oscillations of AST in Wistar rats. A-normal; B-NDEA treated; C-NDEA+ SAC treated; D-SAC treated. Other details as in Fig. 1

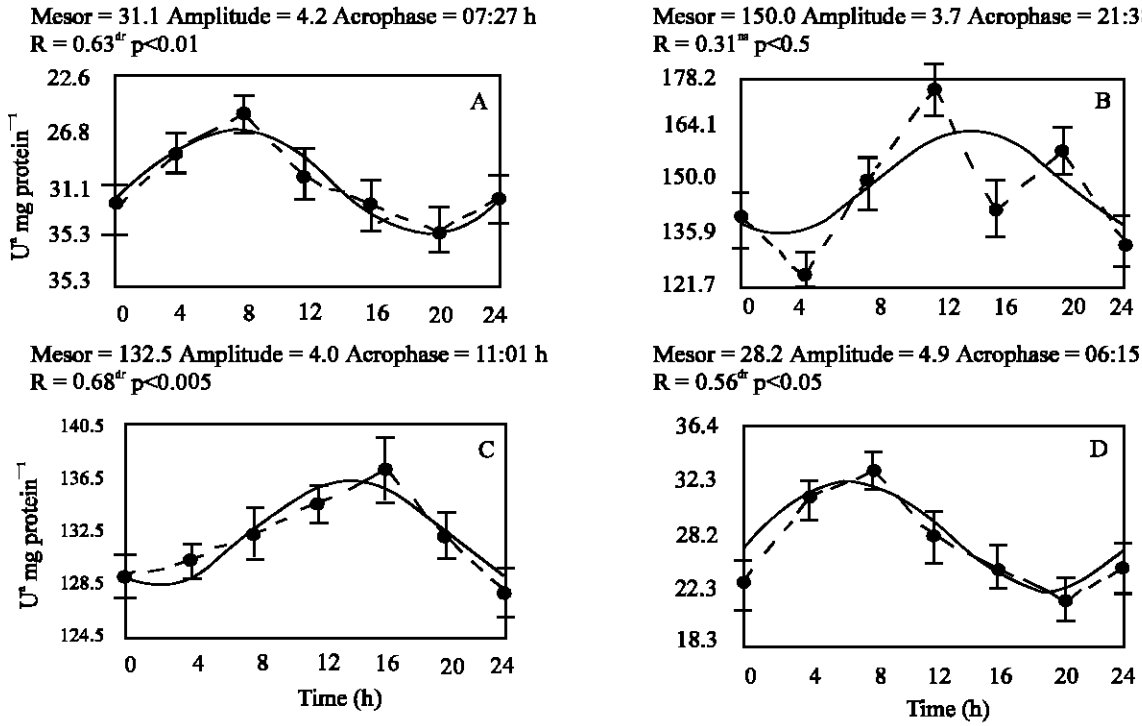

Fig. 4: Rhythms of GGT in Wistar rats. A-normal; B-NDEA treated; C-NDEA+ SAC treated; D-SAC treated. Other details as in Fig. 1

species shows that SAC is early absorbed from the gastro intestinal tract and distributed in plasma, liver and other organs with a bio availability of 98% in rats[6]. Although allyl sulfur compounds were not examined by Shenoy and Choughuley[22] they suggested that cysteine and several other sulfhydryl compounds might act as nitrite scavengers. SAC, recognized as a significant sulphur containing compound in garlic, was previously shown to depress the binding of precursors of the carcinogen nitrosodiethylamine to rat liver cell DNA[23]. Experiment carried out in mice showed that SAC and S-allyl mercapto cysteine (SAMC) were potent inhibitors of liver toxicity induced by industrial oxidant CCl_4 and by commonly used analgestic agent acetaminophen[24].

Interest in rhythms of experimental tumors and different kinds of human cancers began more than 30 years ago. Knowledge of the circadian rhythms in normal and in pathological (including cancer) conditions can be used to improve the understanding of pathophysiological process and therapeutic approach to illness. As to conclude, this study indicates the necessity of more research to reveal the temporal interplay between the central biological clock (suprachaismatic nucleus), peripheral tissue (liver) based oscillators and cancer processes.

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