Full Length Research Paper

Biochemical profile of sodium selenite on chemically induced hepatocarcinogenesis in male Sprague-Dawley rats

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Despite the success of experimental, clinical and epidemiological studies on selenium as an anti-cancer agent, basic studies on the effects of selenium are still scanty. This study was designed to investigate the biochemical effects of sodium selenite using preventive and therapeutic approaches on chemically induced hepatocarcinogenesis in rats. Rats were divided randomly into 6 groups: negative control, positive control [Diethyl nitrosamine (DEN) + 2-Acetylaminofluorene (2-AAF)], preventive group, preventive control group (respective control for preventive group), therapeutic group and therapeutic control group (respective control for therapeutic group). The activities of plasma alanine transaminase (ALT), aspartate aminotransferase (AST), gamma- glutamyl transferase (GGT), alkaline phosphatase ALP and concentrations of total protein, albumin and globulin were determined by an auto-analyzer. GGT and ALT activities were significantly higher in the positive control, preventive and therapeutic groups when compared with the negative control. Globulin concentration was significantly lower in the positive and therapeutic group controls and higher in the therapeutic group and its respective control when compared with the negative and positive controls, respectively. Plasma GGT enzyme marker could be used as an early marker for liver neoplasm in rats. The effect of selenium on globulin, as an indicator of immunity status, needs to be clarified.

Key words: Alanine transaminase, gamma-glutamyltransferase, liver neoplasm, selenium.

INTRODUCTION

Since the past decades, there has been increasing interest in the role of selenium in the pathogenesis of cancer including liver neoplasm. The poor prognosis and current limited treatment for liver neoplasm are still a major concern as it has been noted that the incidence of liver neoplasm is on the rise worldwide (Parkin et al., 2005). Thus, other preventive approaches such as chemoprevention have been highly emphasized (Kensler et al., 2003).

Since the report of Scharauzer and his colleagues (1977), stating that selenium is a potential human cancer protective agent, worldwide, research on selenium as an anti-cancer agent has escalated. In addition, the publication of Clark's clinical study (Clark et al., 1996) has attracted more researchers in this field. In the Clark's study, selenium was found to dramatically reduce the incidence of cancer, in addition to improvements in the prognosis of prostate cancer, colorectal cancer and lung cancer patients. Furthermore, no signs of selenium toxicity were reported in the Clark's study. In addition, other works have emphasized the anti-cancer activity of selenium (Raymond, 2001).

Selenium is an essential micronutrient mineral, which

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could have a high clinical value in some cancer patients (Valkoo et al., 2006). As a part of the preventive health strategy 'Prevention rather than treatment', dietary selenium intake is an essential element in the protection from many diseases (Raymond, 2001). The dietary reference intakes (DRIs) for selenium has been set at approximately 55 µg per day for adults (IOM, 2000).

It has been reported that the analysis of liver enzymes in plasma reflects cellular damage (Tazi et al., 1980). Liver function enzymes such as alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP) and other biochemical parameters such as total protein, albumin and globulin, are generally used in humans and animals as indicators of liver injury as well as liver response to medicine (Kim and Park, 1994) . In addition, basic studies on the effects of selenium are still scanty. Hence, this study aimed at investigating the effects of selenium on some liver enzymes and biochemical markers on chemically induced hepatocarci-nogenesis in rats.

MATERIALS AND METHODS

Chemicals

Sodium selenite, DEN and 2-AAF were obtained from Sigma Chemical Co, Germany. All enzymes and biochemical reagents were obtained from Randox Laboratories Ltd, U.K.

Animals and diet

Male Sprague-Dawley rats (6 – 8 weeks old) were obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, University of Kebangsaan Malaysia, Kuala Lumpur, Malaysia. They were housed in plastic cages (3 – 4 rats per cage) with wood chips for bedding. The animals were acclimatized to standard laboratory conditions [temperature (22 – 25°C), humidity (55 ± 10%) with a 12 h light-dark cycle] for one week before the commencement of the experiments. During the entire period of study, the rats had free access to food and water. The rats were maintained on a basal diet (22% crude protein, 5% crude fiber, 3% fat, 13% moisture, 8% ash, 0.85 - 1.2% calcium, 0.6 - 1% phosphorus and 49% nitrogen free extract) (Mouse pellet 702-P from Gold Coin Co, Limited, Malaysia).

According to the manufacturers of basal diet, mouse pellet 702-P contains 0.2 mg/kg of selenium, which is within the recommended reference range. The recommendations of the University of Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) for the care and use of animals were strictly followed throughout the study (UKMAEC No: FSKB/2006/Jamaludin/22- August/170-December-2006).

Experimental design

Fourty-four rats were randomly divided into 6 groups, 6 or 8 rats in each group, as follow: Group 1 (negative control): rats were given normal rat chow and drinking water. Also, a single intraperitoneal (I.P) injection of saline (0.9%) was given. Group 2 (positive control): liver tumors were induced with a single I.P injection of DEN at a dose of 200 mg/kg body weight in saline (Solt and Farber, 1976).

Two weeks after DEN administration, the carcinogen effect was promoted by 2-AAF (0.02%). The promoter was incorporated into the rat chow for 10 weeks. Group 3 (preventive group): 4 weeks before DEN administration, rats were fed with sodium selenite (4 mg/L) through drinking water and stopped at week 4 (the day of commencement of DEN administration). Group 4 (preventive group control): rats in this group served as controls for group 3. Rats were given sodium selenite for 4 weeks only. No DEN or 2-AAF was given instead a single I.P injection of saline (0.9%) was given. Group 5 (therapeutic group): 4 weeks after the start of DEN administration (as in Group 2), the rats were treated with sodium selenite (4 mg/L) through drinking water and this continued until the completion of the experiment (8 weeks). Group 6 (therapeutic group control): rats in this group served as controls for group 5. Rats were given sodium selenite for 8 consecutive weeks. No DEN or 2-AAF was given instead a single I.P injection of saline (0.9%) was given. 16 weeks after the initiation of the experiment, all the rats were fasted overnight and then killed by cervical dislocation under ether anesthesia. Sodium selenite supplementation in drinking water and normal drinking water was renewed every 2–3 days. Diet with 2-AAF was freshly prepared and wood chips for bedding were changed weekly.

Collection of blood and liver tissues

Under ether anesthesia, the blood of all experimental rats was taken by cardiac puncture using 21 G needle and 10 ml syringe. Samples were then collected in EDTA plastic tubes. Plasma was prepared by centrifuging at 3000 g for 15 min. The plasma, in the supernatant, was then pipetted into 2.0 ml Eppendorf cups. In addition, portions of the livers were fixed in 10% neutral buffered formalin for routine histopathological examination.

Enzyme analysis

The activities of ALT, AST, GGT, ALP, total protein and albumin were determined by an auto-analyzer (Selectra E, Vital Scientific N.V, Netherlands). The mean of duplicate reading was taken. Globulin activity was measured as the difference between total protein and albumin.

Statistical analysis

Data were expressed as means ± standard deviation (SD). The data were analyzed using Statistical Package for Social Sciences (SPSS) version 13. Shapiro – Wilk test was used to check the normality of the variable. Accordingly, Student's t and Mann – Whitney's U tests were used to analyze data that follow normal or non–normal behavior of distribution pattern, respectively (Mahajan, 1997). Differences in statistical analysis of data were considered significant at $P < 0.05$.

RESULTS

Histopathological examination of the liver in the positive control showed a completely disrupted architecture. The normal liver cords were displaced with variably-sized neoplastic nodules. The hepatocytes were more than 2 cells thick, paler and showed enlarged vesicular nuclei with prominent nucleoli (Figure 1). However, the preven-tive and therapeutic groups revealed that the liver was
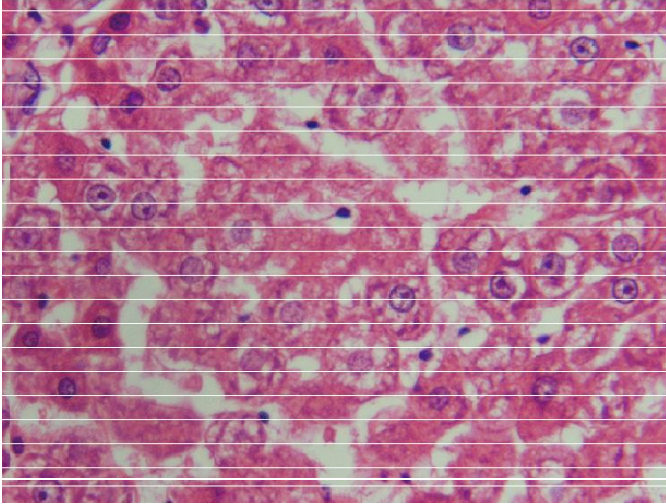
Figure 1. Neoplastic liver, showing enlarged nuclei with prominent nucleoli, as seen in the positive control (received a single I.P injection of DEN at a dose of 200 mg / kg body weight in saline and two weeks later, the carcinogen effect was promoted by 2-AAF (0.02%) and continued for 10 weeks). Hematoxylin and eosin (X 60).
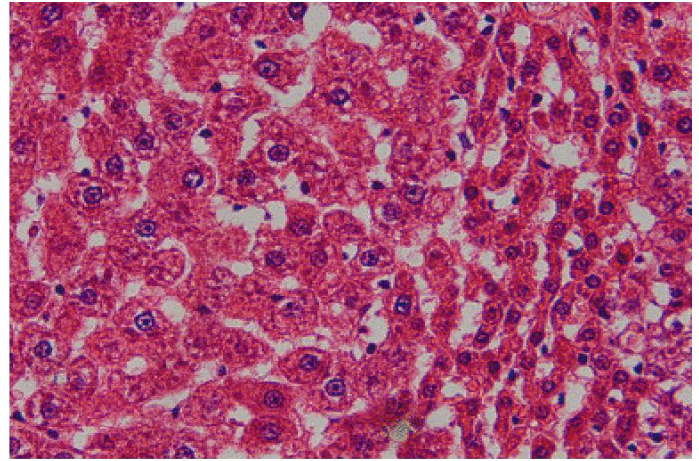

Figure 2. Hyperplastic liver with largely preserved architecture as seen in the preventive group (received sodium selenite and stopped at week 4, the day of commencement of DEN administration as in group 2) and therapeutic group (received a single I.P injection of DEN as in group 2 and 4 weeks later, rats were treated with sodium selenite for 8 weeks). Hematoxylin and eosin (X 40).

nodular but with largely preserved architecture. The majority of varying-sized nodules were hyperplastic (1 cell thick) (Figure 2). The negative, preventive and therapeutic controls were free of any abnormality (Figure 3).

The activities of ALT, AST, ALP and GGT are shown in Table 1. The activity of GGT was significantly higher in the positive, preventive and therapeutic groups when compared with the negative control. On the contrary, GGT activity was significantly lower in the preventive and therapeutic controls (selenium treated groups without carcinogens) when compared with the positive control. Interestingly, the therapeutic group (group 5) showed a significant higher activity when compared with the positive control. In addition, the preventive (group 3) and therapeutic groups showed significantly higher activities of GGT when compared with their respective controls, 4 and 6, respectively. The preventive group showed significantly higher activity of ALT when compared with the negative (group 1), positive (group 2), and respective controls (group 4). On the other hand, the therapeutic control (group 6) showed significantly lower activity of ALT when compared with the negative, positive controls and its treated group. Interestingly, the activity of ALT in the positive control was not affected by DEN and 2-AAF when compared with the negative control. The preventive group showed a significant higher activity of ALP when compared with the negative control. However, all other experimental groups showed no significant activity of ALP when compared with either negative or positive controls. The activity of AST was not affected in all the experimental groups, including the positive control and
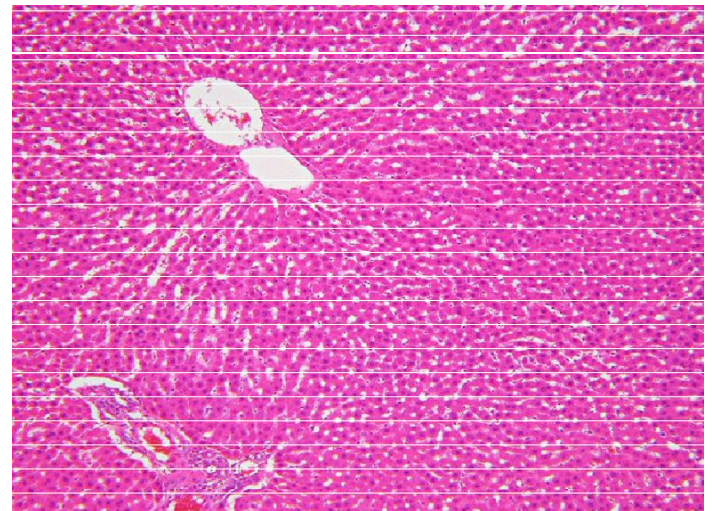

Figure 3. Normal liver architecture as seen in the negative (received normal rat chow and drinking water), preventive (treated with sodium selenite alone for 4 weeks and served as control for group 3) and therapeutic controls (treated with sodium selenite alone for 8 weeks and served as control for group 5). Hematoxylin and eosin (X 10).

selenium treated groups when compared with the negative control.

The concentration of globulin was significantly lower in the positive control when compared with the negative control. Also, the therapeutic control showed a significant lower concentration of globulin when compared with the negative control but not with the positive control or its treated group. On the other hand, the preventive group and its respective control showed significantly higher

Table 1. Effect of selenium on ALT, AST, GGT and ALP on chemically induced hepatocarcinogenesis in rats.

Group	ALT (U/L)	AST (g/L)	GGT (U/L)	ALP (U/L)
Group 1	48.73 ± 3.86	107.25 ±41.63	6.33 ± 0.68	3.67 ± 0.52
Group 2	44.67 ± 13.52	94.25 ± 23.04	11.83 ± 2.21 [a]	3.92 ± 1.46
Group 3	59.64 ± 6.53 [a,b,c]	100.29 ± 14.67	12.79± 2.02 [a,c]	5.29 ± 1.22 [a]
Group 4	49.23 ± 2.59	98.43 ± 24.57	7.57± 2.42 [b]	5.57 ± 2.52
Group 5	47.63 ± 5.66 [d]	92.13 ± 15.14	15.75 ± 1.83 [a,b,d]	4.13 ± 0.74
Group 6	32.07 ± 3.93 [a,b]	75.50 ± 15.32	6.29± 2.69 [b]	3.79 ± 0.70

Results are expressed as means ± S.D. Values were analyzed using Student's t and Mann – Whitney's U tests. [a] significantly different from the negative control (1) ($P< 0.05$); [b] significantly different from the positive control (2) ($P< 0.05$); [c] significantly different from the respective group (4) ($P< 0.05$) and [d] significantly different from the respective group (6) ($P< 0.05$).

concentrations of globulin when compared with the positive control. There is no significant concentration of total protein and albumin among all the experimental groups, including the positive control when compared with the negative control (Figure 4).

DISCUSSION

The measurement of concentrations and activities of various biochemical markers and enzymes in the plasma plays a significant role in disease investigation and diagnosis as well as in response to a toxic drug (Malomo, 2000). Previous studies have shown that high levels of AST, ALT and ALP in serum or plasma are usually indicative of liver injury in humans and animals (Spracklin et al., 1996; Yabubu et al., 2003) whereas the lower levels of these enzymes could indicate a degree of liver protection (Manna et al., 1996). This study is in line with other previously reported study (Nakaji et al., 2004), where it was reported that the activities of AST, ALT and ALP were not significantly affected between the control

group (rats were given 200 mg/kg of DEN, two weeks later, they were fed via gastric tubes with 10 mg/kg of 2-AAF for two weeks and partial hepatectomy was performed one week later) and Interferon – alpha treatment. Plasma liver enzymes are good indicators of liver injury but they are not always elevated during liver injury. They could be elevated at a particular stage of the disease and return to their normal values at a certain point (Simonsen and Virji, 1984).

Sodium selenite at the dose of 4 mg/L did not maintain the activity of GGT and ALT in the preventive group (selenium for four weeks only, then DEN and 2-AAF) and therapeutic group (four weeks of DEN injection, then selenium until the end of experiments). In fact, selenium significantly increased the activity of GGT in the preventive and therapeutic groups when compared with their respective controls. The elevation in ALT and GGT activities seen in the preventive and therapeutic groups could be due to hepatocellular damage that was initiated by DEN and further damage by 2-AAF. GGT, which is found in all cells of the body except myocytes with particularly high concentrations found in hepatocytes and the kidney, is a liver enzyme involved in the transport of amino acids and peptides into cells (Hanigan and Pitot, 1985). ALT, which is mainly produced in the hepatocytes, is more specific for liver injury (Thomson, 1998). It has been reported that ALT is generally increased in situations where there is damage to the liver cell membrane (Schumann et al., 2002). Thus, when the liver is injured, the levels of ALT in plasma usually rise.

However, the positive control (DEN and 2-AAF only) showed that the ALT activity was not significantly increased when compared with the negative control. This shows that ALT activity maybe worsened by the addition of sodium selenite. On the other hand, the other aminotransferase enzyme (AST) was not affected by either DEN + 2-AAF treatment or selenium supplementation. In addition, other markers such as ALP, total protein and albumin were not affected by the same treatment. This might suggest that the activity of ALT alone is not specific for liver cancer in rat model.

The findings of this study disagree with other previous study (Ozardali et al., 2004). They reported that the activities of plasma AST, ALT and ALP were significantly increased with carbon tetra chloride injection whereas GGT activity was not significantly affected. However, they also reported that selenium treatment showed that the levels of AST, ALT and GGT were decreased to nearly the enzyme values in control group but ALP activity was significantly increased.

Thirunavukkarasu and Sakthisekaran (2003) cited the decreased levels of total protein and albumin and increased levels of globulin in rats treated with DEN and Phenobarbitol when compared to control rats. The findings of this study disagreed with the above mentioned study. It was observed that the concentrations of total protein and albumin were not affected by neither

carcinogens nor selenium treatments. In addition, a slight decrease in globulin concentration was observed in the positive control and therapeutic group in comparison with the negative control. Also, it was noted that the globulin concentrations in the preventive group and its respective control were significantly higher when compared with the positive control.

Despite the fact that albumin is a major protein formed by the liver and together with the total protein level in the blood reflect the protein function of the liver (Benoit et al., 2000), the positive control, preventive and therapeutic selenium treated groups showed almost similar concentrations to that of the negative control. This could be due to the long half life of albumin, which has been reported to be around 20 days (Halsted and Halsted, 1991), and a decrease in plasma albumin is usually not seen early in hepatocarcinogenesis (Cheesbrough, 1998). Albumin levels in these groups were normal and this could be also due to the rat's ability to compensate for protein losses.

Recent study on selenium and copper supplementation on blood metabolic markers in male buffalo calves showed that high levels of globulin are beneficial, as it directly correlates with the immune status of the animals (Mudgal et al., 2008). Based on the histopathological examination along with the increased concentration of globulin, it can be concluded that supplementation of selenium in the preventive group and its respective control may have enhanced the immunity status of these rats. On the contrary, supplementation of selenium in the therapeutic control group (selenium for eight weeks but no DEN or 2-AAF) may have reduced the immunity status of those rats. The conclusion that selenium may reduce or enhance the immunity in rats needs to be clarified.

Based on the findings of this study, plasma GGT enzyme marker could be used as an early marker for liver neoplasm in rats. Supplementation of selenium in the preventive and therapeutic experimental groups has increased the activity of GGT and ALT. The effect of selenium on globulin, as an indicator of immunity status, needs to be clarified.

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