The Role of Nitrosamines in Food*

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Introduction

In the twentieth century, cancer has become one of the most important killer diseases in the world and has being studied as an important biomedical research area over the past few decades. Approximately a hundred types of human cancer have been recognised and epidemiological studies have showed about 35% of cancer are related to the diet. It is well documented that different types of carcinogenic stimuli, including chemicals, viruses and radiation, induce cancer development in organs and tissues. In the modern industrialized world, cancer has become the most feared of all diseases. It is a progressively fatal disease for which no treatment has yet been discovered. Studies have been carried out histologically and histochemically but only a few studies have attributed correlation between biochemical assay and histological effects. Thus the carcinogenic process in mice liver and kidney with intra-peritoneal injection with N-Nitrosodimethylamine (NDMA) at sub-acute, acute and chronic doses were analysed by monitoring the marker enzymes of liver cancer namely gamma glutamyl transpeptidase, Phase II enzymes glutathione S-transferase and uridyl diphosphoglucuronyl transferase. Their activities are significant in evaluation of disease progression and promotion during treatment and developments of cancer that makes them as an important tool for cancer research especially for liver cancer. By using N-Nitrosamines as a cancer agent, understanding of carcinogenesis as a multistage process and evaluation in developments can establish an ideal way of detecting this disease at an earlier stage which is one of the objectives beside analysis of the damages to liver cells caused by this potent chemical. Hepatocarcinoma are detected in most cases only after it enters the chronic stage. This is due to unavailability of any symptoms at earlier stages. Therefore it is important to obtain early markers during hepatocarcinogenesis in order to save and/or prolong life or for earlier treatments. Thus nitrosamine such as NDMA serve the purpose due to its metabolic activity being mainly centralized in liver as a major metabolic organ and participation of these detoxifying enzymes are shown to be sensitive to changes during this series of treatment.

Materials and Methods

Male adult mice Mus musculus with an average weight of 26-32gm were purchased from the Institute of Medical Research (IMR). NDMA were purchased from Sigma Chemical. Co.USA. Animals were maintained with free access to food and water *ad libitum*, and in a well-ventilated room. For intraperitonial treatment, NDMA solutions were prepared by dissolving in 0.9% saline and administered to mice on the ratio of 0.5, 2.5 and 0.05mg/kg of body weight at different times of exposure. The i.p injection was given based on LD50 value for mice ie: 7-14 mg/kg of body weight to produce renal tumour and hepatocellular carcinoma (Frei, 1970). For long-term oral administration on mice LD50 23-40mg/kg of body weight of NDMA was used as standard in preparing sub-necrogenic doses for mice. Cytosolic and microsomal fractions were prepared using the method of Speir and Wattenburg (1975). Animals were sacrificed by neck dislocation and the organs were homogenised in volume of 1:3 buffer 1.15% KCL and then centrifuged further at 9000 x g for 60 minutes at 4C. The homogenate were centrifuged again at 105,000 x g after saving some homogenate for ALP analysis. The supernatant obtained contain the cytosol fraction and the pellet contains the microsomes. The cytosolic fraction was used for assay of GSH-Px, GST and GSH-R and microsome fraction was used for assay of UDPGT and GGT enzymes. GGT was assayed according to the method of Jacobs et al. (1971). L-glutmyl-carboxyl-nitroanilide was used as substrate and glycylglycine was used as gamma glutamy acceptor. The absorbance of the final mixture was measured at 405nm. The assay of GST activity in liver and kidney cytosol was done according to the method of Habig et.al, 1974. The reaction mixture consists of 200ul of sample, 1mM of CDNB, %mM GSH and 0.1M phosphate buffer in a total volume of 3ml. The absorbance of the sample was read against the blank at 340nm and a unit of enzyme activity is defined as the amount of enzyme activity that catalyses the formation of 1umol of the substrate with GSH per minute at 29°C.

Results and Discussion

Acute doses given at a single dose of 0.5mg/kg of body weight had significantly increased the GST activity in liver but not in kidney. During sub-chronic and chronic dose treatment of 0.25 and 0.05mg/kg of body weight had significantly increased the liver and kidney GST respectively. Glutathione reductase and Glutathione peroxidase are two enzymes that are involved in glutathione metabolism and in this study their activity shows a significant increase in liver during acute chronic and sub-chronic exposure and not highly expressed in kidney during i.p injection of NDMA in mice. During acute exposure GGT shows marginal decrease in specific activity both in liver and kidney compared to control. Another metabolizing enzyme UDPGT was significantly increased at three different levels of exposures in both metabolic organs suggesting their involvement in NDMA metabolization during i.p injection to male mice.

When histological lesion studies were done, liver tissues show a higher degree of cellular degeneration in all treated groups with enlarged Kupffer cells and the presence of neutrophiles. The metabolic activation of N-Nitrosocompounds are known to take place predominantly in the liver and kidney and especially NDMA are known to exert cytotoxic, mutagenic and carcinogenic effects in various tissues in animal species. GSH is known as a very important substrate in the liver for the detoxification processes of xenobiotic substances. It is well recognised that GSH and GSH related enzymes play important roles in the protection of mammalian cells against the harmful effects of chemical carcinogenesis and other alkylating agents (Lotlikar *et,al.*, 1980; Ketterer et al., 1988). When cytosolic GST activities were measured in kidney and liver, its activities are significantly increased in treated groups of chronic, sub-chronic and acute groups. The placental form of GST is absent from normal liver but found in kidney and in hepatic preneoplastic lesions (Ogita and Markert, 1990). The GST activity are reported to be increased during reversion of cells to the fetal-like state and therefore this result can be an indication of treated cell being reverted to those in embryonic state which serve the purpose as a useful marker during hepatocarcinogenesis. The active metabolites possibly exert their toxic effects in the liver or might be transported from liver via the blood elsewhere e.g kidney. GSH-R, is an important enzyme in protection of mammalian cells against toxicants and in this work it has been shown that elevated level of activities are only found in liver but not in kidney. This has been suggested to be an adaptive response in order to compensate for the depleted level of GSH by reduction of oxidized to GSH. Since liver is the major organ in GSH cycle, thus the presence of this enzymes resemble the importance of liver as a metabolic organ. The increase of GSH-Px activity, another GSH cycle enzyme are due to increased oxidized glutathione formation. Kitahara et.al, in 1984 had shown that certain molecular forms of GST such as GST-A, GST-AA, and GST-B possess GSH-Px activity. It has been reported that GST-B acts like GSH-Px in hyperplastic nodules partly to compensate for the decreased selenium dependant GSH-Px activity during hepatocarcinogenesis. Another important enzyme which catalyses the breakdown of GSH in tissue is GGT, which possess high activity in fetal and in hepatoma but very low activity in the adult rat liver (Taniguchi et.al, 1975). Since its usefulness as an early marker of early neoplasia and severity of hepatocarcinogenesis this enzyme are expected to increase in treated liver sample. The result obtained shows that the percentage of enzyme activities in both liver and kidney decreased at acute stages and gradually increased at sub-chronic period and again increased at chronic stages. The first increase of the enzyme protein is a reflection of appearance of pre-neoplastic lesion and the second increase of the protein is the reflection of hepatoma cells (Sato et.al, 1983). Previous studies reported the changes of enzyme contents are consistent with those determined by enzyme inmmunoassay. Another microsomal enzyme UDPGT in treated liver increases significantly compared to control and also compared to kidney. The results in this study agrees with Bock et.al, 1982, where he has shown an increase in liver UDPGT in preneoplastic liver after treatment with 2-Acetylaminofluorene. This is perhaps due to glucuronidation of the toxic compound during the tumour promotion phase II by the carcinogen. The bio-activation of NDMA are found to be greater in liver and in histological sectioning it has been shown that a single acute doses of NDMA could cause necrosis in liver but not in kidney.

Conclusions

Majority of tumour marker enzyme activities (GST, GSH-R, GSH-Px, GGT and UDPGT) increased in liver and kidney induced by NDMA. In the liver, acute, sub-chronic and chronic exposure to NDMA showed increase enzyme activities in comparisons with control group. In the kidney however the effect of NDMA exposure was rather inconsistent. For instance all enzyme activities increase in acute exposure. In the sub-chronic and chronic exposure some enzymes activity increased (for sub-chronic: GST, GGT and UDPGT) and some enzyme activities did not significantly change (for e.g: subchronic and chronic: GSH-R). Hence kidney and liver appear to be the main target organs of the carcinogenic action of NDMA in mice. Prolonged exposures of mice to NDMA produce a high incidence of hepatocellular adenoma tumour. This study demonstrated that there is very clear correlation between enzyme activity and cell damage, therefore it is possible that these enzymes activities can be used as a tumour marker in future research.

Benefits from the study

By measuring he biochemical parameters GGT, GST, UDPGT and GSH-r together with GSh-Pr, provides an opportunity to identify any disease prone in liver at earlier stage. This will enhance the capability of metabolic organs to minimize or eliminate the toxicity of carcinogen when absorbed in body. The disruption of these biochemical markers during clinical assessment is significant in monitoring the progress of any liver related treatment. Beside, these biochemical parameters are useful when studies are conducted on drugs and medicinal products in order to look at the binding capability or induction property in metabolic organs by measuring these enzymes. Any accidental exposure also can be prevented by analyzing the compounds toxicity by looking at laboratory animals on these liver markers. Induction and activation of these enzymes can provide good susceptibility against any liver disorders thus maintaining healthy cycle of blood, which yield better health condition.

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