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Original Article

Investigation of the Effects of Octreotide Agent on Oxidative Stress, 8-Hydroxy Deoxyguanosine in Experimental Hepatic Carcinogenesis Rat Model

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Abstract

Introduction: 2-AAF and DEN are well-known liver toxicants commonly used to stimulate tumors in laboratory animals.

Aim: The aim of this study was to investigate the effect of octreotide on DEN-induced and 2-AAF-supplemented hepatocarcinogenesis in Wistar albino rats.

Materials and methods: In this study, 64 Wistar albino rats were divided into 8 groups. DEN (175 mg/kg) initiated and 2-AAF (20 mg/kg) promoted liver carcinogenesis in rats. The tumor growth inhibitor octreotide (300 μg/kg) was used. Rats were sacrificed at the end of experiment and their liver tissues were taken for the study. SOD, GSH-Px, CAT activities, NO and MDA levels were measured spectrophotometrically. Also, Hsp70 and 8-OHdG was measured by the ELISA method.

Results: In group 7, MDA, 8-OHdG, and Hsp70 levels were significantly increased. In addition, SOD, GSH-Px activity was significantly reduced in this group. MDA, 8-OHdG and Hsp70 levels were significantly reduced in Group 8, which received octreotide for treatment.

Conclusion: DEN and 2-AAF cause very serious liver damage. Octreotide protects the liver from carcinogenesis, increases the activity of cellular antioxidant enzymes and helps reduce DNA damage. Therefore, octreotide may be an inhibitor in tumor cells and may reduce oxidative stress.

Keywords

2-AAF, DEN, Hsp70, octreotide, oxidative stress

INTRODUCTION

Hepatocellular carcinoma (HCC) is a widespread malignant tumor. Animal models are seen as very important tools in the study of liver cancer. Because of the physiological and genetic similarities between rodents and humans, short lifespan, reproductive capacity and the diversity of manipulation methods, animal models are frequently used in cancer research. 2-AAF exhibits its carcinogenic effect through the formation of DNA adducts, over the manufacture of oxidative DNA damage and reactive oxygen species (ROS). DEN is one of the most significant environmental carcinogens, mainly inducing tumors in the liver. DEN is available in frequently consumed foods (salted fish, meat, alcoholic beverages, pesticides, cigarettes). Overproduc-

tion of ROS is an oxidant/antioxidant imbalance. If oxidants are favoured, oxidative stress will occur which alters and damages many intracellular molecules, including DNA, RNA, lipids and proteins.[1-2] There is a strong link between hepatocarcinogenesis and oxidative stress. Oxidative stress plays an important role in the progression of hepatocarcinogenesis. Oxidative stress markers such as 8-OHdG and lipid peroxidation such as MDA are generally elevated in patients with chronic HCV infection and correlate well with the viral infection and inflammation scores, which are known risk factors for HCC.[3] Heat shock proteins (HSPs) are a family of extremely protected proteins, which are expressed at low levels under normal conditions, but induced in reaction to cellular stresses, including heat shock, hypoxia, nutrient starvation, genotoxic agents and overexpression of oncoproteins.[4-5] Heat shock proteins are often named according to their molecular weight. Hsp70 is a large member of the Hsp family. Hsp70 is normally retained at low levels, but can be induced under protein-damaging conditions. Hsp70 is overexpressed in many human cancer types including liver, colon, prostate carcinomas.[6-7] Octreotide is semisynthetic somatostatin analog used for the management of neuroendocrine tumors. In some studies it has been shown that octreotide inhibits the growth of many tumors such as colon carcinoma, pancreatic carcinoma, gastric carcinoma, hepatic carcinoma.[8-9] We aimed to investigate the effects of the chemotherapeutic agent octreotide on the NO, MDA, Hsp70, 8-OHdG levels and SOD, CAT and GSH-PX activities in experimentally induced hepatocellular carcinoma by DEN and 2-AAF in rats.

MATERIALS AND METHODS

Chemicals and reagents

DEN, 2-AAF and other chemicals were obtained from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA). ELISA kits are the brand of Sunredbio. 8-OH dG of kit Cat No: 201-11-0032, Hsp70 of kit Cat No: 201-11-0523

Animals and experimental design

All rats were housed in standard cages with a 12 hour light/dark cycle. Randomly selected 64 rats were divided into groups of 8 rats in each group. Processing was done between 08:00 and 12:00 in the morning. In this way, the results were obtained without being affected by daily changes. The rats were starved 12 hours before the operation. No rat was given antibiotics. Rats were sacrificed at the end of experiment and their liver tissues were taken for samples.

Dose and experimental groups

The system consists of short-term dietary exposure to 2-AAF, which suppresses the growth of almost all normal hepatocytes from a single dose of genotoxic carcinogenic DEN (**Table 1**).[10]

Biochemical evaluation

Liver tissues from the rats were first weighed, placed in 1.15% KCl solution, and homogenized for 35 minutes at 12,000 rpm. The resulting homogenates were placed in aliquots, centrifuged for 30 minutes at 10,000 rpm and the supernatants were analyzed for MDA, NO levels and CAT, SOD and GSH-Px activities. The concentration of lipid peroxidation (total MDA expressed in terms of nanomolar per kilogram of protein) was determined using the Ohkawa method with simple modifications.[11] Protein measurement was made according to the Lowry method.[12] SOD activity was determined according to the Fridovich method.[13]

The CAT activity was spectrophotometrically measured by the extinction of H_2O_2, at 230 nm.[14] GSH-Px activity was measured spectrophotometrically at 340 nm by an enzymatic reaction initiated by the addition of H_2O_2 to the reaction mixture containing reduced glutathione, nicotine adenosine dinucleotide phosphate (NADPH) and glutathione reductase.[15]

The determination of nitrite, the steady last product of NO radicals, is most frequently used as a measure of NO generation. The nitric acid measurement was performed

Table 1. Experimental groups and study protocol

Groups	N	Chemicals	(7, 8 and 9 days)	Treatment (16.23., and days)
Group 1: Control	8		Olive oil	-
Group 2: Olive oil	8	DMSO	-	-
Group 3: DMSO	8	-	-	-
Group 4: OCT	8	-	-	OCT
Group 5: DEN	8	DEN	-	-
Group 6: 2-AAF	8	-	2-AAF	-
Group 7: DEN+AAF	8	DEN	2-AAF	-
Group 8: DEN+AAF+OCT	8	DEN	2-AAF	OCT

DMSO: dimethyl sulfoxide, DEN: diethyl nitrosamine, 2-AAF: 2-acetylaminofluorene, OCT: octreotide

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using the Griess procedure for the detection of nitrite levels.[16]

Statistical analysis

Statistical analysis was done with Statistical Social Science Package (SPSS) version 22.0. The data were at first tested for normal distribution using the Kolmogorov-Smirnov test and were found to be normal ($p>0.05$). One-way analysis of variance test was used for statistical analysis of biochemical data between groups. Multiple comparisons among the groups were made using Tukey's Honestly Significant Difference test. The data were expressed as means ± SD. Statistical significance was described as ($p<0.05$)

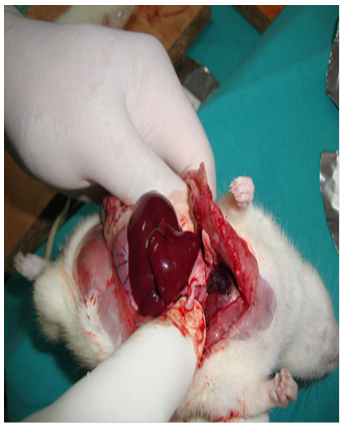
RESULTS

DEN and 2-AAF caused significant changes macroscopically as shown in **Fig. 1**. In **Table 2**, values of SOD, CAT, GSH-Px, MDA, NO, 8-OHdG and Hsp70 were given in the liver tissue. There was a significant increase in MDA (3.07±0.85), 8-OHdG (3.45±0.15) and Hsp70 (154.86±44.17) levels in group 7. Group 8 showed a significant decrease in MDA (2.21±0.17), 8-OHdG (2.95±0.09) and Hsp70 (67.9 ±18.97) levels. CAT (55.4±20.2), GSH-Px (1.25±0.2) activity was significantly reduced in group 7 when compared with Group 1

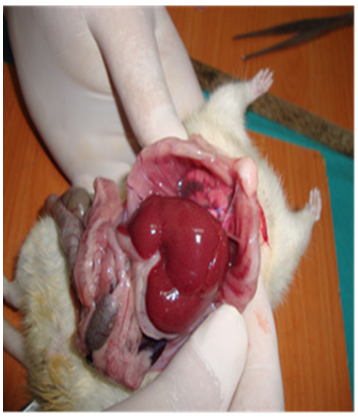
(CAT: 116.5±32, GSH-Px: 3.11±1.00). CAT (99.75±12.8) and GSH-Px (2.2±1.00) activities of Group 8 increased compared to CAT (55.4±20.2) and GSH-Px (1.25±0.2) activities of Group 7. SOD activity of Group 8 (4.21±1.32) increased compared to Group 7 (3.81±0.91).

DISCUSSION AND CONCLUSION

Hepatocellular carcinoma is one of the most common cancers. DEN initiates the preneoplastic liver lesion while 2-AAF is the trigger.[1] Nitrosamines are widely accepted as carcinogenic compounds, but require metabolic activation to perform their cytotoxic and carcinogenic activities. DEN is a nitrosamine compound that induces hepatic carcinogenesis. It has also been shown in other studies that DEN



Group 1. Macroscopic view. No significant change was observed in the livers of Group 1 animals.



Group 5. Macroscopic view. DEN caused significant damage to the liver.

Group 7. Macroscopic view. DEN+2-AAF caused significant damage to the liver.

Figure 1. Macroscopic presentation of liver tissue of some groups.

Table 2. Mean±SD values of oxidative stress parameters of experimental groups

Liver Tissue	Group 1 Mean±SD	Group 2 Mean±SD	Group 3 Mean±SD	Group 4 Mean±SD	Group 5 Mean±SD	Group 6 Mean±SD	Group 7 Mean±SD	Group 8 Mean±SD
SOD (U/mg protein)	2.46±0.6	2.78±0.8	2.81±0.81	4.42±2.4	4.16±1.56	5.48±2.48	3.81±0.91	4.21±1.32
CAT (U/mg protein)	116.5±32.6	87.06±31.8	75.05±32.80	95.9±36.1	52.8±18.9	62.3±16.57	55.4±20.2	99.75±12.8
GSH-PX (U/mg protein)	3.11±1.00	1.25±0.2	2.04±0.75	1.8±0.47	2.1±1.0	2.04±0.75	1.25±0.2	2.2±1.00
MDA nmol/mg protein	2.21±0.17	2.46 ±0.12	2.41±0.40	2.16 ±0.33	2.74±0.24	2.29±0.35	3.07±0.85	2.16±0.65
NO (U/mg protein)	0.39±0.037	0.38±0.030	0.38 ±0.033	0.41±0.044	0.42±0.038	0.43±0.049	0.47±0.028	0.42±0.042

Table 3. Mean±SD values of experimental groups Hsp70 and 8-OHdG

Liver Tissue	Group 1 Mean ± SD	Group 2 Mean ± SD	Group 3 Mean ± SD	Group 4 Mean ± SD	Group 5 Mean ± SD	Group 6 Mean ± SD	Group 7 Mean ± SD	Group 8 Mean ± SD
8-OHdG (ng/ml)	2.95±0.09	2.95±0.05	3.03±0.13	3.22±0.207	3.36±0.11	3.42±0.22	3.45±0.15	3.30±0.14
Hsp70 (ng/ml)	67.9±18.97	68.19±18.31	91.41±15.34	67.65±27.34	129.95±55.97	117.45±42.41	154.86±44.17	103.67±33.93

increases lipid peroxidation and that an increase in lipid peroxidation product may result in a tumor.[17] The discussions on octreotide's treatment in hepatocellular carcinoma are still ongoing: octreotide has been shown to inhibit carcinoid tumor cells in several clinical trials and in various experimental models. Animal tumor model studies have shown the inhibition of somatostatin and tumor growth in human tumor cell lines. Furthermore, some studies have demonstrated that octreotide possesses antiproliferative anti-lipid peroxidation properties.[18,19] MDA is the last major product of lipid peroxidation; it is mutagenic and carcinogenic. The manufacture of MDA and its reaction with DNA to form mutagenic adducts provides a connection between lipid peroxidation.[20] Previous studies have reported that 2-AAF and DEN increase MDA levels. We observed that our study increased MDA levels more when given with DEN + 2-AAF. [21]

Octreotide has been determined that cardiomyocytes increase the antioxidant capacity by activating the Nrf2 pathway. As shown in **Table 2**, we observed that octreotide reduced MDA levels. This was concordant with the previous study. This may be due to the fact that octreotide is a free radical scavenger. SOD, CAT and GSH-Px are significant endogenous antioxidant enzymes. SOD catalyzes the breakdown of superoxide anion into H_2O_2 and oxygen; CAT and GSH-Px are the enzymes playing role in the reduction of H_2O_2 to water.[22]

Indeed, an important feature of tumor inducers such as DEN and 2-AAF stimulates the production of ROS. These enzymes reduce or clear ROS. For example, SOD is an important antioxidant defense in almost all living cells exposed to oxygen.[23] In one study, DEN and 2-AAF determined that SOD activity increased in rat liver cells.[24] We think that SOD activity increases in Group 6 and Group 8 are the response to free radicals produced by DEN and 2-AAF excessively. There are many studies on the reduction of CAT and GSH-Px activities in DEN and 2-AAF induced groups. However, the activity of these enzymes has increased in the treatment groups (Vitamin C, L-carnitine, blueberry).[25-26]

Octreotide may have triggered these enzymes at low doses. NO reacts with peroxy and oxyradicals generated pending the process of lipid peroxidation. NO is known to inhibit the repair of DNA damage to proteins. NO is a biological reporter in physiological and pathological disorders. ROS species cause more than 20 oxidative base damage products in DNA. N-nitroso compounds cause single-strand breaks in DNA in liver cells.[27] The 8-OHdG is highly sensitive among the bases that are exposed to this challenge. It is the most common oxidative DNA damage indicator. In our study, DEN and 2-AAF increased the level of 8-OH dG.[28] Nakae et al. reported that the level of 8-OHdG, which reflects oxidative damage to DNA, increased significantly with DEN in a dose-dependent manner in liver DNA.[29]

The accumulation of ROS in cells has been shown to adversely affect cells. In response to ROS, which may lead to oxidative stress, it is not surprising that the highly regulated proteins called "heat shock proteins," protect the cells by increasing the level of expression. However, this comment was developed with the addition of the Hsp70 family. Therefore, it has been suggested that the cytoprotective effects of Hsps may be due to the protection of DNA breaks in response to ROS-induced stimuli. Hsp70 is normally kept at low levels. However, it can be stimulated under conditions that damage the protein. Hsp70 is expressed in many types of cancers such as breast, colon, liver, prostate and esophageal carcinomas.[30]

Hsp70 levels increased significantly in group 5 and group 7. However, OCT did not significantly reduce Hsp70 levels. Nevertheless, in our study, cells leading to tumor growth also support the idea that Hsp70 levels **(Table 3)** may increase and that OCT partially reduces DNA breaks. After administration of DEN and 2-AAF, liver lipid peroxidation levels were confirmed to be elevated and found to alter some antioxidant parameters. At the dose we gave it (300 μg/kg), octreotide has a positive effect on the antioxidant defense system. In summary, octreotide may also be an inhibitor in tumor-bearing cells and may be a factor in reducing oxidative stress. More work needs to be done to understand this.

Conflict of interests

The authors declare that there is no conflict of interest in connection with the work presented.

Ethical Issue

The study protocol was approved by the Animal Ethics Review Committee of the Faculty of Medicine in University of Kahramanmaras Sütcü Imam.

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Изучение влияния октреотидного агента на окислительный стресс, 8-гидроксигуанозин на экспериментальной крысиной модели с печёночным канцерогенезом

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Абстракт

Введение: 2-AAF и DEN являются токсичными агентами, часто используемыми для стимуляции развития опухоли у лабораторных животных.

Цель: Целью данного исследования было изучение влияния DEN-индуцированного и 2-AAF-добавленного канцерогенеза у крыс-альбиносов Wistar.

Материалы и методы: В этом исследовании 64 крысы линии Вистар были разделены на 8 групп. DEN (175 мг / кг) индуцировал, а 2-AAF (20 мг / кг) стимулировал печёночный канцерогенез у крыс. Использовали ингибитор роста опухоли октреотид (300 μг/кг). Крыс умерщвляли в конце эксперимента и их ткани печени использовали для тестирования. Измеряли спектрофотометрическую активность SOD, GSH-Px и CAT, а также уровни NO и MDA. Кроме того, Hsp70 и 8-OHdG измеряли методом ELISA.

Результаты: В группе 7 уровни MDA, 8-OHdG и Hsp70 были значительно повышены. Кроме того, активность SOD и GSH-Px была значительно снижена в этой группе. Уровни MDA, 8-OHdG и Hsp70 были значительно снижены в группе 8, которую лечили октреотидом.

Заключение: DEN и 2-AAF вызывают очень серьезное повреждение печени. Октреотид защищает печень от канцерогенеза, повышает активность клеточных антиоксидантных ферментов и помогает уменьшить повреждение ДНК. Виду этого октреотид может играть роль ингибитора опухолевых клеток и может уменьшать окислительный стресс.

Ключевые слова

окислительный стресс, октреотид, 2-AAF, DEN, Hsp70