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Cytochrome P-450 Reductase Activities in the Liver Microsomes of Diabetic Rats Induced With Some Chemical Solvents

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Abstract: Cytochrome P-450 Reductase enzyme is an important enzyme providing electrons to the microsomal cytochrome P-450 enzyme system metabolizing some endogenous subtances forming in the liver, drugs, chemical carcinogens insecticides and food preservation materials.

In this work, it has been attempted to determine the alterations brought about in the enzyme activities by oral administering acetone, isopropanol and ethanol to chemically induced diabetic rats. The rats in whom diabetes was induced diabetic by 65 mg/kg streptozotocin (stz) were divided into four main groups as a stz alone (group I), stz + aceton (group II), stz + isopropanol (group III), stz + ethanol (group IV) respectively. The control group was given physiological saline. Then liver microsomal fractions were obtained both from the control and experimental groups of rats. The enzyme activities were determined by using Strobel's method.

As a result of these inverstigations the cytochrome P-450 reductase activities in the controls was determined to be 2,41 U/mg protein. It was observed that compared to that of the control group the enzyme were activated by 95 % in the first group, 286 % in Group II, 205 % in Group III and 97 % in Group IV.

Key Words: Cytochrome P-450 Reductase, Diabetic Rats, Chemical Solvents.

Introduction

Cytochrome P-450 and Cytochrome P-450 Reductase are components of the Monooxigenase enzyme system. This enzyme system takes place in most tissues, particularly the liver. The enzymes are localized embedded in the endoplasmic reticulum membranes of the cells. It has been reported that the liver microsomal monooxigenase system plays an important role in the biotransformation of steroids, fatty acids, drugs, carcinogens and other xenobiotics (1).

Cytochrome P-450 Reductase enzyme is an enzyme containing FAD and FMN, 78.000 in mol. weight, as a prosthetic group. The function of this enzyme in the microsomal system is catalyzing the electron transfer from NADPH to cytochrome P-450 or another acceptor protein. The electron flow takes place in the direction of NADPH $\rightarrow$ FAD $\rightarrow$ FMN $\rightarrow$ Cyt P-450 or acceptor protein (2, 3).

It has been reported that the microsomal monooxigenase system activity is determined by measuring the Cytochrome P-450 Reductase and mass concentration of P-450 (4). Cytochorome P-450 Reductase is also a basic component of Nitrosodimethylamine Demethylase (NDMAd) playing a role in the nitrosoamine metabolism, member of monooxigenase (4, 5, 6). The effects of many chemical inducers on the NDMAd enzyme have been investigated. Yang et al. have shown that the microsomal NDMAd activity was increased by fasting, diabetes, acetone, isopropanol and ethanol, (7).

The aim of this work was to investigate the activity of the Cytochrome P-450 Reductase enzyme, a microsomal NDMAd component, in the presence of different inducers in rats with experimentally induced Diabetes Mellitus.

Materials and Methods

Animals and Injections

Thirty Wistar Albino species rats, each weighing 150-260 gr. were used in our experiments. Of these rats, 6 constituted the control group and were subjected to physiological saline injections i.p. As for the remaining 24 rats, they were given 65 mg/kg streptozotocin (STZ) (pH: 4.50 in 0.05 M citrate buffer) i.p. in order to develop experimental diabetes. Glucose concentrations in

Table 1. Physiological Parameters of Rats Injected STZ.•

Injection	No.of rats	Body Weight Inital	Body Weight Final	Liver Weight (gr)	Blood Glucose (mg/dl)
Control	6	208±11	230±10	7,45±0,35	90±08
I. Streptozotocin(Stz)	6	220±22	235±24	7,15±0,65	262±40
II. Stz.+Acetona	5*	264±03	275±05	7,85±0,45	256±05
III. Stz.+İsopropanol	6	183±09	197±13	7,10±0,15	344±08
IV. Stz.+Ethanol	6	150±03	162±03	5,75±0,85	334±41

*One of the rats died beyond our control

• All values are given as ±SD

Table 2. Cytohcrome P-450 reductase Activities (U/mg protein)

Control	Group I Stz	Group II Stz+Acetone	Group III. Stz+İzopropanol	Group IV Stz+Ethanol
2,84	4,76	9,42	7,58	4,48
1,91	4,48	8,28	8,00	5,14
2,99	5,04	10,20	6,74	4,83
2,85	4,67	9,90	7,24	5,52
2,38	4,10	8,62	7,43	4,43
1,91	5,24	-	7,24	4,28

the plasma obtained from the blood taken from the tail veins of the rats were measured to control the development of diabetes. Ten days after this application, development of diabetes was determined and the rats were divided into 4 separate groups was made of 5 rats. The control group and the first group of diabetic rats were decapitated during this period of ten days. The second group of rats were administered 2.5 ml/kg isopropyl alcohol 24 hours before decapitation by gavage. For three days, 15 % ethanol was added to the drinking water of 5 rats making the last group of rats and the animals were decapitated at the end of this period (7, 8, 9).

Microsomal Pellet

The rats were anesthesized by ether after fasting for 8 hours and blood was obtained from their hearts to determine their blood-glucose levels (based on Glucose oxidase method). The liver was extirpated, 3 ml 154 mM KCl, 10 mM EDTA added per fresh tissue gram and homogenized in B.Broun tissue homogenerator in four strokes. It was centrifuged for 30 minutes at +4°C at 15.000 g in order to remove the membrane fragments and mitochondria. The supernatant obtained was centrifuged for 60 min at +4°C at 105.000 g using Beckman L5-75 B Ultracentrifuge. In order to obtain purer microsomal pellet, the pellet was resuspended using fresh buffer. It was centrifuged again at 105.000 g (10). The determination of protein in microsomal pellet was performed by Coomassie Brillant Blue method (11). Bovine serum Albumine was used as the protein standard.

NADPH-Cytochrome P 450 Reductase Activities

The enzyme activities were determined by Strobel Method(12). The method is based on the spectrophotometrical measurement of the reduction speed of cytochrome c in a medium containing NADPH at 50 nm. 75 µM Cytochrome c in 0.3 M phosphate buffer (pH:7.8), 0.1 mM NADPH and microsomal fraction with known mg/ml protein value as enzyme source were added to 1 ml reaction medium. The decrease in the absorbance at 550 nm and 37°C was followed up for 5 min. 1 unit (U) activity was given as 75 µM amount of Cytochrome c recducing enzyme per mg protein in 1 min at 37°C. Extinction Coefficient: 21.0 mM^{-1} cm^{-1}.

Results

Producing diabets in rats

It was observed that fasting blood glucose levels of rats administered STZ. increased 2.5-4 times compared to those of the control group. The blood glucose level in the control group was measured to be 90 mg/dL on the average. It was noted that the increase in the

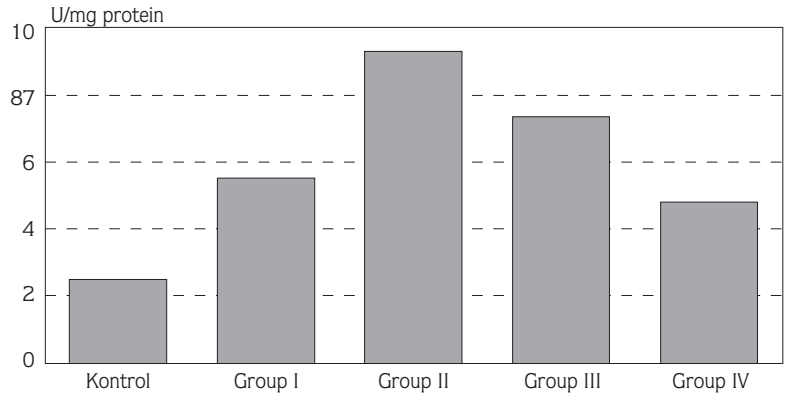

Figure 1. Cytochrome P-450 Reductase Activities

bodyweights of diabetic rats before and after injections was less than that of the controls (Table 1).

The Effect of chemical Inducers on the Cytochrome P-450 Reductase Activity

The reductase activity in the control group was found to be 2.41 U/mg protein on the average. In rats in which diabetes was induced by only administering STZ., the reductase was determined to be 4.75 U/mg protein (Table II). This shows 95% increase in the activity when compared with that of the controls. After the development of diabetes, further increases were determined in the reductase activity in groups administered chemical solvents. An increase of 286 % was observed in the reductase activity in Group II administered STZ. + Acetone, 205 % increase in Group III administered isopropanol and 97 % increase in Group IV administered STZ. + Ethanol compared to that of the control group (Table II, Figure)

Discussion

The interaction of toxic-acting chemicals entering our body by foodstuffs or environmental pollution in our daily life with the liver tissuse microsomal monooxygenase enzyme system has been the subject of many investigations. The main aim of these investigations we have undertaken is to study the interaction between the nitrosocompounds having a carcinogenous effect and the monooxygenase enzyme system.

In this stage of this very comprehensive field of investigation, the activity of NADPH-reductase functioning as an electron carrier in the enzyme system is being investigated in the presence of various chemical stimulants.

The effects of parametres such as fasting and development of diabetes together with chemicals such as aceton, isopropanol, benzene and ether on the NADPH-Reductase activity have been investigated (13.14.15). A low increase has been determined in the enzyme activities of Spraque Dawley rats given aceton and isopropanol (16). As for the rats consuming chronic alcohol a ligh level of increase has been observed in the microsomal ethanol oxidation system, cytochrome P-450 and reductase activities (17). Following phenorbarbital and pyrethrum injections, reductase activity in guinea-pigs increased but after 2,4,5-T isoctylesther injection no significant changes were observed in the liver, lung and kidney enzyme activities (18).

It has been demonstrated that diabetes induced with STZ in the rats increased NDMAd activity and cytochrome P-450ac (an acetone/ethanol-inducible from) in rats. Chemical diabetes developed using STZ and alloxan in rats and diabetes developing by it self were observed to affect all the three parameters in the results obtained. These were NDMAd activity, cytochrome P-450 level and cytochrome P-450 level and cytochrome P-450 mRNA level (8).

The investigations made earlier have demonstrated the relation between the experimental diabetes and reductase activity. Our aim in this work undertaken is to investigate to what extent the chemicals the diabetic living beings are confronted with bear an effect on some toxic compounds. For this pourse, the reductase activity carrying electrons to the monooxigenase has been examined under the experimental conditions mentioned. Previous reference were used in the application of the chemicals in the our experiments. The ethanol application method has been considered to play a role in preventing an expected increase in the activity.

In conclusion, it has been observed that the chemicals applied on diabetic rats have caused a greater increase in the enzyme activities. These findings indicate that toxic radicals on a large scale. For this reason, it has been

considered that the exposure of diabetic organisms to alcohol and isopropanole, particularly in diabetes with aceton production, carries more risks in health.

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