J. Korean Soc. Food Nutr.
20 (3), 203~208 (1991)

한국영양식량학회지

Effect of Pretreatment with Nicotinamide on Changes in the Hepatic Metabolizing Enzyme System Induced by Streptozotocin

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Abstract

The present study was undertaken in order to elucidate the effect of pretreatment with nicotinamide on changes in the hepatic metabolizing enzyme system induced by streptozotocin (STZ). In rats, STZ(50mg/kg) administered by tail vein caused a significant rise in hepatic aniline hydroxylase and a decrease in aminopyrine N-demethylase when compared to control ($p<0.05$). Pretreatment with nicotinamide inhibited these effects ($p<0.05$). Similarly, STZ induced changes in hepatic microsomal cytochrome P-450 activity were inhibited by pretreatment with nicotinamide($p<0.05$). However, changes in UDP-glucuronyl transferase and sulfotransferase activity were not significantly different($p>0.05$). Pretreatment with nicotinamide also prevented STZ induced increases in glutathione S-transferase activity when compared to the control($p<0.05$). These results suggest that nicotinamide pretreatment suppresses STZ-induced changes in the hepatic metabolizing enzyme system.

Key words : nicotinamide, streptozotocin (STZ), mixed function oxidase, glutathione S-transferase

INTRODUCTION

Streptozotocin(STZ) is an antineoplastic antibiotics produced by *Streptomyces achromogenes*. The commercially available drug is prepared synthetically. STZ is a glucosamine-1-methyl-nitrosourea compound which is chemically similar to other antineoplastic agents (e.g., carmustine), but which differs structurally from other nitrosourea derivatives in the absence of a chloroethyl side chain and the presence of a D-glucopyranose moiety. This agent has antineoplastic and diabetogenic effects in several animal species including mice, dogs and monkeys. Thus it is used to produce an experimental model of diabetic animal[1,2].

Recently, many investigators[3,4] have shown that hepatic drug-metabolizing enzyme systems are changed in STZ-induced diabetic animal models especially the cytochrome P-450 mixed function oxidase and several other kinds of enzyme systems. STZ induced hepatotoxicity has been reported[5].

In general, all detoxification reaction can be assigned to one of 2 major categories: Phase I reactions involve the hepatic microsomal mixed function oxidase system, and Phase II reactions involve conjugation reactions[6].

Nicotinamide has been reported to inhibit the diabetogenic effect of STZ[7,8]. We examined the effect of nicotinamide on STZ-induced toxicity by measuring changes in hepatic metabolizing enzyme activity and other enzymes participating in Phase II reactions.

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MATERIALS AND METHODS

Male Sprague-Dawley rats (200-250g), with free access to commercial rat chow (Cheil Foods & Chemical Inc.) and water, were divided into four groups. Group 1 (7 rats) served as control; Group 2 (6 rats) received STZ only; Group 3 (6 rats) received nicotinamide only (Nico group); Group 4 (6 rats) received pretreatment with nicotinamide followed by STZ administration.

Nicotinamide (200mg/kg) was administered orally twice daily for 7 days to Groups 3 and 4. To simulate nicotinamide administration in Group 2(STZ only) saline was administered orally twice daily for 7 days prior to STZ administration.

Freshly prepared STZ (50mg/kg) in 0.01M citrate buffer, pH 4.5, was administered through the tail vein in Group 2; this same dose was administered to Group 4 following the 7 days of pretreatment with nicotinamide.

Groups 1 and 3 went through the same procedure, but only citrate buffer was administered. Two weeks after beginning the nicotinamide treatment (7 days after STZ administration) for 24 hours and anesthetized by carbon dioxide gas, blood samples were drawn from the heart, and the livers were perfused *in situ* with isotonic saline. Subsequently the livers were removed and microsome and cytosol fractions were prepared. Animals with blood glucose levels greater than 200mg/dl were judged to be diabetic. Cytosolic sulfotransferase and glutathione S-transferase activity were measured using the method of Dawsen et al.[9] and Habig et al.[10], respectively. Microsomal cytochrome P-450, aniine hydroxylase, aminopyrine demethylase, UDP glucuronyl transferase activity were measured using the method of Omura et al.[11], Imai and Sato[12], Nash et al.[13] and Reinke et al.[14], respectively. The protein was measured using the methods of Lowry et al[15].

Data was analyzed by analysis of variance with Duncan's new multiple range test for multiple comparison against the control. A probability(p) value less than 0.05 was judged to be significant.

RESULTS AND DISCUSSION

Change in microsomal cytochrome P-450 activity

Fig.1 presents the influence of nicotinamide pretreatment on changes in hepatic cytochrome P-450 levels induced by STZ.

In the group that received only STZ (Group 2) the P-450 activity was increased to 0.753±0.049 n moles/mg protein. This is a significant increase when compared with the control group (0.343±0.049 n moles/mg protein) ($p<0.05$).

Nicotinamide pretreatment inhibited this increase in P-450 activity (0.372±0.041 n moles/mg protein) following STZ administration.

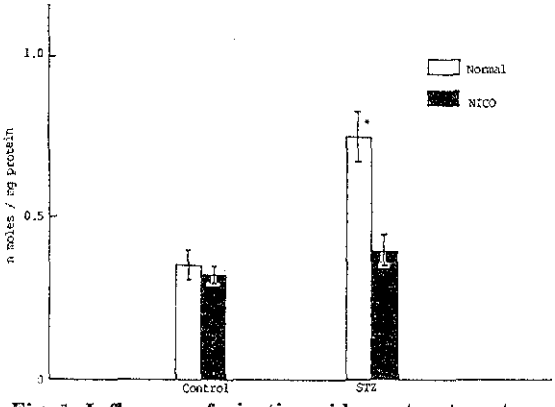

Fig. 1. Influence of nicotinamide pretreatment on the hepatic cytochrome P-450 levels in STZ-induced diabetic rats. Rats were orally administered nicotinamide (200mg/kg) twice a day for seven days, and then STZ(50 mg/ kg, tail vein) was injected. The rats were sacrificed seven days later for enzyme activity. Values are expressed as mean± SD for groups of six rats. * ; p< 0.05.

Changes in aniline hydroxylase and aminopyrine N-demethylase activity

Table 1 presents the changes in hepatic microsomal aniline hydroxylase and aminopyrine N-demethylase activity.

STZ administration increased the activity of aniline hydroxylase significantly by 127%, but decreased the activity of aminopyrine N-demeth-ylase by 45%, Nicotinamide pretreatment normal-ized these effects of STZ.

In the present study, we observed that STZ administration increased the activity of hepatic microsomal cytochrome P-450. Nicotinamide pretreatment inhibited this STZ-induced effect.

On the other hand, STZ administration reduced the activity of aminopyrine N-demethylase (which metabolizes Type I drugs) by 45%. Nicotinamide

pretreatment normalized this inhibitory effect of STZ. However, STZ administration increased the activity of aniline hydroxylase, which metabolizes Type II drugs, by 250% when compared to the control.

Nicotinamide pretreatment also normalized the changes in aniline hydroxylase activity induced by STZ.

Free radicals including electrophilic materials produced by exogeneous factors can induce toxicity *in vivo* and are rendered nontoxic by hepatic detoxification mechanism[16~18].

We studied the effect of nicotinamide on changes in free radical production and enzyme activity followed by STZ administration. Changes in the hepatic microsomal enzyme system also have been reported. Xenobiotics with toxicity are detoxified or inactivated by the metabolizing enzyme system of liver smooth endoplasmic reticulum and then eliminated[19]. The cytochrome P-450 mixed function oxidase system participates in this process and is divided into Type I and Type II reactions based on drug binding type and site existence[20].

Our results on STZ induced changes in hepatic enzyme activity are in agreement with previous reports[21]. These authors postulated that diabetes in rats induces the appearance of a unique cytochrome P-450 population that includes a diabetes-dependent 52,000 M.W. band. They suggest that this P-450 isozyme causes the increased metabolism of aniline.

Changes in UDP-glucuronyl transferase and sulfotransferase activity

Table 2 presents the changes of hepatic microsomal sulfotransferase and cytosolic UDP-glucuronyl transferase activity.

STZ administration tended to increase the activity of UDP-glucuronyl transferase by about 15% compared with the control, which was not statistically significant.

In rats treated only with nicotinamide, with or without STZ, UDP-glucuronyl transferase activity was not significantly different from that in the controls. There were no significant differences between groups on the level of sulfotransferase activity.

Changes in cytosolic glutathione S-transferase activity

Fig. 2 presents the changes in the activity of hepatic cytosolic glutathione S-transferase.

Table 1. Effect of nicotinamide on the hepatic microsomal metabolizing enzyme in STZ-induced diabetic rats

Treatment	Aminopyrine N-demethylase* (HCHO n moles/mg protein)	% of Control	Aniline hydroxylase* (P-aminophenol n moles/mg protein)	% of Control
CON	6.80±0.44(7)	100	0.72 ±0.09(7)	100
STZ	3.72±0.08*(6)	45	1.64±0.18*(6)	227
NICO	7.10±0.19(5)	104	0.74±0.11(5)	102
NICO + STZ	6.47±0.19(6)	97	0.87±0.05(6)	117

* : Each value is the mean ± S. D. , the number of observation is given in parenthesis
*: $p < 0.05$ vs normal (control) group

Table 2. Effect of nicotinamide on the hepatic microsomal UDP-glucuronyl transferase and cytosolic sulfotransferase activity in STZ-treated rats

Treatment	UDP-glucuronyl transferase*	Sulfotransferase**
CON	14.20±0.83(7)* NS	1.04±0.10(7)* NS
STZ	16.88±1.12(6)	1.28±0.06(6)
NICO	15.48±0.13(5)	1.36±0.08(5)
NICO + STZ	15.11±0.98(6)	1.67±0.11(6)

* : Each value is the mean ± S. D. , the number of observation is given in parenthesis
* : Activity of p-aminophenol glucuronide n moles / mg protein / min
**: Activity of p-nitrophenol sultate n moles / mg protein / min
NS : not significant

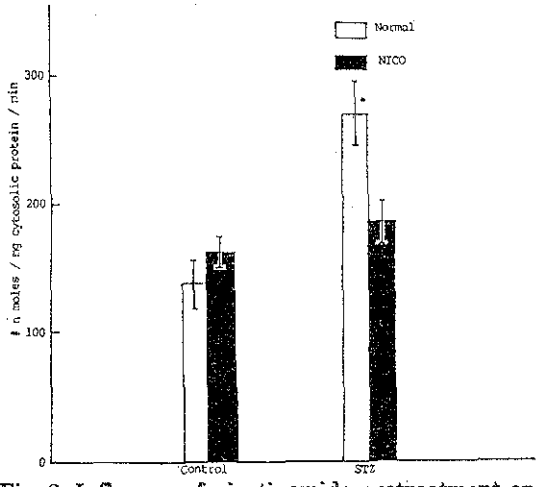

Fig. 2. Influence of nicotinamide pretreatment on the hepatic cytosolic glutathione S-transferase activity in STZ-induced diabetic rats. Rats were orally administered nicotinamide (200mg/kg) twice a day for seven days, and then STZ(50mg/ kg, tail vein) was injected. Rats were decapitated seven days after the last injection of STZ. Values are expressed as mean±SD for groups of six experiments. *; Significantly different from control, p < 0. 05.
 # ; conjugated 1, 2-dichloro-4 nitrobenzene.

Administration of STZ increased glutathione S-transferase activity to 274.1±73.2 n moles/mg protein from a control value of 145.2±16.0 n moles/mg protein($p<0.05$). Pretreatment with nicotinamide followed by STZ administration limited the increase of glutathione S-transferase activity to a value 179.3±31.2 n moles/mg protein.

It is our opinion that nicotinamide pretreatment has a specific pharmacologic effect on changes in hepatic microsomal metabolizing enzyme activity induced by STZ. Xenobiotics are primary eliminated by Phase I reactions and Phase II reactions of glucuronic acid, sulfate and glutathione[22, 23].

There was a slight increase in UDP-glucuronyl transferase activity following STZ administration but the difference was not statistically significant. STZ did not appear to effect the activity of sulfotransferase.

The administration of STZ significantly increased the activity of glutathione S-transferase (which participates in conjugation reactions) when compared to the control. Pretreatment with nicotinamide tended to limit this response. This increased activity of glutathione S-transferase by STZ administration is the same as previously reported. This increased activity is reported to be associated with nitroso derivatives because this increase is induced by chlorozotocin and diethyln-itrosamine[24]. These authors suggested that the increase of glutathione S-transferase is not induced by the STZ-induced diabetic state but is a direct effect of STZ itself.

Further investigation is needed to clarify whether the apparent supression of hepatic metabolic changes by nicotinamide pretreatment is more relted to a direct or indirect STZ induced effect.

The increased activity of glutathione S-transferase induced by STZ results from the detoxification of the toxic metabolites via the cytochrome P-450 system. The toxicity is due to an imbalance between free radical production and detoxification[18, 25, 26].

The results of this study suggest that nicotinamide pretreatment may regulate this imbalance.

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(Received March 12, 1991)

Streptozotocin에 의해 유도된 간 대사효소계의 변화에 미치는 Nicotinamide의 영향

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요 약

Streptozotocin (STZ)에 의해 야기되는 대사효소활성의 변동에 nicotinamide의 전처리가 어떠한 영향을 주는가를 알아보기 위하여 aniline hydroxylase, aminopyrine N-demethylase, cytochrome P-450 및 포합반응에 관여하는 UDP-glucuronyl transferase, sulfotransferase, glutathione S-transferase에 미치는 활성변동을 관찰하였다. STZ투여로서 간 microsomal aniline hydroxylase 및 cytochrome P-450의 활성이 증가되던 것이 nicotinamide 전처리로 대조군 수준으로 억제되었으며 aminopyrine N-demethylase의 활성은 STZ투여로 억제되던 것이 nicotinamide전처리로 도리어 대조군 수준으로 증가되었다. Microsomal UDP-glucuronyl transferase 및 cytosolic sulfotransferase의 활성에서는 STZ투여군에서나 nicotinamide전처리군에서도 대조군과 비교하여 별다른 영향이 없었다. Cytosolic glutathione S-transferase의 활성은 STZ투여로 증가되던 것이 nicotinamide전처리로 억제되었다.