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Cysteine antagonism of captafol induced toxicities in rats

1. Effects on hematological and serum biochemical values

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랫트에 있어서 captafol의 독성에 대한 cysteine의 방어 작용

1. 혈액학 및 혈청 생화학적 성상에 미치는 영향

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초록 : 과일 및 채소류의 곰팡이 억제제로 개발된 captafol은 인간 및 동물에서의 독성작용은 많이 보고된 바 있으나 이들의 독작용을 완화시키거나 치료할 수 있는 제제에 대한 연구는 별로 알려진 바 없다. captafol에 의한 혈액학 및 혈청 생화화적 변화에 따른 cysteine의 방어 작용을 확인할 목적으로 랫트에 5mg/kg 용량의 captafol을 1회 복강 투여하고 동시에 5, 58, 290 및 580mg/kg cysteine을 각각 투여하고 체중의 변화, 부검소견, 절대장기중량, 혈액학 및 혈청 생화학적 소견을 비교 분석 하였다. captafol 단독 투여군에서는 체중 및 간 장기중량 감소, 복강내 장기들의 섬유소에 의한 유착 및 복수 등이 현저하였으며 적혈구 수, hemoglobin 농도, 혈청 AST의 증가가 인정되었으며 또한 혈청 인지질 함량 감소가 확인되었다. 한편, cysteine 58mg/kg 이상의 용량군에서는 복강내 장기들의 유착 및 복수가 확인되지 않았으며 장기중량, 혈액학 및 혈청 생화학적 소견에서도 정상을 나타내었다. 이 연구 결과는 captafol의 중독증상에 대해 cysteine 및 sulfhydryl군을 가진 물질은 그 증세를 감소시키거나 완화시킬 수 있는 효과가 있음을 보여주고 있다.

Key words : captafol toxiciy, cysteine antagonism, hematology, serum biochemical value, rat

Introduction

Captafol, N-1,1,2,2-tetrachloroethylthio-tetrahydrophthalimide, is a phthalimide derivative which has been available for a number of years for the control of several fungus diseases of seeds, fruits, vegetables and ornamentals. The fungitotoxic action of phthalimide derivatives has been extensively studied, for example, captan disturbs calcium metabolism, uncouples oxidative phosphorylation and inhibits microsomal and lysosomal enzymes as well as DNA polymerase of liver nuclei[1-3].

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The mechanism of action of the phthalimide derivatives, particulary captan and folpet, has been intensively investigated and reviewed[4]. It has been suggested that when RN-$SCCl_3$ compounds react with thiols, the fungicide splits at the RN-S bond, the free imide is formed, and the thiol is oxidized to its disulfide. The liberated thrichloromethylthio($SCCl_3$) moiety can be transferred directly to thiol sites, or undergo dechlorination to yield thiophosgene($S=CCl_2$). Thiophosgene is highly reactive and can undergo rapid hydrosis in aqueous solutions to yield carbonyl sulfide HCl, and H_2S, or it may react with thiols and other cellular groups to form various additional products. Oxidation of thiols and reactivities of the $SCCl_3$ moiety and thiophosgene have been postulated to be bases of toxicity for these fungicides. However, not much is known about the biochemical effects of captafol other than inhibition of fungal growth.

Acute toxicity of captafol is relatively low in rats due to its degradation in the gastrointertinal tract(oral LD_{50}, 6500mg/kg); however, it is the highly toxic compound if administered intraperitoneally(ip the lethal dose, 6.2mg/kg in rats). Absorbed captafol given ip has been found to be very toxic to liver as measured by the inhibitions of liver microsomal enzymes and serum enzymes[5].

Captafol's closely related analogue captan is rapidly detoxified after incubation with S-9 fraction of rat liver homogenate, cysteine or rat blood[6]. The in vitro incubation of captan with rat liver microsomes has demonstrated that it is a strong inhibitor of microsomal cytochrome P-450, but the inhibition is almost completely prevented if a reduced glutathione(GSH) is included in the incubation media[7]. Form these results, we assumed that compounds which contain thiol group might also protect captafol toxicity in vivo. Therefore, we assumed that compounds which contain thiol group might also protect captafol toxicity in vivo.

The purpose of the this study was to examine whether ip administered cysteine which also contains thiols such as glutathione can afford protection against the toxicity of captafol on the liver and the hematological and serum biochemical values in rats.

Materials and Methods

Materials : Captafol, 94.5% purity, was obtained from Hannong company. Cysteine was purchased from Junsei chemical company(Japan).

Animal maintenance : SPF male Sprague-Dawley rats(KRICT, toxicology center breeding facility) were housed in a fully air-conditioned animal facility where the temperature(23±3℃) and relative humidity(50±10%) were kept constant, and the room was ventilated in rate of 13~18 times per hour and was lighted for 12 hours from 07:00~19:00. The animals were fed irradiated(2.0 Mrad of Co 60) laboratory rodent dies(Jeil feed Co, Daejeon Korea) and received sterilized water *ad livitum* during the experimental period. The rats(200~230g) were sperated and housed five to each polycarbonate cage.

Experimental procedure : Captafol was suspended in corn oil(Sigma chemical Co) using grinder and voltex and was injected intraperitoneally at a dose level of 5mg/kg body weight. Cysteine was dissolved in saline and injected intraperitoneally at a dose level of 5, 58, 290 and 580mg/kg body weight immidiately after captafol. The control group received both corn oil and saline. The animals were sacrificed by CO_2 at 24 hr following treatment. Blood samples were collected for hematology and serum biochemistry through the posterior vena cava before a complete autopsy is carried out at the termination of the study.

In hematology, erythrocyte count(RBC), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), and mean corpuscular hemoglobin concentration(MCHC) were determined by a hematological autoanalyzer(S-880 Coulter counter, Coulter electronics). Serum biochemistry was conducted to quantity serum levels of glutamic oxaloacetic transaminase(GOT), glutamic pyruvic transaminase(GTP), creatine phosphokinase(CPK), total bilirubin(TBIL), creatinine(CRE), blood urea nitrogen(BUN), total protein(TP), albumine(ALB), and phopholipid(PL) by biochemical autoanalyzer(JCA VX-1000, Jeol Co), sodium(Na) and potassium(K) by flame photometer(IL 943, Instrumentation laboratory) and chloride(CL) by Chloridemeter(C-200, AP, Jookoo

Co).

Organ weights were determined for liver and spleen.

Body weights were recorded at intervals of 3, 6, 12 and 24 hrs after treatment.

Statistical analysis : Mean and standard deviations were calculated. For control and treatment group the statistical significance was tested using the Student t-test. Especially, statistical significance of serum biochemical data was tested using the Dunnett's test.

Results

Body weights : As can be seen from table 1, ip administered captafol and captafol +5mg/kg cysteine caused significant depression of body weights 24 hours after treatment. But no captafol-related depressions of body weights were noted at cysteine dose level over 58mg/kg group(Table 1).

Necropsy findings : Ascites and adhesion of liver lobe were observed in both all animals of group I and II, but this findings of group II were less severe than group I. Fibrin clot in abdominal cavity was only found in group I. But no necropsy finding associated with the captafol was found at cysteine dose level over 58mg/kg group(Table 2).

Organ weitht : Absolute weights of liver were significantly decreased in group I and II. However, no captafol-related depressions of liver weights were noted at cysteine dose level over 58mg/kg group. No spleen weight change associated with captafol and captafol+cysteine treatment was observed(Table 3).

Table 1. Mean body weight change in male rats treated with captafol and cysteine+captafol

Groups	0 hr	3 hrs	6 hrs	12 hrs	24 hrs
Control	224.57[a)] ± 7.23	220.00 ± 8.24	217.50 ± 7.14	221.07 ± 8.20	228.58 ± 9.36
I	227.24 ±11.49	221.17 ±10.14	219.33 ±10.27	212.94 ±10.87	208.91* ± 9.88
II	225.26 ± 9.43	221.35 ± 9.78	219.39 ±9.85	213.25 ±11.62	209.25* ±11.86
III	227.74 ±10.37	224.41 ±10.13	222.02 ± 9.06	223.24 ±11.36	222.85 ± 8.75
IV	228.46 ±10.43	224.94 ± 9.81	222.31 ± 9.58	227.21 ±11.29	233.38 ±10.71
V	228.49 ± 9.20	227.77 ±11.00	225.32 ±10.24	228.71 ± 9.73	234.97 ±11.39

Control: Corn oil and saline were injected as a vehicle.

I : 5mg/kg captafol

II : 5mg/kg captafol+5mg/kg cysteine

III : 5mg/kg captafol+58mg/kg cysteine

IV : 5mg/kg captafol+290mg/kg cysteine

V : 5mg/kg captafol+580mg/kg cysteine

* : Significantly different from control value at $P<0.05$

a) : mean±SD(n=5)

Table 2. Necropsy findings in male rats treated with captafol and cysteine+captafol

Groups	Ascites	Adhesion of liver lobes	Fibrin clot in abdominal cavity
Control	0	0	0
I	100	100	100
II	100	100	0
III	0	0	0
IV	0	0	0
V	0	0	0

Results are expressed as the percentage of animals tested(n=5).

Control: Corn oil and saline were injected as a vehicle.

I : 5mg/kg captafol

II : 5mg/kg captafol+5mg/kg cysteine

III : 5mg/kg captafol+58mg/kg cysteine

IV : 5mg/kg captafol+290mg/kg cysteine

V : 5mg/kg captafol+580mg/kg cysteine

Table 3. Absolute liver and spleen weights in male rats treated with captafol and cysteine+captafol

Groups	Control	I	II	III	IV	V
Liver	11.17[a)] ±0.88	7.91** ±0.31	8.09** ±0.47	10.28 ±0.95	11.62 ±0.83	11.73 ±0.87
Spleen	0.53 ±0.07	0.48 ±0.05	0.50 ±0.07	0.66 ±0.12	0.60 ±0.10	0.60 ±0.05

Control: Corn oil and saline were injected as a vehicle.

I : 5mg/kg captafol

II : 5mg/kg captafol+5mg/kg cysteine

III : 5mg/kg captafol+58mg/kg cysteine

IV : 5mg/kg captafol+290mg/kg cysteine

V : 5mg/kg captafol+580mg/kg cysteine

** : Significantly different from control value at $P<0.01$

a) : mean±SD(n=5)

Hematology : The effect of cysteine on the hematologcial parameters male rats induced by captafol are shown in Table 4. The number of RBC in group I and II was significantly decreased compared to the control group. The concentration of hemoglobin in group I was significantly increased from that of control group. Though that of group II was increased, it was not significant compared to the control group. The percentage of HCT was significantly increased in group I and II compared with the control group.

However, no changes in the WBC, MCV, MCH, and MCHC were observed under this experimental conditions.

Serum biochemistry : The protective effect of cysteine on the serum biochemical parameters induced by captafol are shown in Table 5. The activities of GOT in the group I and II wendsigniticantly increased from that of control group. The concentrations of phospholipid in the group I and II were significantly decreased from that of control. Though the concentration of inorganic phophorus in group I and II was increased, it was not significant compared to the control group.

However, no changes in the GPT, BUN, CRN, TP, ALB, CPK and CL were observed under this experimental condition.

Serum potassium and sodium : Significant decreament of K^+ concentration was observed in group V. No change in the Na^+ concentration was observed under this experimental conditions(Table 6).

Table 4. Hematological values in male rats treated with captafol and cysteine+captafol

Groups	WBC ($\times 10^3/\mu l$)	RBC ($\times 10^6/\mu l$)	HGB g/dl	HCT %	MCV fl	MCH pg	MCHC g/dl
Control	11.84[a)] ±0.87	6.93 ±0.33	14.34 ±0.69	46.68 ±1.00	63.04 ±1.85	20.68 ±0.44	32.86 ±1.00
I	12.34 ±1.30	8.18** ±0.17	16.76** ±0.31	51.02** ±1.29	62.30 ±0.56	20.48 ±0.11	32.88 ±0.42
II	10.96 ±4.28	7.64** ±0.23	15.24 ±0.65	47.46** ±1.55	62.10 ±1.09	19.92 ±0.33	32.08 ±0.61
III	12.18 ±1.65	6.86 ±0.23	14.28 ±0.42	43.74 ±1.24	63.20 ±3.36	20.90 ±1.08	32.74 ±0.42
IV	11.40 ±3.53	6.72 ±0.31	13.62 ±0.19	42.46 ±0.93	63.30 ±1.62	20.32 ±0.69	32.12 ±0.29
V	9.82 ±1.32	6.87 ±0.33	13.84 ±0.65	42.70 ±1.79	62.18 ±2.31	20.22 ±0.73	32.48 ±0.58

Control: Corn oil and saline were injected as a vehicle.

I : 5mg/kg captafol

II : 5mg/kg captafol+5mg/kg cysteine

III : 5mg/kg captafol+58mg/kg cysteine

IV : 5mg/kg captafol+290mg/kg cysteine

V : 5mg/kg captafol+580mg/kg cysteine

** : Significantly different from control value at $P<0.01$

a) : mean±SD(n=5)

Table 5. Serum biochemistry in male rats treated with captafol and cysteine+captafol

Groups	GOT IU/l	GPT IU/l	BUN mg/dl	CRN mg/dl	TP g/dl	ALB g/dl
Control	88.56[a] ±16.10	58.76 ±5.99	16.78 ±0.80	0.15 ±0.03	5.70 ±0.46	4.05 ±0.22
I	163.49** ±28.70	44.26 ±8.90	17.49 ±2.18	0.15 ±0.05	6.19 ±0.16	4.08 ±0.15
II	137.74** ±16.10	43.09 ±2.94	15.08 ±2.52	0.18 ±0.04	6.14 ±0.19	4.13 ±0.21
III	101.73 ±17.86	54.60 ±16.34	17.29 ±3.22	0.14 ±0.08	5.90 ±0.90	4.08 ±0.70
IV	91.47 ±19.15	77.72* ±15.13	18.02 ±2.87	0.19 ±0.03	6.39 ±0.25	4.53 ±0.20
V	81.95 ±4.40	66.40 ±8.06	17.54 ±1.59	0.15 ±0.02	6.38 ±0.15	4.45 ±0.18

Groups	IP mg/dl	CPK IU/l	PL mg/dl	CL mmol/l
Control	12.50 ±1.86	276.83 ±78.81	178.42 ±16.97	91.8 ± 5.32
I	14.47 ±1.09	226.52 ±51.56	140.78* ±15.22	92.8 ± 1.91
II	14.73 ±0.74	207.32 ±50.27	142.47** ±19.66	95.4 ± 2.76
III	12.59 ±3.00	207.66 ±88.46	182.70 ±30.84	88.3 ±19.68
IV	12.91 ±0.87	191.07 ±24.41	190.72 ±15.44	96.3 ± 3.44
V	12.30 ±0.90	205.00 ±36.40	195.64 ±14.76	96.4 ± 0.63

Control : Corn oil and saline were injected as a vehicle.

I : 5mg/kg captafol

II : 5mg/kg captafol+5mg/kg cysteine

III : 5mg/kg captafol+58mg/kg cysteine

IV : 5mg/kg captafol+290mg/kg cysteine

V : 5mg/kg captafol+580mg/kg cysteine

* : Significantly different from control value at $P<0.05$

** : Significantly different from control value at $P<0.01$

[a] : mean±SD(n=5)

Table 6. Concentration of serum K^+ and Na^+ in male rats treated with captafol and cysteine+captafol

Groups	Potassium	Sodium
Control	7.16±0.80[a)]	138.8±2.86
I	6.60±0.93	137.2±1.79
II	7.26±1.14	139.6±2.19
III	7.00±1.21	131.8±0.84
IV	6.42±1.38	140.6±1.82
V	5.82±0.31**	141.2±1.48

Control: Corn oil and saline were injected as a vehicle.
I : 5mg/kg captafol
II : 5mg/kg captafol+5mg/kg cysteine
III : 5mg/kg captafol+58mg/kg cysteine
IV : 5mg/kg captafol+290mg/kg cysteine
V : 5mg/kg captafol+580mg/kg cysteine
** : Significantly different from control value at $P<0.01$
a) : mean±SD(n=5)

Discussion

Previous experiment has demonstrated that a single intraperitoneal dose of captafol(5mg/kg) given to rats caused a toxic liver injury as manifestied by inhibition of the hepatic microsomal drug metabolizing enzyme system and serum enzymes[5].

As can be seen from Table 2, 3 and 5, i.p. administred captafol caused ascites, adhesion of liver lobes, significant depression of absolute liver weights and significant depression of GOT activity. From these results, it was suggested that captafol caused the liver damage.

As can be sen from Table 4, the significant increment of the number of RBC and hematcrit value might be related with ascites, one of the toxic signs of phthalimide derivatives. It was postulated that these results arised from leaking of some of plasma component from blood vessels.

There was significant increment of hemoglobin (Table 4). It has been reported that the erythrocyte membrane contains multiple classes of sulfhydryl groups and the alteration of any of these has a varous of effects on cell permeability[8]. Sutherland et al[9]. suggested that SH reagents react with the sulfhydryl groups of mechanism, captafol might be causing RBC cell damage to result the leakage of hemoglobin into the blood.

As shown in Table 5, captafol significantly decreased serum plasma phospholipid level. Peeples and Dalvi[10] suggested that phthalimide derivatives and its metabolites may be causing liver damage through lipid peroxidation of microsomal membranse. From this report, we presumed that capafol may be causing phopholipid peroxidation to result the decrement of phospholipid concentration in serum. Kumer et al[11]

reported that captafol caused an increase in osmotic fragility of the cells and caused a rapid increase in the efflux of intra cellular potassium. We also presumed that captafol caused a increase of potassium concentration in serum. But our findings in this study, no change in the serum potassium concentration was observed. This result might be related with ascites, that is, captafol caused leaking of plasma component espacially potassium from blood vesseles.

From our results in the study, captafol caused the liver and hematological damages. This toxic action of captafol may be attributed to its reaction with insoluble cellular thiols[12].

By contrast administration of cysteine immidiately after captafol dramatically prevented the captafol induced liver injury decrement of body weight and hematological abnormalities as reflected in normal or near normal values of these parameters at cysteine dose level over 58mg/kg. The work of Lukens and Sisler[13] showed that the toxicity of captan, a compound structurally related to captafol, can be reversed by sulfhydryl containing compounds such as cysteine, homocysteine and glutathione. Though 5mg/kg of cysteine the same amount of captafol was administer with captafol, it was not enough to prevent the captafol toxicity. Owens[14] reported that four sulfhydryl groups were disappeared for each molecule of captan reacted. From this report, we postulated that 4 moles of cysteine can completely ract with 1 mole of captafol. Our findings support this hypothesis. Collectively, the results reported here indicate that ip administered captafol is toxic to liver, hematological and serum biochemical parameters but when cysteine was immidiately injected ip after captafol no significant changes in these parameters were observed in the treated animals as against the control animals.

This suggests that cysteine may have detoxified captafol protecting the liver and blood especially RBC from its toxicity. However, further studies are needed to establish the mechanism of ascites induced by captafol and analysis the ascites whether serum components leak from blood.

Summary

This experiment was carried out to study the preventive effect of cysteine on the toxicities of captafol to the hematological and serum biochemical values. A single dose of captafol(5mg/kg BW, ip) was given to male Sprague-Dawley rats and its toxicities were evalutated by body weitht changes, autopsy findings, absolute organ weight, and hematological and serum biochemical parameters.

The single dose of captafol caused significant decreases in body weitht, and absolute liver weight, ascites, fibrin clot in abdominal cavity, adhesion of liver lobes significant elevation of number of RBC, hemoglobin concentration and serum AST activity, and decreased of serum phospholipid level. Where as cysteine(over 58mg/kg BW) given immediately after captafol appeared to prevent the ascites, fibrin clot in abdominal cavity and liver lobe adhesion. It also prevented the liver and blood, especially RBC toxicites.

The results suggest that cysteine and other compounds containing sulfhydryl groups may protect the subjects from captafol-induced toxicity.

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