CHAPTER 4

DISCUSSION AND CONCLUSION

In a previous study, Hazai et al. (16) demonstrated that disulfiram reduced toxic metabolite formation of acetaminophen, NAPQI. In the current study, we examined the therapeutic effect of disulfiram with *N*-acetylcysteine given after acetaminophen overdose and its potential for clinical application.

4.25 g acetaminophen/ kg body weight was given to rats. This dose corresponded to LD_{10} and was accelerated with liver cell necrosis (101). The rats were fasted 12 hours before acetaminophen administration in order to maximum absorption of acetaminophen.

Administration of NAC by oral route often produces vomiting and requires antiemitic to complete therapy. Avoidance use antiemitic drug, vomiting, and ensure absorption (102) then, 140 mg NAC/ kg body weight, should be given by intraperitoneal (I.P) every 4 hours for 7 times was chosen to treat the rats that received acetaminophen overdose (103).

Stripp et al. found that cytochrome P-450 reductase activities of rats treated with 100 and 200 mg/kg disulfiram were decreased (104). Zemaitis et al. found that cytochrome P-450 levels and NADPH-cytochrome P-450 reductase activity of the rats, treated with 100 and 400 mg/kg of disulfiram were decreased (105). Therefore, 100, 200, and 400 mg/kg doses of disulfiram were chosen to treat with NAC.

In this study, we determined liver function by the levels of hepatic enzyme, alanine transferase (ALT) and aspatate aminotransferase (AST) which primary reflects the degree of liver damage and have been commonly used as a diagnostic marker for hepatotoxicity (106,107) and the hepatotoxicity was confirmed with hepatic necrosis score.

Experimental evidence suggests that the role of CYP2E1, CYP2A6 and CYP2D6 are more significant in biotransformation of acetaminophen in man and rodent (51). Disulfiram, which is the treatment of choice in alcoholic patients, is also a known specific CYP2E1 inhibitor.

Diethyldithiocarbamate, metabolite of disulfiram, is a known inhibitor of CYP2E1 and CYP2A6 that is responsible for NAPQI formation. But disulfiram does not inhibit CYP2A6, CYP3A4 and some other isozyme in rats and humans (108).

The study had postulated that if catalytic activities of CYP2E1, CYP2A6 and CYP 2D6 are inhibited, the NAPQI formation and the hepatotoxicity will be decreased. But if catalytic activities of CYP2E1, CYP2A6 and CYP 2D6 were increased, the NAPQI formation and the hepatotoxicity will be increased.

Previous studies on the mechanism of acetaminophen induced hepatotoxicity have shown that glutathione plays a key role in the detoxification of the reactive toxic metabolites of acetaminophen. Decreasing of glutathione levels in the liver caused of hepatotoxicity. Then, the GSH levels in the blood and the liver were decreased, hepatotoxicity would be increased. But, if the GSH levels in blood and the liver were increased, hepatotoxicity would be decreased.

From this study was found that AST and ALT levels in the groups that received disulfiram in both male and female rats decreased significantly when compared with the group treated with acetaminophen and NAC alone. These results indicated that the rat had less hepatotoxicity.

The AST and ALT levels were the highest level at 24 and 48 hours respectively. These indicated that at 24-48 hours the rats had more hepatotoxicity than the other time. The combination of NAC and disulfiram produced a markedly greater reduction of AST and ALT level than that obtained with NAC alone.

CYP2E1 and CYP2A6 activities in the groups that received disulfiram were significantly decreased when compared with the control group, acetaminophen overdose and NAC alone.

Radzialowski et al. (109) showed that a change in cytochrome P-450 activities was an important role in the temporal variation of acetaminophen-induced hepatotoxicity. It has been observed that the role of drug metabolism measured in vitro in subcellular fractions of liver is not constant during the day. The cytochrome P-450 activities usually show maxima during the early morning and minima during the afternoon. Moreover, cytochrome P-450 activities are increased after 8 hours of fasting (110-111).

However, it is postulated that not all the subtypes of cytochrome P-450 family would follow an identical rhythm in the catalytic activity. Rhythmicities in the activity of each isozyme

of cytochrome P-450 have scarcely been examined, but a limited number of studies have been revealed that the activities of different cytochrome P-450 isozymes exhibit dissimilar time-dependent variations (112).

Ducharme et al. (113) and kharsch et al. (114) suggested that short-term treatment with disulfiram 200 mg had no effect on CYP2D6 activity. This study found that CYP2D6 activity in the group that received 100 and 200 mg/kg disulfiram was not significantly different but 400 mg/kg disulfiram was significantly decreased when compared with the control, acetaminophen overdose and NAC alone groups. The characteristic of decreased CYP2D6 activity may correlate with dose-response relation ship of disulfiram concentration.

The reduced glutathione level in liver of male and female rats in the group which received with acetaminophen and groups which received 100, 200, and 400 mg /kg disulfiram was not significantly different when compared with the control group. But, the reduced glutathione level in the group which received NAC alone was significantly decreased.

Theoritically, the reduced glutathione level in liver of the group which received only acetaminophen would be decreased. But in the experiment, the reduced glutathione level was not lower than the control group. Abraham et al. (115) found that protein thiol was increased in the liver when acetaminophen induced liver damage. Protein thiols are physiological free radical scaventures and may serve as antioxidants by several mechanisms. Protein thiols in rats' liver would be produced to combat the NAPQI more than the reduced glutathione. Then, the reduced glutathione level in rats' liver was not decreased.

Theoritically, the reduced glutathione level in the liver of the group which received only NAC would increase beyond the group which received with only acetaminophen. But the experiment found that reduced glutathione levels were lower than the group received with only acetaminophen. Skrzydewska et al. (116) found that NAC was shown to prevent the decrease of liver glutathione concentration and NAC can react directly with NAPQI. Wang et at. (117) suggested that NAC, S-allyl cysteine and S- methyl cysteine has no inhibition on cytochrome P-450 activities. NAPQI was trapped with NAC and reduced glutathione. Then, the reduced glutathione levels were decreased more than the other group. Bertram et al (118) showed that disulfiram induced glutathione and glutathione-S-transferase in the liver and the esophagus. Russell et al (119) suggested that oxygen radicals were scavenged by NAC and NAC caused a

shift in the redox balance of glutathione to a more reduced state rather than causing synthesis of glutathione.

In the group which received disulfiram and NAC, disulfiram and its metabolite, diethyldithiocarbamate inhibited the CYP2E1 and CYP2A6. NAPQI formation decreased and was trapped with NAC. Then, the glutathione in the liver was not decreased when compared with the control group.

Glutathione levels in the blood of male and female rats in the group treated with 100, 200-mg/kg disulfiram and NAC were significantly higher than in the group treated with NAC alone, and the group treated with 400 mg/kg and NAC. And at 48 hours in male and 24 hours female rats, the glutathione level in blood was the highest after treatments.

Glutathione in the groups treated with disulfiram and NAC decreased when compared with the control group. Ploeman et al. (120) shown that disulfiram and diethyldithiocarbamate inhibit glutathione-S-transferase and glutathione. A single oral dose of disulfiram in animals and humans produced rapid inhibition of CYP2E1 and diethyldithiocarbamate inhibit CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP3A3, and CYP3A4. Hu et al. (121) suggested that disulfiram rapidly reduced glutathione reductase in the blood to diethyldithiocarbamate and glutathione was used in the metabolism of disulfiram and acetaminophen detoxicfication.

Pharmacokinetic of acetaminophen overdose after treatment with disulfiram and NAC was studied on plasma acetaminophen half life and acetaminophen clearance. From this study, it was found that plasma acetaminophen half-life in male rats was increased. But, acetaminophen clearance was decreased when disulfiram dosage was enhanced, while plasma acetaminophen half-life in female rats decreased. But, acetaminophen clearance was increased when disulfiram dose was enhanced. Jorgensen et al (122) showed that clearance of acetaminophen was decreased after administration of 200 mg/kg disulfiram. Schiodt et al (123) showed that acetaminophen half-life increased porportion to the severity of hepatotoxicity in acetaminophen overdose in-patients treated with NAC. The acetaminophen half-life increased with increasing degrees of the hepatotoxicity, reflecting a limited hepatic capacity for acetaminophen metabolism and clearance.

These results have described how sexes, the hormone cycle, the pathology of liver and idiosyncratic reaction affect to the plasma acetaminophen half-life and acetaminophen clearance.

The hepatic necrosis score was indicated to severity liver necrosis. Data were collected 168 hours after acetaminophen administration like in the study on induced mortality rate. The liver necrosis occurred around the central vein (zone 3) which low oxygen content, high capacity for glucuronidation and sulfation and high cytochrome P-450 (CYP2E1).

In this study we found that fatty change (steatosis) occurred before liver necrosis. In the group which received acetaminophen alone and the group treated with NAC alone, the hepatic necrosis score were higher than the group treated with disulfiram.

Hepatic necrosis score of the control group in both male and female rats were not found. In male rats group 2, hepatic necrosis score was the highest (100%) at 24 hours after acetaminophen administration. It correlated with increasing of CYP 2E1, CYP2A6 and CYP2D6 activities. The AST and ALT levels were the highest at 24 hours. The GSH levels in the liver and blood decreased at 24 hours too.

In male rats group 3, hepatic necrosis score was the highest (100%) at 48 hours after acetaminophen administration. It correlated with increasing of CYP2E1, CYP2A6 activities, the AST and ALT levels at 48 hours. The GSH level in the liver and blood were decreased at 48 hours too.

In male rats group 4, hepatic necrosis score was the highest (50%) at 48 hours after acetaminophen administration. CYP 2E1, CYP2A6 and CYP2D6 activities was inhibited by disulfiram then liver necrosis would not occur but liver necrosis still generated. It suggested that some cytochrome P-450, beside CYP2E1, CYP2A6 and CYP2D6 can generate NAPQI. The GSH level in the liver was the highest at 24 hours after treatment with NAC. The AST and ALT were the highest at 12 and 24 hours respectively. It was found that hepatotoxicity from NAPQI occur before GSH in the liver started.

In male rats group 5, hepatic necrosis score was the highest (66%) at 24 hours after acetaminophen administration. It correlated with decreasing of the GSH levels in blood and the liver and increasing of the AST and ALT levels at 24 hours.

In male rats group 6, hepatic necrosis score was the highest at 72 hours. The AST and ALT levels increased at 24 and 48 hours respectively. The GSH levels in blood and liver decreased because the GSH was used in eradicate disulfiram. The CYP2E1, CYP 2A6 and CYP2D6 activities decreased at 48 hours.

In female rats group 2, hepatic necrosis score was high (100%) at 12 to 48 hours. It correlated with increasing of the CYP 2E1 and CYP2A6 activities, the AST and ALT levels. The GSH levels in blood and the liver decreased at 12 to 48 hours too.

In female rats group 3, hepatic necrosis score was the highest (50%) at 72 hours. The CYP2E1 and CYP2A6 increased and the GSH levels in the liver decreased. The AST and ALT were the highest level at 48 hours too.

In female rats group 4, hepatic necrosis score was high at 12 and 48 hours. Liver necrosis occurred from high activities of CYP2E1 and CYP2A6 correlated with decreasing of GSH level in the liver and blood. Hepatotoxicity at 24 hours was the highest it can be seem the AST and ALT level which were the highest at this time.

In female rats group 5, hepatic necrosis score was the highest at 48 hours. The CYP2E1 and CYP2A6 activities were the highest at 12 hours and correlated with decreasing of GSH level in the liver at 24 to 48 hours.

In female rats group 6, hepatic necrosis score was 33% every time. The CYP2E1, CYP2A6 and CYP2D6 activities were the highest at 48 hours and correlated with decreasing of GSH level in the liver.

Some rats died before the treatment started. They were not included into the mortality rate because heart failure or apnea might have been cause of death. The mortality rate of male rats which treated disulfiram with NAC 140 mg/kg every 4 hours for 7 times was no significantly difference when compared with the control group and significantly lowers than the group received acetaminophen alone and treated with NAC alone. But the mortality rate of female rats which received 100 and 200 mg/kg disulfiram with NAC 140 mg/kg every 4 hours for 7 times were higher than the control group and were not significantly lowers than the group treated with NAC alone. Outing to there were many variation in female sex such as hormonal, anatomical and physiological differentiation.

Increasing of the AST and ALT levels in the blood and catalytic activity of CYP2E1 in the liver are correlated with hepatic necrosis. Then, prediction severity of hepatic necrosis score could determine these enzymes. Catalytic activity of CYP2E1 is the best marker of hepatic necrosis but it difficult to determine because of using liver tissue. Practically, the AST and ALT levels in the blood could bring to predict severity of hepatic necrosis. Before and after treatment

acetaminophen overdose should determine the AST and ALT levels every 12 hours 2 to 3 times to assess hepatic necrosis.

Efficacy of treatment in male rats which received acetaminophen overdose by disulfiram with NAC 140 mg/kg every 4 hours for 7 times is seem better than treatment with NAC alone. But, efficacy of treatment in female rats which received acetaminophen overdoses by disulfiram with 140 mg/kg every 4 hours for 7 times seem increased mortality rate. Side effects of disulfiram when used treatment with NAC 140 mg/kg every 4 hours for 7 times was decreasing of glutathione in blood.

Acetaminophen-induced hepatotoxicity is dependent on at least 3 factors: the capacity of conjugation with either glucuronic acid or sulfate, hepatic glutathone content and cytochrome P-450 activity. Disulfiram is a drug that inhibits cytochrome P-450 activity and enhances hepatic glutathione. Thus, treatment of acetaminophen by accomparying disulfiram with NAC may be better than using NAC alone. And effective dose of disulfiram is 200 mg/kg in experimental model. However, the further research and investigation will be set to clarity the use of disulfiram on acetaminophen overdose.