CHAPTER 1

INTRODUCTION

1.1 State of the problems

Acetaminophen (paracetamol) is the most popular analgesic in most countries in the world. It is available without prescription from shops and pharmacies and it is kept in marketed under at least 50 brands name and contained in over 200 proprietary drug combinations (1). Therefore, not surprising that it is often involved in episodes of accidental or deliberate self-poisoning (2).

In 2000, a huge health-care burden to the accident and emergency, medical and psychiatric services, estimated 70,000 cases acetaminophen overdose per year in the United Kingdom (3). In the United State, the American Association of Poison Center reported 112, 809 cases in 2001 (4). In Thailand, Maharaj Nakorn Chiang Mai hospital reported 30 cases in 2000 (5).

Acetaminophen is mainly eliminated at therapeutic dose through glucuronidation and sulfation and small fraction is oxidized by cytochrome P 450 enzymes to *N*-acetyl-*p*-benzoquinone-imine (NAPQI). A highly reactive metabolite future conjugated with glutathione and excreted in urine. After acetaminophen overdose, the glucuronidation and sulfation pathways are saturated and the increased NAPQI production, which causes hepatic injury.

Inhibition of cytochrome P 450 enzymes responsible for NAPQI formation might be useful beside *N*-acetylcysteine treatment, a specific antidote of acetaminophen overdose. In this way the organism may take time to produce cofactors that are necessary for detoxifying pathways. Although, cimetidine is often used (6-7) in management strategy of acetaminophen poisoning as a cytochrome P 450 inhibitor to reduced NAPQI formation. But cimetidine appears to have no role in management of acetaminophen overdose in human (8-10).

Disulfiram is a therapeutic drug to alcoholism patient. It can inhibit cytochrome P 450 and reduces NAPQI formation. There are not reports which treat disulfiram with *N*-acetylcysteine detoxified acetaminophen overdose. This study has investigated the effects of disulfiram in addition to *N*-acetylcysteine on acetaminophen overdose and determine effective dose of disulfiram.

1.2 Literature reviews

1.2.1 Pharmacological properties of acetaminophen

Acetaminophen (paracetamol, 4-hydroxyacetanilide, *N*-acetyl-*p*-aminophenol, tyrenol 4 amidophenol,) has analgesic and antipyretic effects that do not differ significantly from those of aspirin. However, as mentioned, it has only weak antiinflamatory effects. Minor metabolites contribute significantly to the toxic effects of acetaminophen. The structural formula of acetaminophen is shown in Figure 1

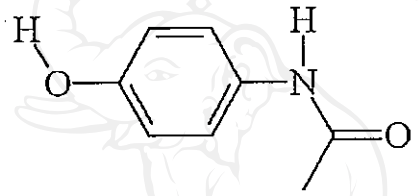

Figure 1 The structure of acetaminophen

The failure of acetaminophen to exert anti-inflammatory activity may be attributed to the fact that acetaminophen is only a weak inhibitor of cyclooxygenase in the presence of the high concentrations of peroxide that are found in inflammatory lesions. In contrast, its antipyretic effect may be explained by inhibiting cyclooxygenase in the brain, where peroxide tone is low. Further more, acetaminophen does not inhibit neutrophil activity as other NSAIDS.

Single or repeated therapeutic doses of acetaminophen have no effects on the cardiovascular and respiratory systems. Acid-base imbalance, produce the gastric irritation, erosion or bleeding does not occur but may occur after administration of sylicylates. Acetaminophen has no effects on platelets, bleeding time, or the excretion of uric acid (11).

1.2.2 Pharmacokinetics

Following oral ingestion, acetaminophen are rapidly absorbed and have a time peak of approximately 45 minute of table and liquid acetaminophen and 30 minutes respectively (12). But extended-release acetaminophen has a time to peak of 1 to 2 hours, and 95 percent of the drug is absorbed in 5 hours. Time of peak of this is delayed by food or drugs. Acetaminophen bioavailbility is 60 to 98 percent. Peak blood level after recommended dose ranges from 8 to 32 μ g/ ml. After administration of rectal suppositories in children, the time to peak ranged from107 to 288 minutes with bioavailability of 30 to 40 percent. Peak after single 20 mg/kg doses given rectally varied from 8.8 to 11 mg/L.

Acetaminophen has total protein binding of 10 to 30 percent with therapeutic dose. It crosses both the plasma and the blood brain barrier (13). Acetaminophen is metabolized almost exclusively in the liver. Once absorbed, approximately 90 percentage of acetaminophen normally undergoes hepatic glucuronide (40 to 67 percent) and sulfate (20 to 46 percent) conjugation and change to inactive and harmless metabolites, which are eliminated in urine. A small fraction of unchanged acetaminophen (less than 5 percent) and other minor metabolites reach the urine.

The remaining fraction, usually ranging from 5 to 15 percent, is oxidized by the cytochrome P 450 mixed function oxidase system (CYP), CYP2E1, CYP1A2, CYP3A4, CYP2A6 and CYP2D6 (14-16), resulting in the formation *N*-acetyl-*p*-benzoquinone-imine (NAPQI). Glutathione quickly combines with NAPQI; the resulting complex is coverted to nontoxic cysteine or mercaptate conjugates, which are eliminated in urine. The metabolic pathway of acetaminophen is shown in Figure 2.

The elimination half-life of acetaminophen is 2 to 4 hours. Biliary excretion is normal. Breast milk contains less than 2 percent of the material dose.

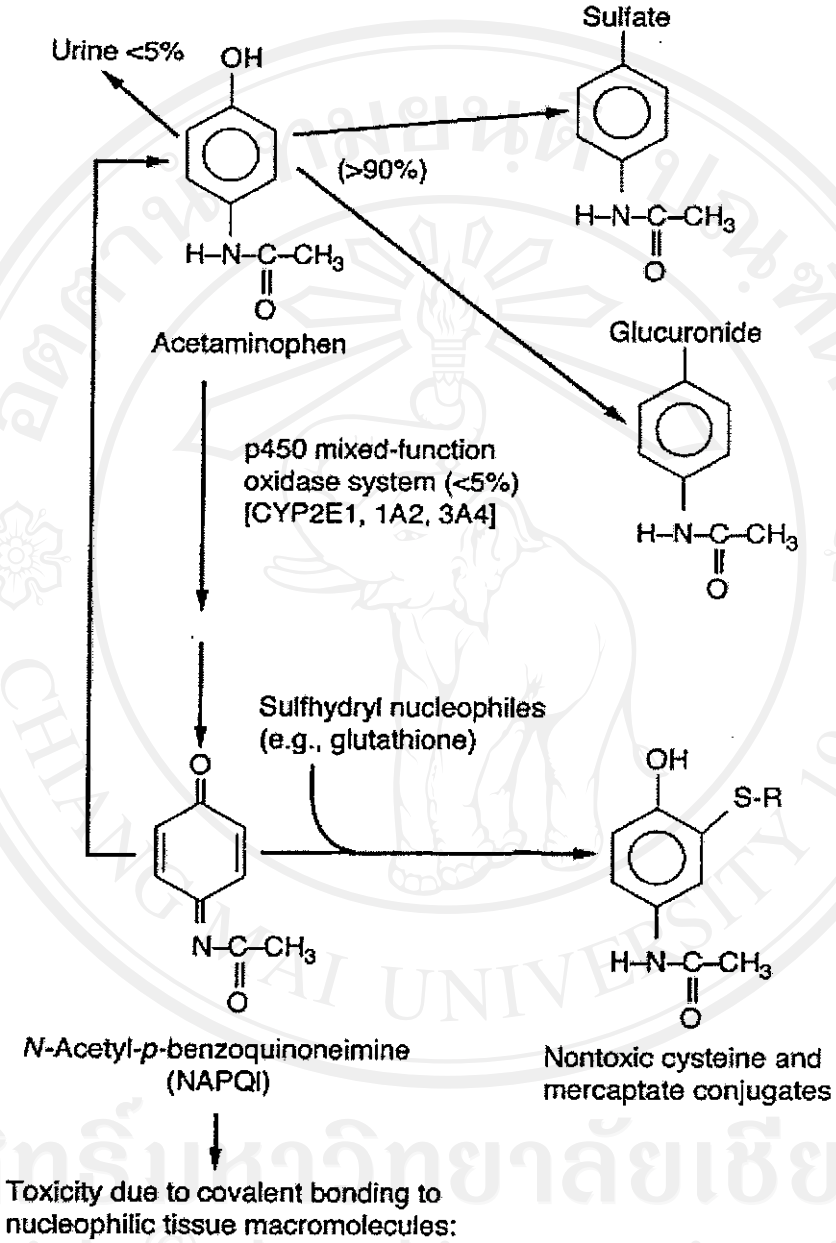

Figure 2 Acetaminophen metabolism and NAPQI formation (17)

1.2.3 Toxicology

Acetaminophen was first introduced into medicine in the late 19th century. Toxicology of acetaminophen was performed a little before the drug went into widespread use. Some animal studies was performed to establish whether acetaminophen, like its precursors phenacitinand acetanilide, might cause analgesic nephropathy after repeated use, but no evidence of this was found. Hepatic necrosis after repeated exposure was reported in cats and in rats. The early death usually due to respiratory failure, between 1 and 7 days after ingestion of toxic dose and hepatic necrosis (18).

The first report of acetaminophen poisoning in human with describing hepatic necrosis (19) provoked a large number of animal studies to establish the mechanism. These demonstrated that acute centrilobular hepatic necrosis could be produced in many species, although there were differences in susceptibility, shown in Figure 3.

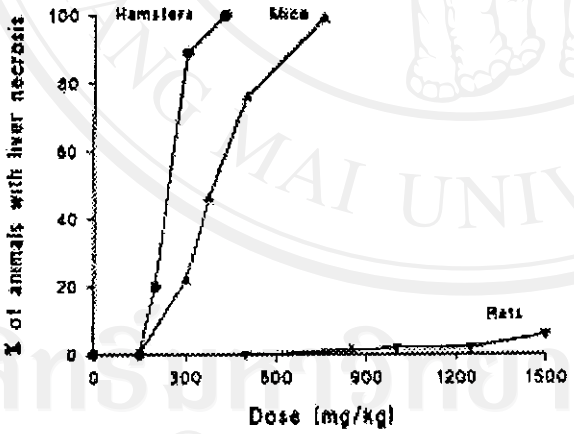

Figure 3 Species differences in the dose-response relationship for acetaminophen-induced liver damage. (20)

There were also species difference in the effects of poisoning; for example, methemoglobinemia occurs in dogs, cats, mice and pigs, but these are not features of poisoning in man (21-22).

In man, the dose required to produce hepatic damage can not accurately be estimated from the history of patients taking overdoses as the dose taken is often not accurately reported. Although death has been reported after as litter as 2.5 g. acetaminophen (23). Hepatotoxicity is not expected to develop unless at least 10 to 15 g. has been taken. A more accurate estimate of the dose taken can be made from the 3 hours blood level if the volume of distribution is assumed. The threshold for liver damage is approximately 250 mg/ kg to 350 mg/ kg usually associated with severe hepatotoxicity (24)

1.2.4 Mechanism of Toxicity

The biochemical basis of acetaminophen toxicity was largely studied by Mitechell and his colleagues (25-28). In therapeutic dose, most acetaminophen is conjugated with glucuronide or sulphate in the liver. However, a small amount is convertd by CYP2E1, CYP2D6 and CYP2A6 (16) to the reactive metabolite alkylating metabolite NAPQI.

This occurs either via oxygenation of acetaminophen to *N*-hydroxyacetaminophenol with subsequent dehydration to NAPQI. The NAPQI may also be produced by single-electron oxidation. The NAPQI generated by therapeutic dose of acetaminophen is rapidly metabolized to non-toxic cysteine and mercapturic acid conjugates or back to the parent compound (29) in glutathione-dependent reactions. These conjugates are excreated in the urine and to lesser extent in the bile.

When acetaminophen is taken in toxic dose: hepatic glutathione becomes to depletion and the formation of the reactive intermediate metabolite outstrips their detoxification. These metabolites could bind to vital cell constituents and the extent of binding is proportional to the amount of cellular damage that occurs over the next 1 to 2 hours. In mice, damage occurs when hepatic glutathione is depleted to 20 to 30 percent of its normal value (26) and species susceptibility to acetaminophen toxicity is related to the rate of acetaminophen activation and glutathione depletion and is consistent with the capacity of each species for *N*-hydroxylation (30) and sulphation.

Human toxicity also occurs via binding of acetaminophen metabolites to cell constituents , as evidenced by 3-(cysteine-S-yl)-acetaminophen adducts. Whose serum levels increase in proportion to the degree of acetaminophen-induced hepatic damage (31).

Arylation and oxidation by NAPQI of cellular enzymes, such as glutathione peroxidase and thiol transferase, inhibits their activity (32). Although in animals, at least, there appears to be a delay before irreversible changes occur. As a result, the cell is susceptible to exogenous activated oxygen radicals, which cause further oxidation of protein thiols.

Reduced mitochondrial sequestration of calcium resulting from impairment of Ca^{2+} dependent ATPase activity results in an increased influx of calcium (33-34), calcium cycling and oxidative stress (35). Calcium-dependent catabolic process are activated, including phospholipids and protein degradation and disruption of the cytoskeleton and DNA occurs (36). Further injury results from microcirculatory changes, including periacinar ischemia or infarction, and from invasion of tissue by activated neutrophils and macropharge. Mechanism of toxicity shown in Figure 4.

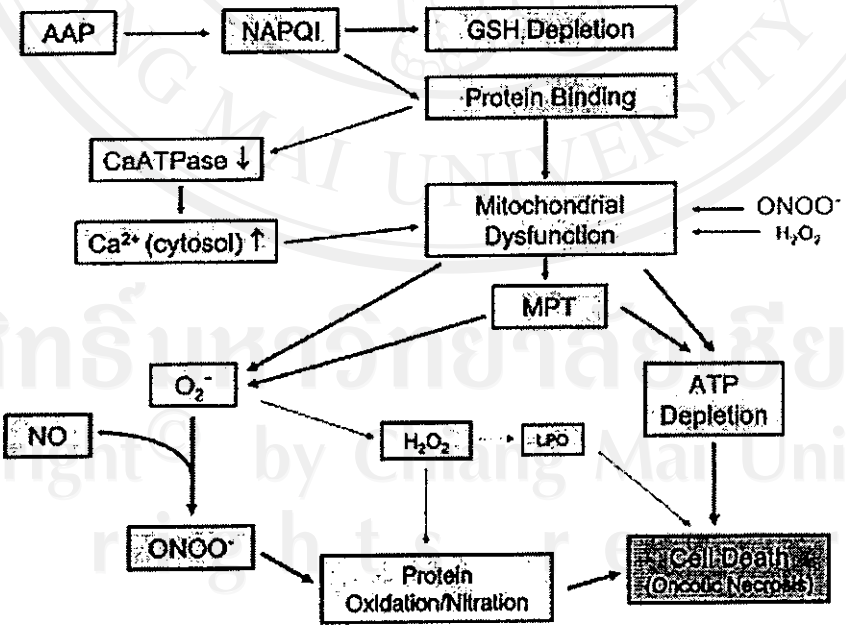

Figure 4 Mechanism of acetaminophen toxicity induced liver injury.(35)

1.2.5 Pathology

In toxic dose, acetaminophen causes acute centrilobular hepatic necrosis with collapse of the reticulin framework (21). Sequential electron microscopy studies in animals exposed to acetaminophen show depletion of glycogen, loss of ribosome, cytoplasmic matrix swelling, hydropic vacuolation of the endoplasmic reticulum, sinusoidal congestion and coagulative necrosis (38). Increases in serum levels of hepatic transaminase reflect the extent of the histological damage in both animal and human and in survivals there is rapid regeneration with no progression to cirrhosis.

Biopsies after on interval of 3 months are usually normal unless the acute liver damage was severe (34). Repeated exposure to acetaminophen toxicity in man is comparatively uncommon, but in a man who took 31 separate overdoses involving acetaminophen, a liver biopsy demonstrated disturbance of architecture, fibrous extension of portal triads and fibrous septa with the suggestion of regenerative changes and progression to cirrhosis. Chronic aggressive hepatitis with the development of cirrhosis has also been described in a patient who took 3 g. daily for over a year.

The most frequent non-hepatic feature of acetaminophen poisoning is renal failure, which occasionally occurs in the absences of hepatic necrosis. Renal biopsies have demonstrated a predominantly distal tubular necrosis with focal coagulation and sloughing of the tubular cells into the lumen of the nephron as far as the collecting ducts (41-42).

Electron microscope shows normal glomeruli, loss of the tubular brush border, loss of the basolateral mitochondrial array and the presence of envaculated electron-dense material in the cytoplasm and tubular lumen. It appears probable that, as with hepatic damage, renal tubular damage is caused by local metabolic activation of acetaminophen and subsequent covalent binding of NAPQI to vital cell constituents. As in the liver, covalent binding of acetaminophen metabolites in renal tissue and subsequent toxicity is enhanced by glutathione depletion and reduced by cobaltous chloride and piperonly butoxide, which inhibit renal cytochrome P 450 activity.

Less common pathological features of acetaminophen poisoning include thrombocytopenia (43), subendocardial necrosis associated with hemorrhage, fatty degeneration and focal clumping of myofibrils (44-46) and pancreatitis associated with edema and area of

hemorrhage and fat necrosis. A focal necrotizing myopathy has been found at post-mortem in patients dying from hepatic necrosis of various causes, including acetaminophen poisoning, but it is unclear if this is a direct effect of poisoning or a secondary effect of liver failure.

1.2.6 Clinical manifestrations

Early recognition and treatment of patient with acetaminophen poisoning are essential in order to minimize morbidity and mortality. This task is made difficult by the lack of predictive clinical findings early in the cause of acetaminophen poisoning, and clinicians should not feel reassured by a patient's lack of symptoms soon after ingestion. The first symptom after acetaminophen overdose may be those of hepatic injury, which develop many hours after the ingestion, when antidotal therapy is already less effective Table 1. Summarized the clinical course of acute acetaminophen toxicity.

State I of toxicity, hepatic injury has not yet occurred and even patients who ultimately develop hepatotoxicity may be asymptomatic treatment at this stage. Clinical findings, when present are nonspecific, such as nausea, vomiting, malaise, pallor and diaphoresis. Laboratory indices of liver function are normal. In extremely rare cases of massive overdose, decreased level of consciousness and metabolic acidosis may be caused directly by the effects of acetaminophen (47-48). These findings are so uncommon that they should never be attributed to acetaminophen alone without through evaluation of other possible causes.

State II represents the onset of liver injury, which occurs in only a fraction of those who overdoses patients. Onset is most common within 24 hours after ingestion, but is nearly universal by 36 hours (49). Symptoms and physical signs during state II vary with the severity of liver injury but mimic the other causes of hepatocellular injury such as infectious hepatitis. Aspartate aminotrasferase (AST) is the most sensitive, widely available measure to detect the onset of hepatotoxicity, and AST abnormalities always precede evidence of actual liver dysfunction (elevated international normalized ratio (INR), elevation bilirubin, hypoglycemia, and metabolic acidosis). Although uncommon, AST elevation may occur as early as 8-12 hours after ingestion in the most severely poisoned patient.

State III defined as the time of maximal hepatotoxicity, is most common between 72 and 96 hours after ingestion. The clinical manifestations vary from absent to fulminant hepatic failure

with encephalopathy, coma, or exsanguinating hemorrhage. Laboratory studies are also variable: AST and alanine aminotransferase (ALT) values above 10, 000 IU/L are common, even in patient without evidence of liver failure. The highest reported ALT caused by acetaminophen toxicity is over 100, 000 IU/L (50). Much more important than the degree of aminotransferase evaluation, abnormality of INR, bilirubin, glucose, and pH indicate the degree of liver failure and essential determinants of prognosis and treatment.

Fatalities from fulminate hepatic failure generally occur between 3 and 5 days after overdose. Death results from either single or combined complications of multiorgan failure, including hemorrhage, acute respiratory distress syndrome (ARDS), sepsis, and most importantly, cerebral edema (51).

State IV defined as the recovery phase. Hepatic regeneration becomes complete in survivors; there are no reported cases of chronic hepatic dysfunction solely because of acetaminophen poisoning. The rate of recovery varies; in the most case, laboratory evaluation in normal by 5 to 7 days after overdose; but recovery may take much longer in severely poisoned patients and microscopic histologic abnormalities may persist for months (52-53)

1.2.7 Factors affecting toxicity

The toxicity of acetaminophen is enhanced by factors that cause glutathione depletion, increase the formation of NAPQI or reduce the antioxidative capacity of the liver. Thus, low protein diet or pretreatment with diethyl maleate cause glutathione depletion and enhanced toxicity, as do hepatic P 450- inducing agents, including alcohol, phenobarbitone and 3-methylcholanthrene. Vitamin E depletion also increases toxicity, probably by imparing the hepatic response to oxidative stress. Conversely acetaminophen toxicity is reduced by glutathione realtors like cysteine and butylated hydroxyanisol, hepatic enzyme inhibitors, such as piperonyl butoxide (26), cimetidine (6-7), cobaltous chloride, disulfiram, diethyldithiocarbamate (16), diallyl sulfide (54) which are inhibitors of cytochrome P 450 production. Toxicity is also reduced by antioxidants and inhibitors of lipid peroxidase, such as diethyldithiocarbamate and anisyldithiothione (55). Reducing agents, such as ascorbic acid, promote the conversion of NAPQI to acetaminophen and reduce covalent binding (56). More recently, calcium- channel

blockers, such as nifedipine, have been shown to reduce hepatic necrosis in rats as do phospholipase A2, cyclo- oxygenase and thromboxane synthetase (57). Antioxidants, such as allopurinol, may reduce toxicity by preventing late microcirculatory changes. Toxicity is increased in subjects with a negative nitrogen balance, including those with cancer.

1.2.8 Assessment of the risk of acute toxicity

Because of the delayed onset of clinical manifestrations after acetaminophen overdose, early recognition of overdose is essential to prevent morbidity and mortality. The currently accepted toxic dose is more than 7.5 g in adults and 150 mg/kg in children (58-60).

Patients'descriptions of acetaminophen exposure are often unreliable, so alternative methods of assessment are required. The most reliable method for assessing the risk of toxicity after acetaminophen ingestion is measurement of the plasma acetaminophen concentration. This determination is necessary when the history indicates ingestion of a potentially toxic dose or after ingestion of an unknown quantity. A single measurement of the plasma acetaminophen concentration at least 4 hours after acetaminophen ingestion can be plotted on the Rumack-Matthew nomogram to assess the risk of toxicity (61-62).

The Rumark- Matthew nomogram

The original nomogram for assessing the risk of acetaminophen toxicity was developed on the basis of outcomes of patients in Edinburgh, Scotland. It was characterized by a " treatment line" defined by a plasma acetaminophen concentration of 200mg/L at 4 hours after drug ingestion and 30 mg/L at 15 hours. Rumack and Matthew (61) extrapolated the treatment line to 24 hours. It is important to realize that the original treatment line was based on liver transaminase elevation rather than hepatic failure or patient death and was intended to be sensitive rather than specific. The original treatment line is still used in the United Kingdom; however, the line used in the United States and Canada has been lowered by 25 percent to add even greater sensitivity (Figure 5). The lowered treatment line is now defined by 150 mg/L at 4 hours and 37.5 mg/L at 12 hours. Bond et al. (63) found that only 0.4 percent of 662 patients with on acetaminophen concentration below the lowered treatment line developed hepatotoxicity (64).

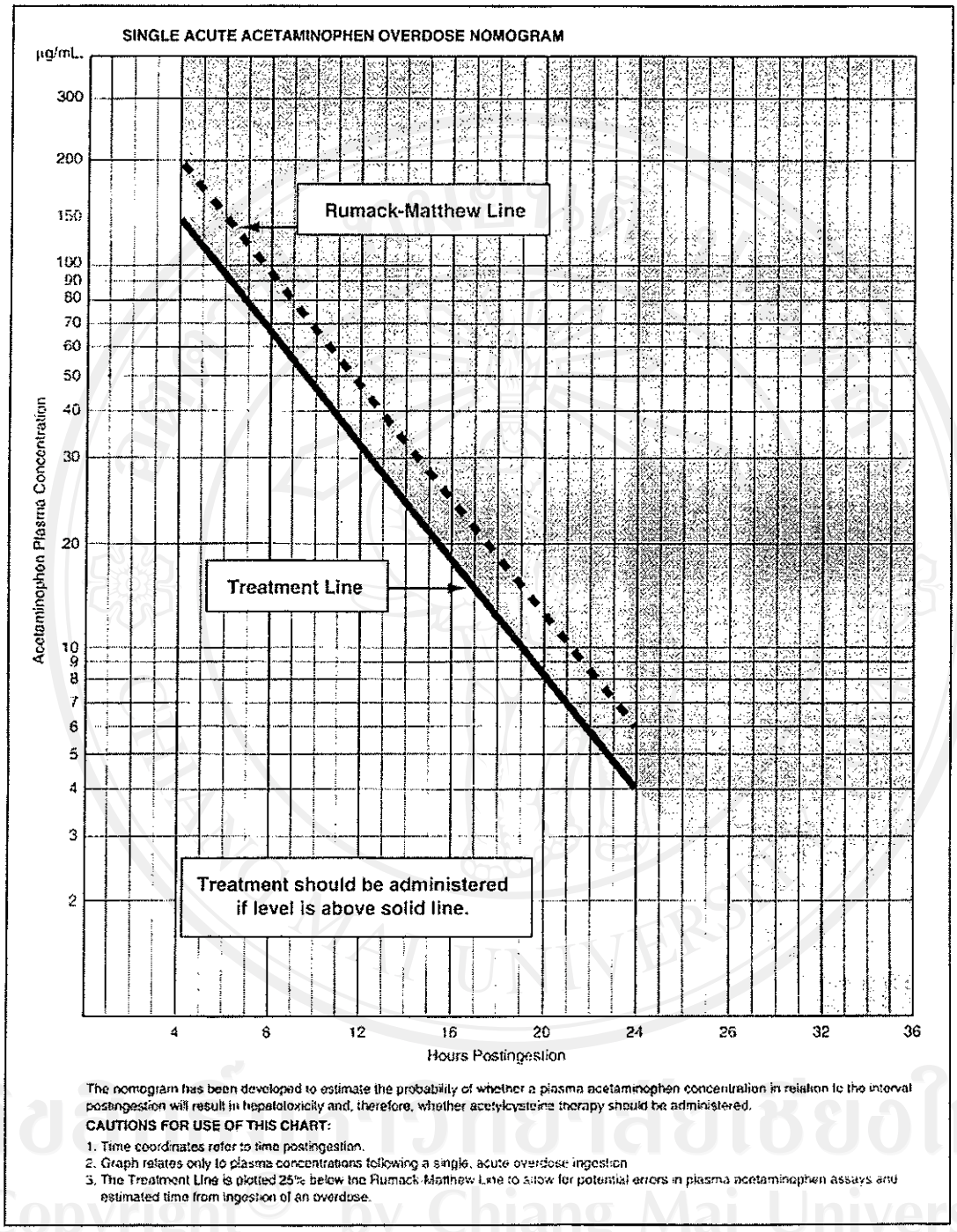

Figure 5 Rumack- Matthew nomogram, used to determine the risk of acetaminophen- induced hepatotoxicity on the basis of plasma acetaminophen concentration and time since ingestion. (10)

Assessment of hepatic injury

Laboratory indicators of hepatic injury should be measured initially and daily during therapy in many patients with a serum acetaminophen concentration above the treatment nomogram line. With progressive hepatic failure, testing should be done every 12 hours. Most patients who will develop liver toxicity have an elevated AST level within 24 hours of ingestion and in one small series, all cases had reported AST elevation within 36 hours after acetaminophen ingestion is sufficient to eliminate the possibility of liver toxicity.

When liver injury occurred following acetaminophen ingestion, additional diagnostic testing is needed to guide treatment and assess prognosis. Severe hepatic injury is associated with extensive disruption of hepatocytes, decreased capacity of synthesize coagulation factors, decreased glycogen and altered glucose homeostasis and impairment of synthesis and excretion of bilirubin. In some cases, by determination of prothombin time, INR, arterial pH, and serum creatinine. Patient with an INR greater than 2 at 24 hours, 4 at 48 hours, or 6 at 72 hours are likely to develop fulminant hepatic failure (FHF) (65). Persistent metabolic acidosis despite intravascular volume repletion also indicates a poor prognosis. This should be differencialed from a lactic acidosis, occurring early in overdose and without evidence of FHF, that is due to a direct effect of acetaminophen on hepatic lactic acid uptake and oxidation. Transaminase levels do not predict the clinical course, they may decline either during hepatic recovery or with progressive FHF. During recovery, it is common for declining serum transaminases to procede the decline of the serum bilirubin. Acute renal insufficiency also occurs and indicates a poorer prognosis when the serum creatinine is greater than 300 mmol/L (3.4 mg/dL) in association with prothrombin time greater than 100 seconds and grade III or grade IV hepatic encephalopathy.

1.2.9 Treatment of acetaminophen poisoning

1.2.9.1 Gastric aspiration and lavage

Gastric aspiration and lavage has been shown to be of value for up the 6 hours after ingestion of acetaminophen overdose. However, because small children of less than 5 years old tend to swallow only small amounts of acetaminophen, gastric lavage is unnecessary and syrup of ipecac is usually required in children.

1.2.9.2 Activated charcoal

Activated charcoal has been advocated as an additional means of preventing absorption of acetaminophen from the stomach. It has been shown, for example, that 10 g of charcoal administered immediately after ingestion of 1 g acetaminophen will reduce absorption by about 70 percent. Similar results have been achieved with cholestyramine. The value of treatment would not be set more than a few hours after ingestion of an overdose of acetaminophen. Furthermore, the ideal charcoal: acetaminophen ratio is 10: 1, and a patient who ingested 50 g of acetaminophen would be required to swallow a half a kilogram of charcoal.

1.2.9.3 Specific antidotes

1. Cysteamine (Mercaptamine)

Although cysteamine increase intracellular glutathione concentrations, its principal beneficial action is probably to prevent the conversion of acetaminophen to NAPQI (67). After cysteamine had been shown to prevent covalent binding of acetaminophen to mice and rat microsomes (68), Prescott and his colleagues (69) gave 3.2 g cysteamine between 4 and 10 hours after overdose to treat 10 episodes of poisoning in 7 patients. Liver damage occurred in only 3 treated patients and this was only mild. These results were compared with those of 11 untreated patients with apparently similar severity of poisoning, all of whom developed liver damage, which was fatal in one case. Using the same dose, Prescott (70) subsequently found that none of 23 treated patients with acetaminophen levels above 300 mg/L treated within 10 hours developed liver toxicity, while 2 of 6 patients treated after this interval developed severe liver damage. Douglas and his colleagues' (71) randomized 38 patients with significant toxicity to receive cysteamine or no treatment. Comparing the two groups, there were no differences in deaths, development of renal failure, PTR or liver histology, but cysteamine- treated patients showed smaller rises in AST and ferritin. However, little consideration was given to the interval between poisoning and treatment in this study. In a further study, Smith and his colleagues (72) found that cysteamine treatment before 10 hours was associated with significant liver damage in 3 out of 23 cases, compared within 8 out of 16 cases treated after 10 hours. However, the acetaminophen assay should spuriously high results, so some of the treated patients would not have been at risk if left untreated. In a prospective controlled study, showed that hepatic damage was reduced in

patients receiving cysteamine (mean peak AST 86 IU/L) compared with those receiving placebo (mean peak AST 1046 IU/L).

Although these clinical trials suggested that cysteamine was of some benefit, particularly if given early, its use was inhibited by its severe and unacceptable adverse effects, including vomiting, flushing, drowsiness, irritability, severe debility and the smell of mercaptans on the breath.

2. Methionine

Action

This amino acid indirectly replenishes hepatic and renal glutathione stores after biotransforamation to cysteine via homocysteine (24). It is possible that this process is impaired in acetaminophen toxicity because of inactivation of the key enzymes cystathione synthetase and cystathionase by NAPQI. Furthermore, methionine is not a thiol and cannot form an adduct directly with NAPQI. For these reasons it dose not inhibit covalent binding of acetaminophen adducts in *vitro* unlike NAC, cysteamine or cysteine (24). It is usually administered by mouth and, thus, its absorption may be impaired in the presence of vomiting or activated charcoal and the treatment is impractical in patients with reduced levels of consciousness

Efficacy

Oral and intraperitoneal therapy with methionine was found to be effective in preventing liver damage in rats. The efficacy of methionine in man was first demonstrated by Prescott (70) . Using an intravenous dose of 5 g with an additional 15 g given over the next 20 hours, only 3 of 10 patients with acetaminophen level over 250 mg/L developed severe hepatic damage. Use of intravenous methionine was not generally adopted because there was no commercially available preparation.

Oral methionine in a dose of 2.5 g every 4 hours for a total of 4 doses has also been assessed : Crome and his colleague (73) reported that, of 30 patients with acetaminophen levels above 200 mg at 4 hours or 80 mg/L at 12 hours treated within 10 hours, only 3 developed hepatotoxicity. This compared favourably with the 55 to 71 percent incidence in similarly poisoned historic controls (70). Peak AST levels were higher in those treated after an interval of 5 to 10 hours compared with those treated within 5 hours. A subsequent report from the same center involved 132 cases of significant poisoning. The 96 patients treated within 10 hours, only 7

developed severe liver damage and none died. The 36 patients treated between 10 and 24 hours fared less well and 17 (47 percent) developed severe liver damage. In patients with severe poisoning (acetaminophen level greater than 300 mg at 4 hours and 75 mg at 12 hours), 6/43 (14 percent) treated within 10 hours and 14/31 (45 percent) treated between 10 and 24 hours developed severe hepatic damage and 2 patients in the latter group died. These figures were an improvement over historical controls. 58 percent of those significantly poisoned and 89 percent of those who severely poisoned developed severe hepatic damage. In a prospective controlled study, oral methionine (10 g over 16 hours) was as effective as intravenous cysteamine and more effective than placebo in preventing subsequent increase in AST and PTR and histological liver damage and was better tolerated than cysteamine.

Adverse effects

Although methionine can cause adverse effects similar to those of cysteamine when given intravenously, no serious adverse effects have been reported when given by mouth and vomiting appears less of a problem than with oral NAC (74). In theory, methionine might precipitate incipient hepatic encephalopathy (75), although in practice this has not been a significant problem.

The addition of methionine to acetaminophen preparations has been suggested as a way of reducing morbidity associated with overdose. One such preparation is available in the United Kongdom.

N- acetylcysteine

N- acetylcysteine (NAC) is a small molecular weight thiol compound (M.W. 163.2) shown in figure 6. It is an acetylated from of the amino acid L-cysteine. As early as 1970, NAC was shown as a source of sulhydryl group. Initially used as a mucolytic agent, NAC now has proven to be a very important therapeutic agent in treatment of acetaminophen poisoning.

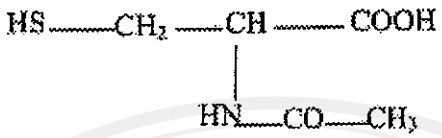

Figure 6 The structure of *N*- acetylcysteine (11)

Action

The use of NAC as an alternative to cysteamine for maintaining hepatic glutathione was first suggested by Prescott and Mathew (76). Glutathione repletion is both a direct effect of NAC and also occurs as an effect of cysteine, which is rapidly produced in the plasma from NAC. Reduced glutathione, in addition to detoxicifying NAPQI, may also reduce enzyme thiol groups oxidized by NAPQI, particularly those involved in calcium homeostasis, such as calcium translocase (77). NAC may have other beneficial actions; it may act as a source of sulphate for conjugation with acetaminophen (78), and there is evidence in rats that it reduces acetaminophen half-life by increasing sulphation (79). It may also reduce or conjugate directly with NAPQI (80). It has been suggested that the antioxidant properties of NAC may protect against microvascular damage produced by neutrophil accumulation (25-27).

Pharmacokinetics

Pharmacokinetic studies with NAC may produce conflicting results as total NAC may be measured or the assay used may be specific for the parent compound, reduced NAC. Reduced NAC is distributed through extracellular water with a volume of distribution that appears dose independent. Its major route of metabolism to cysteine, glutathione, inorganic sulphite and other related compounds. Only about 6 to 10 percent of a 600 mg oral dose reaches the systemic circulation as reduced NAC because of presystemic metabolism in the gut wall and in the liver. However. Portal vein concentrations are probably higher and in any case, the low systemic bioavailability is of questionable importance, as much of glutathione repleting action of NAC is via its metabolite cysteine. Radioactivity appearing in the faeces over 96 hours after oral dosing of rats with ^{35}S-NAC is under 6 percent, comparable with faecal excretion after intravenous

administration and indicating good oral absorption. The half-life of NAC in man is 2.5 to 5.7 hours, although the terminal half-life may be as long as 13 hours. The pharmacokinetic of NAC are not altered in patients with severe hepatic damage.

Efficacy

Preliminary studies in mice indicated that NAC was effective if given within a few hours of acetaminophen poisoning. Fifteen patients with acetaminophen levels above the line connecting 200 mg/L at 4 hours with 60 mg/L at 12 hours on a semilogarithmic plot were, therefore, treated with an intravenous regimen of 150 mg/kg over 15 minutes, followed by 50 mg/kg over 4 hours and 100 mg/kg over the next 16 hours (a total of 300 mg/kg over 20.25 hours). Of 12 treated within 10 hours, 11 had only minor disturbances of liver function; however, each of the 3 patients treated after this interval developed severe liver damage and 1 developed renal failure. In further studies involving 100 episodes of acetaminophen poisoning, the protection of cases developing severe hepatic necrosis (plasma transaminase more than 1000 IU/L) after treatment with the same intravenous regimen after intervals of up to 8 hours, 8 to 10 hours, 12 to 15 hours and 15 to 24 hours were 0, 7, 43, 56 and 82 percent respectively, compared with histological controls, of whom 89 percent developed liver necrosis. These results suggested that NAC was of little value when given after 15 hours; however, only 11 patients were treated after this interval. Severe liver damage occurred in 1 of 62 patients treated with NAC, 0 of 27 treated with cysteamine and 3 of 15 treated with methionine. Abnormalities of liver function were less common after NAC (26 percent) than after cysteamine (59 percent) or methionine (60 percent). Renal dysfunction developed in 13 percent of patients treated with NAC after 10 hours, but no patients treated within 10 hours, compared with a 13 percent incidence in histological control.

In the United States, intravenous NAC has not been approved by Food and Drugs Administration; however, there was early anecdotal evidence suggesting benefit for oral therapy and the drug has since usually been given via the oral route using a dose of 140 mg/kg followed by 70 mg/kg given every 4 hours to a total of 17 doses. In studies involving 100 patients, 10 percent of 49 patients presenting within 10 hours developed important hepatotoxicity compared with 45 percent of those treated after this interval. Further evidence of the importance of the interval between poisoning and NAC was that the proportions of patients developing hepatotoxicity when

treated within 10 hours, 10 to 16 hours and over 16 hours after poisoning were 7, 29, and 63 percent respectively. The largest report on the efficacy of oral NAC was the report of analysis of the National Multicenter Study performed in the United States between 1976 and 1985, which involved 11, 195 cases of suspected acetaminophen poisoning, 2540 of which were treated with NAC. In patients with acetaminophen levels above a line connecting 200 mg/L at 4 hours with 50 mg/L at 12 hours on a semilogarithmic plot (n=2023) 6 percent of those treated within 10 hours developed hepatic toxicity compared with 34 percent treated between 10 and 24 hours after poisoning. Prior to 8 hours, there was no loss in efficacy of NAC, but after this interval there was a stepwise reduction. No patients treated within 16 hours died and of those with a high risk of hepatotoxicity treated between 16 and 24 hours, only 116 of 283 (41 percent) developed severe liver necrosis.

Adverse reaction

The most important adverse effects of intravenous NAC have been anaphylactoid reaction. The common features were rash, often uricarial, angio-oedema, bronchospasm, hypertension and tachycardia. These reactions appear more frequency in patients receiving accidental overdose of NAC and their dose-related nature indicates that they are not true immune reactions. Less common features of NAC overdose include hypertension, DIC and renal failure (81). Oral NAC is rarely associated with serious adverse effects. It may induce vomiting (26) or diarrhea (82) and may be poorly tolerated because of its unpleasant smell and the prolong course required.

Timing of administration

While in theory NAC is expected to be most effective when given early after overdose, there is no evidence that delay in administration up to 8 hours after overdose is associated with a poorer outcome (83). Since there is a small risk of serious adverse effects from intravenous NAC this preparation should not be administered until plasma levels have shown the patient to be at risk, unless levels cannot be obtained until after 8 hours deadline, or this has already expired. Under the circumstances, it is reasonable to give NAC if there is a reliable history that more than 7.5 g of acetaminophen have been taken; indeed, a delay in administration of antidote in patient presenting after more than 8 hours has led to criticism during legal proceedings (84).

1.2.10 Disulfiram

Disulfiram, tetraethylthiuram disulfide, Antabuse, is a commonly used medication in the management of alcoholism because of its effectiveness in maintaining abstinence. The structure formula is shown in Figure 7.

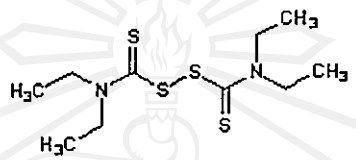

Figure 7 Structure formula of disulfiram (66)

Pharmacokinetic

Absorption

Disulfiram is highly lipid-soluble and very insoluble in water (84). Following ingestion, disulfiram is either absorbed as the parent compound or converted to diethyldithiocarbamic acid (diethyldithiocarbamate) in the acid environment of the stomach. Diethyldithiocarbamic acid is also very unstable in this acid environment and rapidly undergoes absorption and spontaneous decomposition to carbon disulfide and diethylamine, or chelates copper, forming a bis (diethyldithiocarbato) copper complex. The bis (diethyldithiocarbato) copper complex is more stable than diethyldithiocarbamic acid and also can be absorbed as it pass through the upper gastrointestinal tract. In fact, most disulfiram is absorbed from the small intestine as this bis (diethyldithiocarbato) copper complex. Approximately 70 to 90 percent of an ingested therapeutic dose of disulfiram is absorbed. The bioavailability of disulfiram varies with different preparation. In one study, the mean plasma disulfiram concentration in humans following a 250 mg dose was reported to be 0.38± 0.03 μg/ ml (85). Peak serum levels of disulfiram and its metabolites are achieved 8 to 10 hours following a 250 mg dose (86).

Distribution

Approximately 96 percent of disulfiram itself and approximately 80 percent of disulfiram metabolites are protein bound (86). Following absorption disulfiram and its metabolites are uniformly distributed throughout body tissues. A specific volume of distribution for disulfiram is not recognized.

Metabolism

Any absorbed disulfiram is rapidly converted to diethyldithiocarbamate acid by erythrocyte glutathione reductase and endogenous thiols. It is difficult to detect the parent compound disulfiram in blood because of its rapid conversion to diethyldithiocarbamic acid. Diethyldithiocarbamic acid in the blood also chelates copper, forming a bis(diethyldithiocarbato) copper complex. The bis(diethyldithiocarbato) copper complex also undergoes conversion back to diethyldithiocarbamic acid. Diethyldithiocarbamic acid is metabolized by a number of different pathways including glucuronidation, methylation, nonenzymatic degradation, and oxidation. Nonenzymatic degradation of diethyldithiocarbamic acid produces diethylamine and carbon disulfide. Carbon disulfide can be further oxidized to carbonyl sulfide, which can be further oxidized to carbon dioxide. Phase II methylation of diethyldithiocarbamic acid, which is mediated by an S-methlytransferase produces diethyldithiomethylcarbamic acid. Diethyldithiomethylcarbamic acid can be oxidized to diethylthiomethylcarbamic acid. Diethylthiomethylcarbamic acid is further oxidized to sulfoxide and sulfone metabolites and undergoes demethylation to from diethylthiocarbamic acid. Although diethyldithiocarbamic acid can be converted back to disulfiram and carbon disulfide and diethylamine can be converted back to diethyldithiocarbamic acid, these reactions are not clinically significant (87). Disulfiram metabolism is shown in Figure 8.

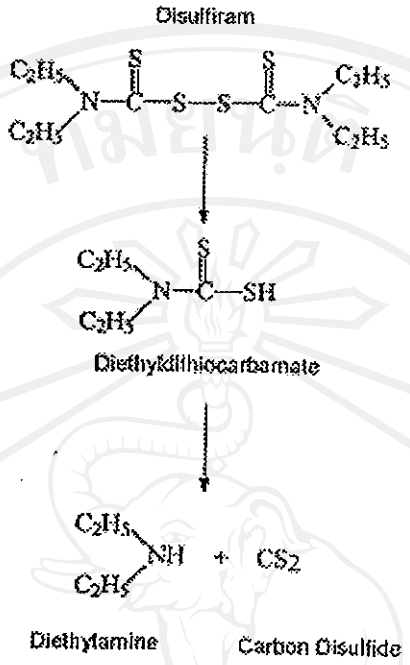

Figure 8 Disulfiram metabolism occurs in the liver and erythrocyte. (66)

Elimination

Following a 250 mg dose, the half-life of disulfiram, diethyldithiocarbamate and carbon disulfide are 7.3 ± 1.5 hours, 15.5 ± 4.5 hours, and 8.9 ± 1.4 hours, respectively (84). Approximately 20 percent or more of disulfiram is excreted unchanged in the feces and another 20 percent or more is excreted by the lungs as carbon disulfide. The majority of disulfiram is excreted in the urine as the glucuronidated metabolite of diethyldithiocarbamic acid (86). At 48 hours following a single 250 mg dose there is a negligible amount of disulfiram and metabolites detectable in the serum (85).

Disulfiram and the Cytochrome P450 system

Disulfiram and its metabolite are known as inhibitors of CYP 2E1 (88). Single doses of disulfiram administered to healthy humans result in 50 percent inhibition of baseline CYP2E1 activity for at least 3 days, with some inhibition for greater than 1 week (89-90). Although animal studies suggest that disulfiram alters acetaminophen metabolism, a human study found that disulfiram did not significantly alter the metabolism of a therapeutic dose of acetaminophen in either healthy patients or those either alcoholic liver disease (91). Disulfiram may be inducer of CYP2B1 and CYP 2A1. Disulfiram does not appear to affect CYP2C9, CYP2C19, CYP2D6 or CYP3A4 activity (86, 88). Disulfiram inhibits the metabolism and/ or decrease the clearance of phenyltoin, theophylline and wafarin. The effects of disulfiram on the cytochrome P 450 system maybe both dose and time dependent.

1.3 Objective

1. To study biochemical and histological changes in antitoxic effects of disulfiram with *N*- acetylcysteine on acetaminophen overdose in rats.
2. To study the effective dose of disulfiram when used with *N*-acetylcysteine to antitoxic acetaminophen overdose.