CHAPTER II

BACKGROUND INFORMATION

Ethylene glycol.

Ethylene glycol (EG), an alcohol containing two hydroxyl groups, is colorless, odorless, relatively nonvolatile liquid with a boiling point of 197°C. It is completely miscible with water, has a low freezing point, and is a principal component of a variety of antifreeze solutions for internal combustion engines and coolant in the lunar module solution.

Metabolism of ethylene glycol.

After ingestion, EG is rapidly absorbed from the gastrointestinal tract and uniformly distributed to the blood and the tissues of the animal (Bachman and Goldberg, 1971). The initial step in the EG metabolism is the oxidation of one of the hydroxyl moieties to an aldehyde, resulting in glycoaldehyde. This oxidation is catalyzed by the hepatic enzyme alcohol dehydrogenase. In human, this enzyme has a rather broad substrate specificity and also catalyzes the oxidation of ethyl glycol or methyl alcohol. Glycoaldehyde is then further oxidized to glycolic acid which is catalyzed by enzyme aldehyde oxidase or aldehyde dehydrogenase. Glycolic acid may accumulate in high concentration in the blood (Hewlett, Ray, and Reagor, 1983). The second hydroxyl group may be oxidized to the aldehyde, resulting in glyoxylic acid.

Glyoxylic acid is more toxic, but its half-life is short. This oxidation is catalyzed by glycolic acid oxidase. The predominant fate of this intermediate is to be oxidized to carbon dioxide and formic acid as demonstrated using various rat tissue homogenates including liver, and it requires nicotinamide adenine dinucleotide (NAD), thiamine pyrophosphate (TPP), manganese chloride, and L-glutamate for maximal reaction (Hagler and Herman, 1973). A small fraction of the glyoxylic acid is converted to oxalic acid which is catalyzed by three enzymes, glycolic acid oxidase, aldehyde oxidase, and lactic dehydrogenase (Turk, Morrell and Avioli, 1986). In dogs, only 2-3% of EG ingested is metabolized to oxalic acid. However, the cat produces more oxalic acid than in other species.

Most of EG and its metabolites are excreted in the urine. Sanyer et al. (1973) discovered that 5.7-23.4% of administered ethylene glycol was found in the urine during the first 24 hours. Since EG is less toxic than its metabolites, the goal of the treatment is to promote the excretion of the unmetabolized EG by using ethanol. Ethanol interferes with ethylene glycol metabolism because the enzyme alcohol dehydrogenase has a higher affinity for ethanol than for EG. The competitive inhibition of alcohol dehydrogenase results in the excretion of unmetabolized EG by the kidneys.

Oxalic acid is not bound by plasma proteins in the physiological pH range. Biological membranes (except mitochondria) are freely permeable to oxalic acid. Most oxalic acid is excreted in the urine. There is no evidence to indicate that oxalic acid is utilized or catalyzed in mammalian tissues. With calcium, excreted oxalic acid forms a practically insoluble salt at neutral or alkaline pH (0.67 mg /100 g H_2O). It is this property which gives oxalate its biological significance because crystallization may occur in the physiological pH range. The solubility of calcium oxalate is increased with decreasing pH, but the required degree of acidity obviates any potential physiological benefit (Hagler and Herman, 1973).

Clinical syndrome of ethylene glycol intoxication.

In animals, early after ingestion of the ethylene glycol, the clinical syndrome of EG intoxication may appear. Common clinical signs are ataxia, depression, vomiting, and hypothermia. In man, the patient may appear to be drunk but lacks the odor of ethyl alcohol on the breath (Turk et al., 1986). The effects of EG intoxication on the central nervous system (CNS) are nystagmus, ophthalmoplegia, hyperreflexia, coma, and seizure.

Sanyer et al. (1973) found that moderate CNS depression and mild ataxia appeared by 30 to 60 minutes after EG was given. Coma occurred by 6 to 12 hours after EG was administered, depending on the dose given. Immediately on regaining consciousness, the dogs were thirsty and drank water.

Dial et al. (1994a) found that the dogs became ataxic and depressed by 3 hours after EG ingestion. After treated with 4-methylpyrazole 5 to 8 hours after ingestion, 2 of 11 dogs remained lethargic throughout the evaluation period, but were not ataxic 12 hours after EG ingestion. Water consumption increased significantly from preingestion values of 36.2±6.4 and 40.0±12.1 ml/kg in 24 hours to 234±12.5 and 255.5±17.4 ml/kg in 24 hours after EG ingestion in the dogs treated with 4-methylpyrazole at 5 and 8 hours after EG ingestion respectively ($P<0.05$).

In case of EG intoxication in cats, Dial et al. (1994b) found that the cats which were given EG and not treated became lethalgic, ataxic, and disoriented by 3 hours after EG ingestion and were hypothermic and nonresponsive by 9 hours after EG ingestion.

Clinical findings were evaluated in 24 dogs and 26 cats admitted at the Colorado State University Veterinary Teaching Hospital by Thrall et al.(1984). The most common intoxicated clinical signs of CNS were depression, ataxia, coma, and

seizures in both dogs and cats. The complications of the digestive system were anorexia and vomiting. In addition, there were nearly equal numbers of animal which showed clinical signs of oliguria and anuria.

Effect of ethylene glycol on the kidney and urinary system.

Toxic metabolites of EG, including glycoaldehyde, glycolic acid, and glyoxylic acid play an important role in renal injury. They can be detected in the serum of intoxicated dogs as early as 3 hours after EG ingestion. Acute renal failure with oliguria was shown by 24 hours or more after ingestion (Parry and Wallach, 1974). Kidneys were swollen and congested. The toxic metabolites of EG damaged the structure of the kidney, especially the renal tubular part. The degrees of tubular damage were ranged from hydropic degeneration to Frank tubular necrosis with karyorhexis, pyknotic nuclei, disrupted tubular epithelium, and scattered mitotic figures (Sanyer et al., 1973).

Smith et al. (1990) studied the early effects of EG on the ultrastructure of the renal cortex in dogs. The light microscopic as well as the electron microscopic alterations were shown. The light microscopic changes were observed at 12 hours after ingestion. Tubular dilatation was the most common change. Other lesions were composed of the hydropic degeneration, the loss of cellular details, and the tubular cell necrosis. Tubular epithelial cells were flat and sometimes ruptured in the areas in which large clumps of crystal filled the tubular lumen. The electron microscopic changes were observed as early as 5 hours after EG ingestion. The distribution of numerous vacuoles through the cytoplasm of the proximal convoluted cells was evident which was prominent at 8 hours after ingestion. The distention of the extracellular parabasal spaces and the rupture of the proximal convoluted tubule cells were observed at 8 hours after ingestion. The increased density of mitochondria was first observed at 12 hours after ingestion. The lesions of distal convoluted tubule cells

were similar to those of the proximal convoluted tubule cells, and were prominently shown at 12 hours after ingestion.

At autopsy, the deposition of calcium oxalate crystals may also be observed histologically in the renal parenchyma and the collecting system. Although dihydrate calcium oxalate crystalluria was found in the report of EG intoxication in man, it was observed in only 18% of the dogs and cats evaluated (Thrall et al., 1984). Monohydrate calcium oxalate crystalluria was occasionally found in normal dogs (Thrall, Dial, and Winder, 1985). Monohydrate calcium oxalate crystalluria was detected as early as 3 hours after EG ingestion and mostly found in nonsurviving cats (Dial et al., 1994b). The serum concentrations of creatinine and urea nitrogen were increased above the reference range by 12 hours after EG ingestion in cats (Dial et al., 1994b; Thrall, Grauer, and Dial, 1995).

Dial et al. (1994b) found that most urine samples obtained at 1 hour after EG ingestion contained large numbers of erythrocytes, leukocytes, and increased protein concentration. Trace glucosuria was observed in cats with increased serum glucose concentration. Significant numbers of granular casts or renal epithelial cells were not observed.

Ethylene glycol produced diuresis in all dogs, but urine specific gravities were within normal limits in first 24 hours after ingestion (Sanyer et al., 1973). In addition, the rate of urine production was also increased significantly in cats (Dial et al., 1994b).

Effect of ethylene glycol on electrolytes and acid-base balance.

Clinical changes of electrolytes are observed in EG intoxicated animals. Hyperkalemia develops with the onset of oliguria and anuria. Hypercalcemia is infrequently observed because the acidosis results in a shift to the ionized.

physiologically active form of calcium. Hyperphosphatemia is caused by the decreased glomerular filtration or by the phosphate which is mixed in the antifreeze solution. If hyperphosphatemia is observed during 3-6 hours after ingestion, the early increase in serum phosphate is attributable to the high phosphorus content of antifreeze solution, meanwhile, acute renal failure usually develops with the onset of azotemia. Dial et al. (1994a) found that serum phosphorus concentrations increased at 9 hours after EG ingestion in cats. Grauer et al. (1984) found a slight increase of serum sodium concentration and serum chloride concentration by 6 hours after EG ingestion in dogs.

Serum osmolality is mostly increased by 1 hour after ingestion. Hyperosmolality occurs because the ethylene glycol is an osmotically active agent (Thrall et al., 1995). Both the osmolal gap and measured osmolality remain significantly high for approximately 18 hours after EG ingestion. Turk et al. (1986) showed the approximate evaluation of the osmolal gap produced by the estimated EG concentration. The EG concentration of 21 mg/dl produced 3.4 mOsm/kg of the osmolality. On the other hand, Thrall et al.(1995) estimated the serum osmolality by multiplying the osmolal gap by 6.2.

The increase of anion gap seen in patients is a result of the addition of the unmeasured anions that are most likely the EG metabolites. Glycolic acid is responsible for the severe metabolic acidosis associated with an extreme elevation of the anion gap. The anion gap is increased by 3 hours after EG ingestion, peaks at 6 hours after EG ingestion, and remains increased for approximately 48 hours. By 3 hours after EG ingestion, total carbon dioxide (tCO_2), plasma bicarbonate concentration, and blood pH are decreased. The pCO_2 often decreases as a result of partial respiratory compensation (Thrall et al., 1995).

Dial et al. (1994b) found that cats given EG and not treated had decreased blood pH and bicarbonate concentration by 6 and 9 hours after EG ingestion. Serum glycolic acid and bicarbonate concentrations in cats given EG and not treated had significant negative correlation ($r^2 = 0.89$, $P<0.01$).

EG metabolites and also lactate could cause metabolic acidosis. It would be expected on the basis that the depletion of the oxidized form of the pyridine nucleotide nicotinamide-adenine dinucleotide (NAD) during the oxidation results in the accumulation of lactate, and the high measured plasma lactate concentration in human patients poisoned with EG has been report (Parry and Wallach, 1974).

Treatments of ethylene glycol intoxication.

Treatment is based on preventing the metabolism of EG to its metabolites by inhibiting the liver enzyme alcohol dehydrogenase. There are two drugs of choice which are commonly used, the ethyl alcohol and the 4-methylpyrazole.

Ethyl alcohol is a competitive substrate for the alcohol dehydrogenase which is administrated intravenously in various concentrations. The common treatment in dog is to administer 5.5 ml/kg of 20% ethyl alcohol every 4 hours for 5 treatments followed by the treatment at every 6 hours for 4 treatments. Ethyl alcohol is also administered orally in concentration up to 50% by volume. More concentrated solution should not be used in order to avoid gastritis (Turk et al., 1986). However, doses of 50% or higher may be required if the patient has been treated with oral activated charcoal before the administration of the ethyl alcohol. During hemodialysis in EG intoxicated patient, the rate of ethyl alcohol administration should be increased to maintain the blood ethyl alcohol level (Peterson, Colling, and Himes, 1981). Alternatively, ethyl alcohol may be added to the dialysis bath. Ethyl alcohol, although not appropriated, remains the recommended antidote for EG intoxication in cats (Dial et al., 1994b).

The disadvantages of ethyl alcohol are the increased plasma osmolality which prolongs diuresis by inhibiting the antidiuretic hormone released, and the created hypoglycemia. In addition, ethyl alcohol enhances many of the metabolic

effects of the EG, such as the central nervous system depression, which results in a near-comatose state of the animals (Bahri, 1991).

Another drug of choice is the 4-methylpyrazole which has a rapid onset and prolongs the inhibitory effect of alcohol dehydrogenase enzyme. A blood concentration of 4-methylpyrazole above 10 μmol/L provides the constant inhibition of the alcohol dehydrogenase activity. The 4-methylpyrazole induces a competition with ethyl alcohol at a 3000:1 ratio of ethyl alcohol to 4-methylpyrazole, and has less effect on the plasma osmolality. Because of these reasons, 4-methylpyrazole is recommended to the EG intoxicated dogs. However, there is an evidence of unsuccessful treatment of EG intoxication in cats at 2 or 3 hours after EG ingestion using the 4-methylpyrazole (Dial et al., 1994b).

The 4-methylpyrazole is initially administered intravenously at a dose of 20 mg/kg, followed by 15 mg/kg at 12 and 24 hours, and finally 5 mg/kg is given at 36 hours (Bahri, 1991). The 4-methylpyrazole should be administered within 8 hours after EG ingestion.

Sanyer et al. (1973) found that administration of the sodium bicarbonate effectively prevented or reversed the acidosis, favored the excretion of the unchanged EG, and reduced the calcium oxalate formation.

Gastric lavage with activated charcoal is indicated within 1 to 2 hours of ingestion (Thrall et al., 1995).

Effect of ethylene glycol on mitochondria.

Because of the great energy requirement, proximal convoluted tubule cells have large number of mitochondria. Smith et al. (1991) found that the kidney mitochondria of EG ingested dog had the increased density. In addition, mitochondria

swelling was not a prominent feature. Bachmann and Golberg (1971) reported the effects of EG and its metabolites on tissue mitochondria. They found that when the EG concentration exceeded 5 mM, the phosphorylation of guanosine diphosphate was inhibited, but there was no adverse effect on the mitochondrial enzyme except for the inhibition of the substrate-level phosphorylation. Glyoxylic acid was found to be a very potent in vitro inhibitor of the mitochondrial electron-transfer activities. The numerous mitochondria in the proximal convoluted tubules and the evidences of mitochondrial changes may be the predisposing causes of the acute renal failure in EG intoxicated animals.

Free radicals and acute renal failure.

Free radicals, the highly reactive molecules, contain an unpaired electron in their outer orbit. Most of them are unstable and react with other molecules. When a free radical reacts with a nonradical molecule, another free radical must be produced. This reaction can propagate in chain reactions.

There are various releasing sources of free radicals, for example, the antibiotics, the phagocytic cells, the radiation, the xenobiotics, the solvents, and the intracellular metabolisms (Freeman and Crapo, 1982).

The oxygen molecule has two unpaired electrons in different orbits. The full reduction of the molecular oxygen within the cells leads to water formation and needs four electrons ($O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$). When the reduction is incomplete, the oxygen molecule can be converted into the reactive oxygen species; such as, the superoxide anion, the hydrogen peroxide, and the hydroxyl radical (Laurent and Ardillou, 1986; Lantz, 1995).

During ischemic hypoxia, the intracellular adenosine triphosphate (ATP) is degraded to hypoxanthine. In normal state, hypoxanthine is oxidized to xanthine by

xanthine dehydrogenase which uses nicotinamide adenine dinucleotide (NAD) as a substrate. Shortly after the onset of ischemia, the intracellular calcium increase is secondary to the ischemia-mediated breakdown of cell membrane. Xanthine dehydrogenase is converted to xanthine oxidase by the stimulated protease. Xanthine oxidase uses oxygen molecules as the substrate, so this enzyme is unable to catalyze the conversion of hypoxanthine to xanthine in the absence of oxygen (Lantz, 1995).

When the reperfusion is performed, and oxygen is reintroduced to the ischemic tissues. Xanthine oxidase can converse hypoxanthine to xanthine resulting in the generation of large amount of the superoxide anion and begins the chain reactions to produce other radicals. Superoxide anion is unstable and spontaneously reacts or dismutates to yield hydrogen peroxide and water. This reaction is catalyzed by the superoxide dismutase enzyme. Hydrogen peroxide itself is not a potent oxidizing agent.

Meanwhile, iron, stored in the protein ferritin, has been released during the ischemic state and is oxidized by the superoxide anion at the time of reperfusion. The reduced ferrous ion (Fe^{2+}) may combine with hydrogen peroxide. The result is the hydroxyl radical formation by the Haber-Weiss reaction in the presence of hydrogen peroxide and ferrous ion (Fe^{2+}) . Hydroxyl radical is the most reactive and destructive radical (Forsyth and Guilford, 1995; Freeman and Crapo, 1982; Lantz, 1995; Laurent and Ardaillou, 1986).

Therefore, free radicals can damage many parts of cell structure; for example, the cell membranes, the structural proteins and enzymes, and the DNA, resulting in cellular dysfunction and cellular damage.

The reactive oxygen species can induce the lipid radicals formation and cause cell injury. There are many evidences of acute renal failure related to the reactive oxygen formation; for example, the gentamicin-induced acute renal failure (Walker and Shah, 1988), the glycerol-induced acute renal failure, and the ischemic acute renal failure (Paller, Hoidel, and Ferris, 1984).