CHAPTER II
BACKGROUND AND LITERATURE REVIEW

2.1 Alcohol Metabolism

Alcohol dehydrogenase (ADH) oxidation, microsomal ethanol oxidizing system (MEOS), and catalase oxidation, in descending order of their contribution to metabolism, are the three primary pathways for ethanol conversion to acetaldehyde. Acetaldehyde formed from the oxidation of ethanol is rapidly converted to acetic acid by irreversible enzymatic reactions by two forms of acetaldehyde dehydrogenase: ALDH1 and ALDH2. A schematic of the reaction pathways is shown in Figure 2.1

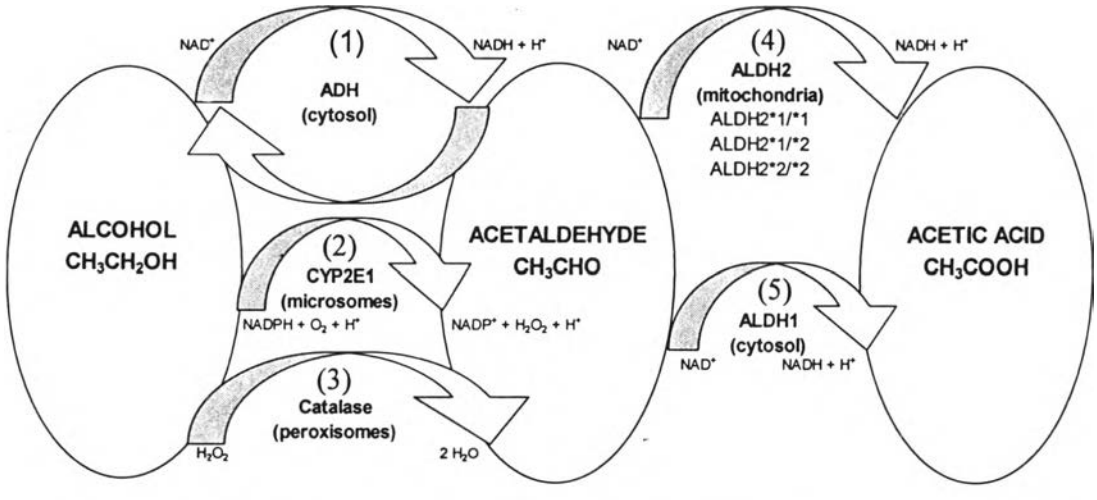

Figure 2.1 Alcohol and acetaldehyde metabolic pathways. The MEOS (CYP2E1) and catalase pathways proceed only from reactants to products while the ADH pathway is reversible.

2.1.1 Alcohol Dehydrogenase Oxidation Pathway

Alcohol dehydrogenase (ADH) is the principal enzyme contributing to the oxidation of ethanol to acetaldehyde in the liver (Lands, 1998). There are six classes of alcohol dehydrogenase in humans that show a wide range of activities and values of the Michaelis-Menten constant, K_m, which corresponds to the concentration

at which the rate of reaction is half of the maximal value. Classes I, II, III, V, and VI occur predominantly in the cytosol of hepatocyte cells in the liver while the high value of K_m in class IV is found in the gastric mucosa of the stomach. All classes of alcohol dehydrogenase follow the generalized reaction given in Equation 1 (Riveros-Rosas *et al.*, 1997).

$$CH_3CH_2OH + NAD^+ \overset{ADH}{\Longleftrightarrow} CH_3CHO + NADH + H^+ \qquad (1)$$

During alcohol metabolism, ethanol is oxidized by ADH in the presence of NAD^+ (oxidized form of nicotinamide adenine dinucleotide) to form acetaldehyde, NADH (reduced form of nicotinamide adenine dinucleotide)and H^+. At physiological conditions, ethanol is thermodynamically favored over acetaldehyde and the reverse rate is 10 times greater than the forward rate. However, acetaldehyde produced from ethanol oxidation is rapidly oxidized by acetaldehyde dehydrogenase (ALDH) into acetic acid. Acetic acid is a common intermediate in metabolic processes such as the Krebs cycle where it is converted into energy, CO_2 and H_2O. The rapid oxidation in normal human beings keeps the concentration of acetaldehyde low, which favors ethanol oxidation and therefore the reverse reaction back to ethanol has negligible effect on ethanol metabolism. The reverse reaction, however, is important in ethanol and acetaldehyde metabolism in individuals where acetaldehyde accumulates due to either an increased ethanol metabolism or a reduced acetaldehyde metabolism.

2.1.2 Microsomal Ethanol Oxidation System

The second pathway for ethanol metabolism is called the ethanol inducible microsomal ethanol oxidizing system (MEOS). The MEOS contains the cytochrome P-450 (CYP) enzymes of which the ethanol metabolizing CYP enzymes CYP2E1 and CYP2A1 belong. This cytochrome P-450 dependent pathway generates acetaldehyde and subsequently acetate while oxidizing biosynthetic reducing power, NADPH, to NADP+. Because it uses oxygen, this pathway generates free radicals that damage tissues.

$$CH_3CH_2OH + NADPH + O_2 \overset{MEOS}{\Longleftrightarrow} CH_3CHO + NADP^+ + H_2O_2 + H^+ \qquad (2)$$

Furthermore, H_2O_2 is efficiently converted to water and oxygen keeping the availability of H_2O_2 far too low for conversion of acetaldehyde back into ethanol.

$$2\,H_2O_2 \Leftrightarrow 2\,H_2O + O_2 \qquad (3)$$

The CYP enzymes, which are induced by ethanol substrate stabilization, contribute to increased ethanol metabolism during periods of heavy drinking. The faster ethanol metabolism rates associated with alcoholics have been attributed to the MEOS pathway (Riveros-Rosas *et al.*, 1997). With the exception of alcoholics, this pathway is much less significant than ADH pathway.

2.1.3 Catalase Pathway

Catalase, another hepatic enzyme, oxidizes ethanol using hydrogen peroxide to form acetaldehyde and water.

$$H_2O_2 + CH_3CH_2OH \overset{Catalase}{\Leftrightarrow} CH_3CHO + 2\,H_2O \qquad (4)$$

However, due to very low concentrations of hydrogen peroxide, the contribution of catalase to ethanol oxidation is quantitatively insignificant.

2.1 Acetaldehyde Dehydrogenase Metabolism

Acetaldehyde dehydrogenase (ALDH) irreversibly oxidizes acetaldehyde into acetic acid as shown in Figure 2.1. There are 5 distinct classes of ALDH enzymes based on their structure (Riveros-Rosas *et al.*, 1997). ALDH converts acetaldehyde to acetic acid in the presence of NAD^+ to produce NADH and H^+ (Riveros-Rosas *et al.*, 1997). Acetic acid is subsequently removed from the body as CO_2 and H_2O via the Krebs metabolic cycle.

$$CH_3CHO + NAD^+ + H_2O \overset{ALDH}{\Longleftrightarrow} CH_3COOH + NADH + H^+ \qquad (5)$$

2.2.1 Acetaldehyde Dehydrogenase II

The enzyme acetaldehyde dehydrogenase II (ALDH2) is located in mitochondria, the cell's primary energy producer. ALDH2 is the primary agent of acetaldehyde metabolism and is responsible for keeping acetaldehyde concentrations 2000 times lower than ethanol exiting the liver during normal ethanol metabolism (Jones *et al.*, 1988). ALDH2 has a very high affinity for acetaldehyde with a value of the Michaelis constant, K_m between 0.2 and 3μM. There are two prevalent forms of the ALDH2 gene, which give rise to three genetic combinations, or genotypes, as shown in Table 2.1

Table 2.1 Punnett Square showing possible genotypes based on two major forms of the ALDH2 gene from heterozygous parents, ALDH2*1 and ALDH2*2. The genotypes are homozygous normal (ALDH2*1/*1), heterozygous deficient (ALDH2*1/*2) and homozygous deficient (ALDH2*2/*2)

Gene	ALDH2*1	ALDH2*2
ALDH2*1	ALDH2*1/*1 Homozygous Normal	ALDH2*1/*2 Heterozygous Deficient
ALDH2*2	ALDH2*2/*1 Heterozygous Deficient	ALDH2*2/*2 Homozygous Deficient

The ALDH2*1 gene is labeled "normal" because the enzyme encoded by it has full oxidizing capacity, whereas the ALDH2*2 gene is labeled "deficient" because the enzyme encoded by it has a lower oxidizing capacity. The ALDH2*1 and ALDH2*2 genes are partially dominant in heterozygous ALDH2*1/*2 =

ALDH2*2/*1 individuals because the overall oxidizing capacity falls between the capacities of both the homozygous normal ALDH2*1/*1 and homozygous deficient ALDH2*2/*2 individuals (Enomoto *et al.*, 1991).

2.2.2 Acetaldehyde Dehydrogenase I

ALDH1 is located in the cytosol of erythrocytes, or red blood cells, and has a K_m value for acetaldehyde of 33μM. The K_m value is significantly larger than acetaldehyde concentrations, which are normally around 3μM in normal human blood during alcohol intoxication. Although ALDH1 does not contribute to acetaldehyde metabolism in normal individuals, ALDH1 may contribute to acetaldehyde metabolism in ALDH2*1/*2 and ALDH2*2/*2 deficient individuals.

2.3 Acetaldehyde Dehydrogenase Deficiency

Mitochondrial acetaldehyde dehydrogenase is encoded by the ALDH2 gene, which is known to exist in two forms ALDH2*1 and ALDH2*2. ALDH2*1 is the active form and ALDH2*2 is a naturally occurring inactive form. ALDH2*2 is caused by an amino acid substitution of glutamic acid (Glu) to lysine (Lys) (Wang *et al.*, 1996). The gene that codes for the inactive form is prevalent in either the heterozygous or homozygous form in a number of populations, for example, Cachi Indians: 7.7%, Shuara Indians 42% (Novoradovsky *et al.*, 1995), and Japanese: 49% (Peng *et al.*, 1999).

Acetaldehyde accumulation leads to a number of symptoms including facial flushing, nausea, anxiety, hypotension (Sellers *et al.*, 1981), and cardiac arrythmias (Condouris *et al.*, 1987). The intensity of these symptoms is most severe in ALDH2*2/*2 individuals, and less severe in ALDH2*1/*2 individuals who have some ALDH2 activity due to the co-dominant nature of the ALDH2 genes. While ALDH2*2/*2 individuals accumulate more acetaldehyde than ALDH2*1/*2 individuals under the same conditions, there is no clear relation between physiological effects and concentration of acetaldehyde.

2.4 Previous Ethanol Pharmacokinetic Models

Each of the previous models provided a step forward in understanding alcohol elimination kinetics, organ functionality (Levitt & Levitt, 1993), first pass metabolism (FPM) and intestinal absorption (Levitt 2002), and distribution kinetics (Norberg, 2001). However, each model is based on criteria that detract from its ability to use or produce physiological results. The main criteria are: (1) whether the elimination follows zero-order kinetics or Michaelis-Menten kinetics, (2) whether the reaction is reversible, (3) the number of separate compartments, and (4) the type of liver model used. The main criteria for each of the alcohol pharmacokinetic models are presented in Table 2.2

Table 2.2 Previous alcohol pharmacokinetic models' authors and assumptions. Compartment is the number of compartments, and Liver is the model used for the liver compartment. In this work the stomach is included as a compartment in human model, however it is treated separate for comparison purposes due to zero blood volume. †Lundquist and Wolthers (1958) investigated the reverse reaction, however it was not used in their final model. ‡M-M: Michaelis-Menten

Date	Author	Rate-Law	Reversible	Compartment	Liver
1932	Widmark	Zero order	No	1	None
1958	Lundquist†	M-M	No	1	None
1977	Wilkinson	M-M	No	1	None
1993	Levitt	M-M	No	2	Well-mixed
2000	Pastino	M-M	No	8	Well-mixed
2001	Norberg	M-M	No	3	Well-mixed
2005	This work	M-M	Yes	4+stomach	FlowLimited