Free Radical Production During Ethanol Metabolism

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Ethanol Metabolism

- Ethanol is oxidized to acetaldehyde through several enzymatic pathways:
  - Alcohol dehydrogenase
    - $CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+$
  - Catalase
    - $CH_3CH_2OH + H_2O_2 \rightarrow CH_3CHO + 2\ H_2O$
  - Cytochrome P-450
    - The “Microsomal Ethanol Oxidizing System” (MEOS)
    - $CH_3CH_2OH + NADPH + H^+ + O_2 \rightarrow CH_3CHO + 2\ H_2O + NADP^+$
- And through a non-enzymatic free radical pathway:

Free Radical Metabolite of Ethanol

- Strong oxidizing intermediates such as the hydroxyl radical can abstract a hydrogen atom from ethanol, preferentially producing the 1-hydroxyethyl radical (also called the α-hydroxyethyl radical)

$$CH_3CH_2OH + {}^{\bullet}OH \rightarrow CH_3C^{\bullet}HOH + H_2O$$

- Hypervalent iron complexes may also catalyze this reaction without the apparent involvement of ${}^{\bullet}OH$[1,2].

[1]Reinke LA, Rau JM and McCay (1994), Free Rad. Biol. Med. **16**: 485-492.

[2]Welch KD, Davis Z and Aust SD (2002), Arch. Biochem. Biophys. **397**: 360-369. And Qian SY, Buettner GR. (1999) *Free Radic Biol Med*, **26**: 1447-1456.

Reactions of the Hydroxyethyl Radical

- Hydroxyethyl radicals may react with oxygen to form a peroxy radical intermediate, which can then rearrange to release acetaldehyde and superoxide.

$$CH_3C^{\bullet}HOH + O_2 \rightarrow CH_3C(OO^{\bullet})HOH$$

$$CH_3C(OO^{\bullet})HOH \rightarrow CH_3CHO + O_2^{\bullet -} + H^+$$

- Hydroxyethyl radicals can also react with proteins to produce antigenic adducts[1] or induce the mitochondrial permeability transition[2].

[1]Clot P, Bellomo G, Tabone M, Arico S and Albano E (1995), Gastroenterology **108**: 201-207.

[2]Sakurai K, Stoyanovsky DA, Fujimoto Y and Cederbaum AI (2000), Free Rad. Biol. Med. **28**: 273-280.

Hydroxyethyl Radical Formation in Liver Microsomes

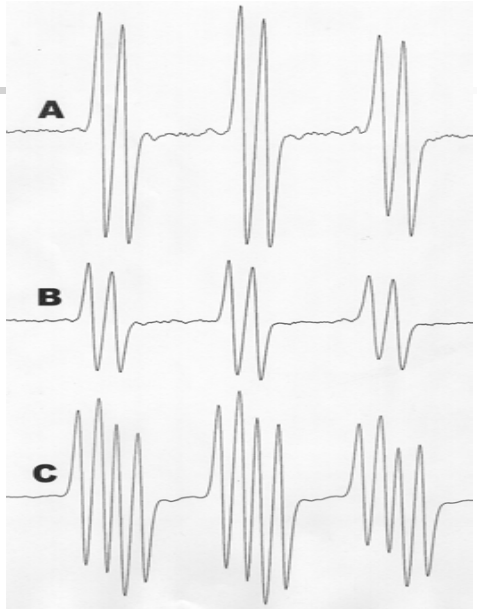
- Spin trapping experiments have demonstrated that hydroxyethyl radical formation by liver microsomes requires a source of reactive oxygen intermediates[1-4].

[1]Albano E, Tomasi A, Goria-Gatti L and Dianzani MU (1988), Chem.-Biol. Interact. **65**: 223-234.

[2]Reinke LA, Rau JM and McCay PB (1990), Free Rad. Res. **9**: 205-211.

[3]Knecht KT, Thurman RG and Mason RP (1993). Arch. Biochem. Biophys. **303**:339-348.

[4]Rashba-Step J, Turro NJ and Cederbaum AI (1993), Arch. Biochem. Biophys. **300**: 401-408.



A. Rat liver microsomes + ethanol, POBN, & NADPH.
B. As A, but + catalase
C. As A, but with 1-^{13}C-ethanol. The 12-line EPR spectrum identifies the 1-hydroxyethyl radical.

Rates of Microsomal Hydroxyethyl Radical Formation are Increased by Ethanol Feeding

- Prior ethanol feeding increases rates of hydroxyethyl radical formation by liver microsomes[1-3].
- The ethanol-inducible cytochrome P-450 2E1 (CYP 2E1) is somewhat uncoupled, and produces substantial amounts of superoxide and hydrogen peroxide[4].

[1]Reinke LA, Lai EK, DuBose CM and McCay PB (1987), PNAS **84**: 9223-9227.

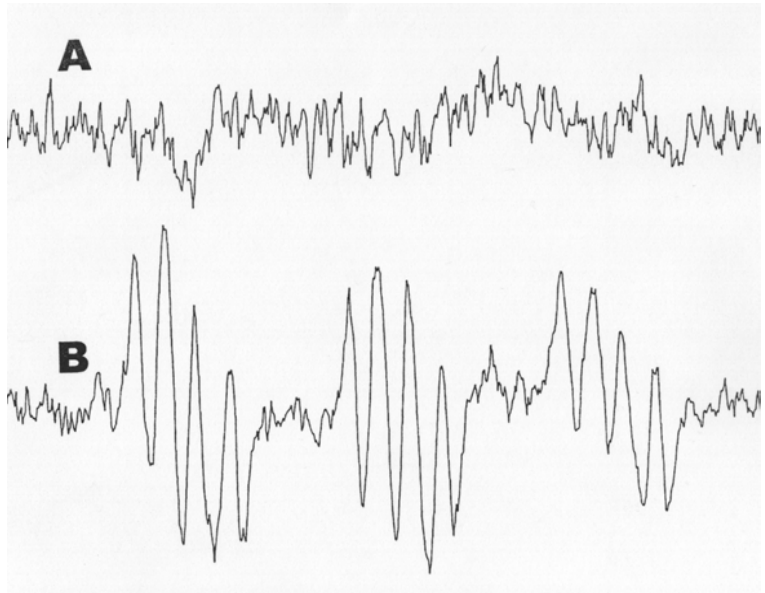
[2]Albano E, Tomasi A, Goria-Gatti L and Dianzani MU (1988), Chem.-Biol. Interact. **65**: 223-234.

[3]Rashba-Step J, Turro NJ and Cederbaum AI (1993), Arch. Biochem. Biophys. **300**: 401-408.

[4]Eksktrom G, and Ingelman-Sundberg M (1989), Biochem. Pharmacol. **38**: 1313-1319.

Hydroxyethyl Radical Formation *in vivo*

- Spin trapping experiments have demonstrated that hydroxyethyl radicals are formed in mice and rats[1,2]
- Radicals of endogenous compounds can also be detected following acute alcohol administration[1,2].



A. Bile sample from chow-fed rat given POBN only.
B. Bile sample from rat in A, but after injecting 1-^{13}C-ethanol

[1]Knecht KT, Bradford BU, Mason RP and Thurman (1990), Mol. Pharmacol. **38**: 26-30.

[2]Moore DM, Reinke LA and McCay PB (1995), Mol. Pharmacol. **47**: 1224-1230.

Alcohol-Initiated Radicals of Endogenous Compounds

- Spin trapping experiments with a deuterated derivative of PBN demonstrated that two types of radicals could be formed from endogenous compounds in liver following a large dose of ethanol[1]:

$$R\text{-}C^{\bullet}H_2 \qquad\qquad RO^{\bullet}$$

- These radicals are likely intermediates of lipid peroxidation initiated by large doses of ethanol[2].

[1]Reinke LA, Kotake Y, McCay PB and Janzen EG (1991), Free Rad. Biol. Med. **11**: 31-39.

[2]DiLuzio NR and Hartman AD (1967), Fed. Proc. **26**: 1436-1442.

Radicals Associated with Chronic Ethanol Consumption

- Radicals likely formed from liver lipids of ethanol-fed rats were first detected *in vivo* in spin trapping experiments with a trimethoxy derivative of PBN[1].
- High levels of dietary fat (as corn oil) given with alcohol intensifies:
  - Liver injury (steatosis)
  - Cytochrome P-450 induction
  - Lipid radical formation

[1]Reinke LA, Lai EK, DuBose CM and McCay (1987), PNAS **84**: 9223-9227.

Enhanced Radical Formation *In Vivo* Following Chronic Alcohol Administration

- Chronic alcohol administration to rats increased *in vivo* formation of hydroxyethyl and lipid radicals, and caused consistent detection of ascorbate radicals in bile[1].

[1]Reinke LA, Moore DR and McCay PB (1997), Alcohol. Clin. Exp. Res. **21**: 642-646.

A. Bile sample from ethanol-fed rat, given POBN only. The six-line spectrum is likely from lipid radicals, with the central doublet from ascorbate radicals.
B. Bile sample from rat in A, but after injection of 1-^{13}C-ethanol.

Role of Polyunsaturated Fat

- Alcohol feeding with saturated fats causes negligible liver injury. Alcohol feeding with corn oil or fish oil produces liver injury that increases with the unsaturated fat content of the diet[1].
- Feeding alcohol with fish oil, but not with saturated fat, also increases lipid radical formation[2].
- Free radicals associated with alcohol feeding persisted for at least 24 h following removal of alcohol from the diet[2].

[1]Nanji AA, Griniuviene B, Sadrzadeh SMG, Levitsky S and McCully JD (1995), J. Lipid Res. **36**: 736-744.

[2]Reinke LA, Moore DR and Nanji AA (2000), Alcohol. Clin. Exp. Res. **24**: 332-335.

Potential Involvement of Kupffer Cells

- Inhibition of Kupffer cells with gadolinium chloride decreased free radical formation and liver injury following chronic alcohol treatment of rats[1].
- When alcohol-fed rats were given a small dose of lipopolysaccharide, hydroxyethyl radical formation was stimulated[2].
- Mice deficient in TNFα receptors were resistant to alcohol-induced liver injury[3].

[1]Knecht KT, Adachi Y, Bradford BU, Iimuro Y, Kadiiska M, Quh-Hui X and Thurman RG (1995), Mol. Pharmacol. **47**: 1028-1034.

[2]Chamulitrat W, Carnal J, Reed NM and Spitzer JJ (1998), Am. J. Physiol. **274**: G653-G661.

[3]Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster, MI and Thurman RG (1999), Gastroenterology **117**: 942-952.

Potential Role of NADPH Oxidase

- NADPH oxidase deficient mice ($p47^{phox}$ knockout) exhibited less liver injury and lower levels of radicals during alcohol feeding[1].
- Treatment of rats with the NADPH oxidase inhibitor, diphenyleneiodonium sulfate, decreased free radical formation and liver injury following chronic alcohol treatment[2].

[1]Kono H, Rusyn I, Yin M, Babele E, Yamashina S, Dikalova A, Kadiiska MB, Connor HD, Mason RP, Segal BH, Bradford BU, Holland SM and Thurman RG (2000), J. Clin. Invest. **106**: 867-872.

[2]Kono H, Rusyn I, Uesugi T, Yamashina S, Connor HD, Dikalova A, Mason RP and Thurman RG (2001), Am. J. Physiol. **280**: G1005-G1002.

Potential Role of CYP 2E1

- CYP 2E1 inhibitors fed with alcohol decreased liver injury in alcohol-fed rats[1].
- Levels of CYP 2E1 correlated positively with the degree of liver injury in alcohol-fed rats[1].
- In contrast, CYP 2E1 knockout mice exhibited similar liver injury and free radical formation as alcohol-fed wild type mice[2].

[1]Morimoto M, Hagbjork AL, Wan YJ, Fu PC, Clot P, Albano E, Ingelman-Sundberg M and French SW (1995), Hepatology **21**: 1610-1617.

[2]Kono H, Bradford BU, Yin M, Sulik KK, Koop DR, Peters JM, Gonzalez FJ, McDonald T, Kilaova A, Kadiiska MB, Mason RP and Thurman RG (1999), Am. J. Physiol. **277**: G1259-G1267.

Liver Mitochondria and Oxidative Stress

- Mitochondria have been implicated as a source of alcohol-induced oxidative stress, and as critical targets of alcohol-associated damage[1,2].

[1]Bailey SM and Cunningham CC (2002), Free Rad. Biol. Med. **32**: 11-16.
[2]Adachi M and Ishii H (2002), Free Rad. Biol. Med. **32**: 487-491.

Summary: Alcohol-Induced Oxidative Stress

- Oxidative stress associated with chronic alcohol administration may be initiated by reactive oxygen intermediates that are most likely generated by:
  - NADPH oxidase found in Kupffer cells and other phagocytes
  - Cytochrome P-450 enzymes
  - The mitochondrial electron transport chain

Summary: Free Radicals and Alcohol Toxicity

- Ethanol is metabolized to a free radical intermediate, the 1-hydroxyethyl radical, *in vitro* and *in vivo*.
- 1-Hydroxyethyl radical formation seems dependent on formation of reduced oxygen species and iron.
- The same oxidizing intermediates that form 1-hydroxyethyl radicals from ethanol likely initiate free radical reactions in lipids and/or other endogenous compounds.