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INTERACTION OF ETHANOL WITH HAEMOGLOBIN: IMPLICATIONS FOR ALCOHOL ABUSE

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Ingestion of large amounts of ethanol results in a variety of toxicological consequences of which reduced oxygen supply is a marked respiratory effect. Experimentation involving the *in-vivo* administration of ethanol to rats, identifies the formation of ferri-haemoprotein species and hydroxyl radicals in the liver. Our work on the *in-vitro* effect of ethanol on oxyhaemoglobin shows the formation of ferri-haemoglobin and the α-hydroxyethyl radical as an intermediate. The impairment of oxygen transport efficiency of haemoglobin may be a result of its oxidation to the non-functional ferrihaemoglobin form.

KEY WORDS: EPR, Ethanol, Haemoglobin, Hydroxyethyl radical.

INTRODUCTION

In the oxidation of ethanol to acetaldehyde by liver microsomes,[1] the participation of cytochrome P-450 in a specifically active form, variously known as cytochrome P450 isozyme 3a,[2] and cytochrome P-450IIE1,[3] has been identified. This active form is induced by the *in-vivo* administration of ethanol in a dietary mode. The haem iron of the cytochrome is oxidised to the Fe^{III} derivative. Oxidation of ethanol occurs via formation of the α-hydroxyethyl radical, $CH_3\dot{C}HOH$ by abstraction of hydrogen from the α-carbon of the alcohol.[4]

Instances of acute ethanol ingestion as in alcohol abuse result in appreciable amounts being present in the circulatory system.[5] Considering the predominance of haemoglobin especially in the physiologically functional oxy-derivative, the effect of alcohol on oxyhaemoglobin may have great significance in alcoholism and respiratory efficiency. Furthermore the induced generation and accumulation of free radicals in the body is potentially dangerous.

We have employed the technique of electron paramagnetic resonance (EPR) spectroscopy with spin-trap protocol *in-vitro* to study the interaction of ethanol with haemoglobin. Low temperature EPR measurements have also been used to determine the resultant product from the haemoglobin.

RESULTS AND DISCUSSION

Addition of small volumes of aqueous ethanol to dilute solutions of oxyhaemoglobin followed by rapid-freezing in liquid nitrogen provided samples for determination of both oxidation and spin states of the iron. Addition of a dilute aqueous

solution of the spin trap, 4-pyridyl-1-oxide-t-butyl nitrone, (4-POBN) to a freshly prepared mixture of ethanol and oxyhaemoglobin, gave a stable radical adduct by which the free radical product was detected.

Our results show clearly the formation of oxidised haem species, Fe^{III}, identified by the spectrum in Figure 1. The spectral feature at $g = 6$ is characteristic of the high spin form of ferrihaemoglobin. The spectrum in Figure 2 shows distinct features attributed to the ethanol-haemoglobin derived α-hydroxyethyl radical adduct of the 4-POBN with the parameters $A_N = 15.6G$, $A_H = 2.5G$.[6] These parameters are clearly different from those of the hydroxyl radical adduct of 4-POBN as listed in Table 1. These results show that the major radical species generated in the interaction between ethanol and haemoglobin is the α-hydroxyethyl radical. In contrast with previous results on liver microsomes of ethanol-treated rats,[7]

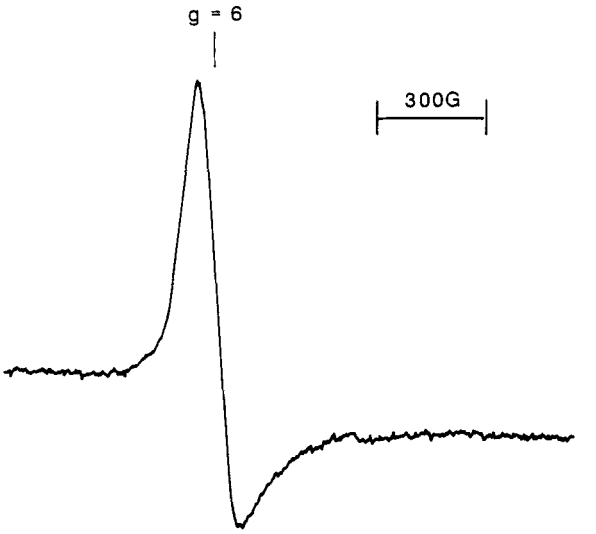

Figure 1 X-Band EPR spectrum at 77K for the oxyhaemoglobin-ethanol reaction showing an intense signal at $g = 6$, characteristic of ferrihaemoglobin in the high spin state.

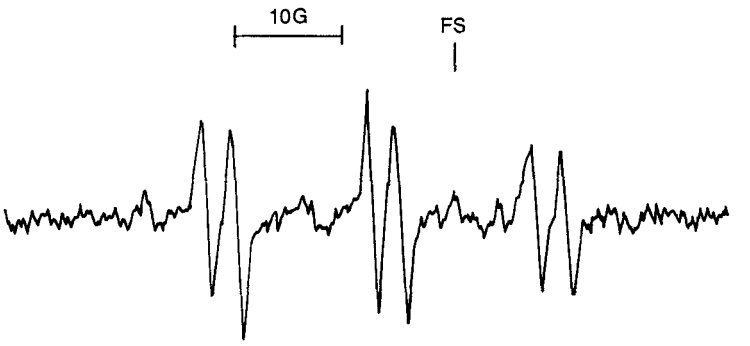

Figure 2 X-Band EPR spectrum at room temperature for the radical adduct of 4-POBN with $CH_3\dot{C}HOH$ radicals.

TABLE 1
EPR parameters for the radical adduct of 4-POBN with hydroxyl radical

A_N/G	A_H/G	Ref.
14.97	1.68	8
14.93	1.69	9
14.95	1.68	10
14.93	1.69	11

hydroxyl radicals were not detected in our system. The reaction of oxyhaemoglobin with ethanol may be represented as (1)

$$HbFe^{II}O_2 + CH_3CH_2OH \rightarrow HbFe^{III} + CH_3\dot{C}HOH + HO_2^- \quad (1)$$

The rapid addition of the spin trap efficiently traps the free radical from which the final oxidation product, acetaldehyde would have been formed.

The intense signal at $g = 6$ is similar to that normally obtained for aquoferri-haemoglobin in which the haem iron is oxidised to +3 and its sixth coordination position is occupied by water. In this form haemoglobin does not bind oxygen. This may provide some explanation for the reduction in oxygen-binding capacity of haemoglobin due to the presence of alcohol.

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