Ethanol Inhibition of Signal Transduction in Superoxide Production by Rat Alveolar Macrophages

A Proposed Mechanism for Ethanol Related Pneumonia*

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ABSTRACT

Alveolar macrophages and neutrophils produce superoxide and other free radicals which are important in killing bacteria. The focus of this paper is the inhibition by ethanol of superoxide production and anti-bacterial activity. The signal transduction pathways leading to superoxide production by phagocytic cells will be reviewed. Our hypothesis is that ethanol alters these signal transduction pathways. Stimulation of superoxide production can be initiated by concanavalin A and phorbol esters. Previously, there were reports by us that ethanol, in vitro, inhibits phorbol ester induced superoxide production in rat alveolar macrophages. Our present report states that concanavalin A induced superoxide production was more sensitive to ethanol inhibition than phorbol ester induced superoxide production. The ethanol induced inhibition of alveolar macrophage superoxide production provides a possible mechanism to explain the increased sensitivity of alcoholics to pneumonia.

Introduction

This paper presents the hypothesis that the susceptibility of alcoholics to pneumonia is due to interference by ethanol in the signal transduction pathways that regulate superoxide (O₂⁻) production in alveolar macrophages. The literature concerning susceptibility of alcoholics to pneumonia will be reviewed followed by a review of information about the signal transduction process, including some recent evidence from our laboratory suggesting a mechanism for...
ethanol modulation of signal transduction in rat alveolar macrophages. Finally, our hypothesis concerning effects of ethanol upon the alveolar macrophage in the context of the general effects of ethanol upon signal transduction will be reviewed.

Susceptibility of Alcoholics to Pneumonia

The increased susceptibility of human alcoholics to bacterial infections and to pneumonia is well documented.2,15,19 The incidence of bacterial pneumonia and subsequent mortality is three times greater in alcoholic populations than in control populations. Studies of the effects of ethanol in humans are complicated by the fact that alcoholics have a multitude of associated problems, including cirrhosis of the liver and malnutrition, which may independently promote infection.

The neutrophilic infiltrate seen in bacterial pneumonia is familiar to all clinicians. It is not surprising that many studies have attempted to link bacterial pneumonia with ethanol induced neutrophil dysfunction. Studies have been performed both in vivo and in vitro. Ethanol inhibits in vivo neutrophil mobilization into skin windows.3,18 Ethanol ingestion reduces bacterial clearance from skin20 and from the lungs.9 Given these in vivo results, isolated neutrophils have been studied in an attempt to determine the site of action of ethanol. At lethal ethanol concentrations, 640 mg per dl, many neutrophil functions are impaired.14,19 However, at lower, more physiologic concentrations of ethanol, impaired neutrophil function is difficult to demonstrate. Adherence of neutrophils to nylon is diminished by 460 mg per dl of ethanol.17 Furthermore, higher levels of ethanol are required in vitro to inhibit neutrophilic chemotaxis, phagocytosis or bacterial killing.3,18

One of the principle sites of infection in alcoholics is the lung. Studies of alveolar macrophage function may be particularly important in understanding the pathogenesis of alcohol related pulmonary infection. The alveolar macrophage is the indigenous inflammatory cell in the uninfected lung and constitutes the first line of defense against pulmonary bacterial infections.11 The best evidence for the importance of the alveolar macrophage comes from the studies by Green and Kass.13 These investigators studied the fate of relatively low concentrations of aerosolized bacteria in murine lungs. Phagocytosis, observed by gram stain, was rapid. Phagocytosis of most of the organisms by macrophages occurred within three minutes. By four hours, viability of the bacteria was reduced 80 percent. Of importance was that no neutrophils were seen in lung sections. Ethanol inhibits mobilization of alveolar macrophages following bacterial challenge.13 In vitro inhibition of bactericidal activity by 400 mg per dl or 87 mM of ethanol has been reported using rabbit alveolar macrophages.21 The literature suggests that in vitro bactericidal functions of the alveolar macrophage are more sensitive to inhibition by ethanol than are those of the neutrophil.

Superoxide Production by Phagocytes

Alveolar macrophages and neutrophils possess bactericidal activity. These cells secrete superoxide and produce other free radicals upon stimulation which are instrumental in bacterial killing. The best evidence for the importance of superoxide generation in anti-bacterial host defense is in those patients with chronic granulomatous disease who lack the ability to produce superoxide and suffer recurrent pulmonary infections.22 Superoxide production in macrophages and neutrophils is regulated by a complex series of reactions that involves
two inter-related pathways. Both of these pathways are coupled to the breakdown of phosphatidylinositol 4,5-bisphosphate \([\text{Ptd Ins (4,5)P}_2]\). Following receptor binding, Ptd Ins (4,5)P\(_2\) is broken down by phospholipase \(C\). Two intracellular second messengers are formed; myo-inositol 1,4,5-trisphosphate \([\text{Ins(1,4,5)P}_3]\) and diacylglycerol. Ins(1,4,5)P\(_3\) is the second messenger that results in the liberation of calcium from intracellular stores. Diacylglycerol is the endogenous activator of protein kinase \(C\). Activation of superoxide production presumably results from subsequent calcium-dependent or protein kinase \(C\)-dependent phosphorylation steps. Superoxide production can be stimulated by adding model antagonists. Concanavalin \(A\) binds to the cell surface and results in superoxide production associated with an elevation of intracellular calcium. Superoxide production can also be stimulated by calcium ionophores. Since the concentration of calcium in the extracellular medium is 10\(^4\) times greater than the concentration in the cytoplasm, an elevation of intracellular calcium can be achieved by adding the calcium ionophore, A23187, which allows influx of calcium from the extracellular medium. An elevation of intracellular calcium is not \textit{sine qua non} for production of superoxide. Phorbol 12-myristate 13-acetate (PMA), which activates protein kinase \(C\), stimulates superoxide production in the absence of a rise in intracellular calcium. Thus, PMA stimulates superoxide production and bypasses Ptd Ins (4,5)P\(_3\) breakdown by activating protein kinase \(C\) directly.

Recent Observations

It has been found in our laboratory that ethanol interferes directly with the phosphatidylinositol related signal transduction pathway in rat alveolar macrophages. Addition of ethanol raises intracellular calcium and stimulates superoxide production. Some of the increase in intracellular Ca\(^{2+}\) was independent of extracellular Ca\(^{2+}\), indicating that at least a portion of the rise in intracellular calcium is the result of release of Ca\(^{2+}\) from intracellular stores. This internal Ca\(^{2+}\) release is probably due to Ins(1,4,5)P\(_3\) production. The amount of superoxide stimulation (11 pmol per min per \(10^6\) cells following 100 mM ethanol) is almost two orders of magnitude less than that obtained following saturating amounts of PMA (750 to 900 pmol per min per \(10^6\) cells). Phorbol 12-myristate 13-acetate is the most potent activator of O\(_2^-\) production in these cells.

Although it has been shown by the present authors that ethanol itself is a weak agonist for superoxide production, ethanol also acts as an antagonist for other more potent agents, such as PMA or concanavalin \(A\) (figure 1). Macrophages were preincubated in various concentrations of ethanol for 15 minutes followed by stimulation by either PMA or concanavalin \(A\). The degree of inhibition of superoxide dismutase inhibitable superoxide production is shown on figure 1. At 50 mM ethanol (230 mg per dl), there is a significant difference between the percentage of inhibition seen with concanavalin \(A\) compared to that observed with PMA. It should be noted that there is more than a 50 percent inhibition of the concanavalin \(A\) induced O\(_2^-\) production following preincubation in 50 mM ethanol.

The effect of alcohol on inhibiting PMA and concanavalin \(A\) stimulated superoxide production is dose dependent, as shown in figure 1. High concentrations of alcohol are required to sustain the inhibitory effect of ethanol on the inhibition of PMA-induced activity. Dilution of ethanol from 300 mM to 25 mM immediately before addition of phorbol ester completely reversed the inhibition of PMA stimulated superoxide production.
Figure 1. Alveolar macrophages were isolated from specific pathogen-free Sprague Dawley male rats as previously described. Macrophages (10⁶ cells per ml for concanavalin A and 0.2 × 10⁶ cells per ml for phorbol 12-myristate 13-acetate (PMA) stimulation) were preincubated in various concentrations of ethanol for 15 minutes at 37°C before addition of maximally stimulating concentrations of PMA (10 ng per ml) or concanavalin A (250 μg per ml). Values are ± S.D., n = 3. Error bars not shown are within the points. *p < 0.01 for inhibition for PMA vs concanavalin A.

Ethanol and Signal Transduction

The continued presence of high concentrations of ethanol may be critical for other effects on anti-inflammatory cells. For instance, Brayton et al³ and MacGregor¹⁸ both showed that ethanol administration to volunteers in a bolus results in decreased mobilization of neutrophils into skin windows. In contrast in an earlier study, Gluckman, Dvorak and MacGregor⁸ allowed individuals to consume an alcoholic beverage during the course of an afternoon, to mimic more closely the natural consumption patterns. Maximal blood ethanol levels were probably lower. In this study, ethanol consumption had no effect on neutrophil mobilization using a similar skin abrasion technique. The individuals in the latter study were alcoholics, while those individuals in the former two studies were non-alcoholic volunteers. The alcoholic patients probably had an increased capacity to metabolize ethanol and would have had lower blood ethanol concentrations. The possibility that neutrophils in alcoholics had adapted to high ethanol concentrations can not be discounted. Therefore, the concentration of ethanol, method of administration and the population studied are important variables in evaluating effects of ethanol.

Alcohol causes a variety of problems such as pneumonia, fetal alcohol syndrome, impotence, cirrhosis, pancreatitis and intoxication itself. Some of these alcohol associated syndromes could possibly be secondary to a disturbance in signal transduction. Evidence has been presented here and elsewhere⁶,⁷ that ethanol interferes with signal transduction systems important in stimulating bactericidal superoxide production. This signal transduction system involves the mobilization of intracellular calcium (through phosphatidyl inositol 4,5-bisphosphate turnover) and activation of protein kinase C. The calcium and protein kinase C pathways are ubiquitous. Many cellular receptor systems use these systems. Some examples are α-adrenergic and muscarinic stimulation, histamine, serotonin, platelet activating factor and vasopressin activation.¹ The sensitivity to interference by ethanol of this signal transduction system may vary from tissue to tissue. This variation in cellular response may explain the inability to demonstrate in vitro cellular defects in chemotaxis, phagocytosis and intracellular bacterial killing by neutro-
phils at physiologically relevant ethanol concentrations. Ethanol has effects upon calcium homeostasis and inhibits signal transduction in a number of other cellular systems. Ethanol stimulates an increase in intracellular calcium in hepatocytes,\textsuperscript{16} inhibits concanavalin A and somatostatin induced histamine release from mast cells,\textsuperscript{12} decreases colony-stimulating activity production by T lymphocytes, suppresses blast formation of lymphocytes, and inhibits natural killer activity \textit{in vitro}.\textsuperscript{19}

\textbf{Summary}

Ethanol modulates the activity of the signal transduction pathways utilized to produce superoxide. It is speculated by the present authors that the ethanol related interference in concanavalin A and PMA-induced superoxide production in alveolar macrophages may provide a mechanism for the increased susceptibility of alcoholics to pneumonia. Furthermore, interference in signal transduction systems by ethanol in other tissues may provide a unifying mechanism to explain the widespread effects of ethanol.

\textbf{References}


10. \textsc{Green, G. M.} and \textsc{Kass, E.}: The role of the alveolar macrophage in the clearance of bacteria from the lung. J. Exp. Med. \textbf{119}:165–175, 1964.


