Available online at www.sciencedirect.com
SCIENCE DIRECT®
Toxicology Letters 156 (2005) 81–93
Toxicology Letters
www.elsevier.com/locate/toxlet

Butoxyacetic acid-induced hemolysis of rat red blood cells: effect of external osmolarity and cations

M.M. Udden*, C.S. Patton

Hematology/Oncology Section, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Available online 26 November 2004

Abstract

Hemolysis is the principal toxicity of acute exposure to ethylene glycol monobutyl ether (EGBE) in rats. EGBE itself is not an active hemolytic agent, but its metabolite, butoxyacetic acid (BAA) formed as a result of dehydrogenase activity is a potent hemolysin. Here we address the role of osmolarity and cation composition of the suspending buffers in the mechanism of BAA-induced hemolysis of rat red blood cells in vitro. Rat erythrocytes were protected from BAA-induced cell swelling and hemolysis by the addition of sucrose to the suspending media. Hemolysis and cell swelling were also reduced by replacing external sodium with potassium. When calcium was not present in the suspending medium or when chelated by EGTA, hemolysis was increased after 2 h incubation with 1 mM or 2 mM BAA. Addition of as little as 0.05 mM $CaCl_2$ reduced hemolysis significantly while the addition of $MgCl_2$ had no effect. The dose-response relationship between BAA concentration and hemolysis determined in the presence or absence of calcium showed an increased effect of BAA in the absence of calcium. BAA-induced spherocytosis and cell fragmentation were more pronounced in the absence of calcium. The time course of BAA-induced hemolysis in the presence and absence of calcium demonstrated that the effect of calcium is to delay the onset of hemolysis. Increased intracellular calcium as a result of exposure to BAA was verified by atomic absorption spectroscopy. Charybdotoxin, an inhibitor of the calcium activated potassium channel, blocked the protective effect of calcium suggesting that the delay of onset of hemolysis in the presence of calcium is due to potassium loss caused by this channel. We conclude that the mode of action of BAA is to cause a colloid osmotic lysis of the rat red blood cell. Hemolysis requires external sodium and is associated with calcium uptake. Calcium appears to delay the onset of hemolysis. We speculate that: (1) BAA causes sodium and calcium to enter the cell; (2) calcium initially has a protective effect via the calcium activated potassium channel which facilitates the loss of potassium thereby, compensating for the osmotic effect of increased cell sodium; (3) calcium subsequently may have other deleterious effects through activation of proteases and externalization of phosphatidylserine in the exterior leaflet of the membrane.

Keywords: Ethylene glycol butyl ether; 2-Butoxyethanol; Butoxyacetic acid; Hemolysis; Rat red blood cells; Calcium; Osmolarity

* Corresponding author. Tel.: +1 713 798 6105; fax: +1 713 798 6677.
E-mail address: udden@bcm.tmc.edu (M.M. Udden).

0378-4274/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2003.09.020

1. Introduction

Ethylene glycol monobutyl ether (EGBE) or 2-butoxyethanol (2-BE) is an important solvent with a wide array of applications. Its toxicity has been reviewed recently by Boatman (Boatman and Knaak, 2001). As first recognized by Carpenter, EGBE is a potent hemolysin of rat red blood cells (Carpenter et al., 1956). Rats exposed to EGBE by gavage, inhalation, or dermal application develop a significant hemolytic anemia (Ghanayem, 1996). Acute exposure results in anemia, hemoglobinuria, kidney and liver toxicity and death (Carpenter et al., 1956; Tyler, 1984). Carpenter proposed that most of the hemolytic activity was the result of metabolism of EGBE via alcohol dehydrogenases to its aldehyde and then to its acetic acid derivative, the latter being the actual hemolytic agent (Carpenter et al., 1956). Ghanayam et al. showed that EGBE, and its aldehyde derivative by themselves were not hemolytic in vitro, but that butoxyacetic acid was a potent hemolysin (Ghanayem et al., 1987b). He also showed that inhibition of alcohol dehydrogenase activity eliminated the hemolysis caused by administration of EGBE in vivo. The hemolysis induced by EGBE has been well characterized, but the underlying mechanism is not understood. Cell swelling (increased mean cellular volume), stomato/spherocytic changes with a variable degree of echinocytosis and red cell ghost formation occur when rat erythrocytes are exposed to BAA (Ghanayem, 1996; Udden and Patton, 1994). The changes in red blood cells observed in vivo after gavage treatment of rats with EGBE are similar to those observed when rat red blood cells were incubated with BAA in vitro (Udden, 2000). Also apparent is a tendency of the affected red blood cells to aggregate (Udden and Patton, 1994). Recently, significant thrombosis has been observed in rats given high doses of EGBE (Ghanayem et al., 2001).

In addition to differing cell targets of the glycol ethers, there are remarkable species differences for the hemolytic activity of EGBE (Ghanayem and Sullivan, 1993). Species have been classified as potentially sensitive or resistant to the effects of EGBE based upon the assessment of in vitro red blood cell sensitivity to BAA (Ghanayem and Sullivan, 1993). Using the expected increase in red blood cell MCV as a guide, the species activity of BAA as a hemolysin can be ranked in the following order: rats > mice > rabbits > baboons (Ghanayem and Sullivan, 1993). Species whose red blood cells appear to be resistant include pigs, dogs, cats, guinea pigs, and humans. The disparity in effects on the two primates is puzzling. Similarly, the resistance of guinea pigs compared to their near relations, rodents and lagomorphs, is also of interest.

Several studies indicate that younger rats are more resistant to the effects of EGBE, and that red blood cells from younger animals are more resistant to the hemolysis caused by BAA (Ghanayem et al., 1987a). At least part of this resistance is due to the presence of relatively young red blood cells in younger animals (Ghanayem et al., 1992). Reticulocytosis follows an EGBE induced episode of hemolysis and it is likely that these very young red blood cells are resistant to subsequent challenges by EGBE in vivo, or BAA in vitro. This resistance has been studied further and appears to be an example of autoprotection (Sivarao and Mehendale, 1995). A mathematical model of EGBE autoprotection has been proposed which suggests that the phenomenon of resistance is a complex phenomenon that cannot be explained by resistance of young erythrocytes alone (Sawant et al., 1999). In vitro studies with BAA show that rat hemolysis ensues after a lag period depending on the concentration of BAA (Udden and Patton, 1994). Others have noted that hemolysis of rat red cells continues after BAA is washed away after an initial exposure (Ghanayem, 1996).

Here we describe experiments to further investigate the mechanism of BAA-induced rat red blood cell hemolysis. We hypothesized that colloid osmotic lysis would occur when red blood cells are incubated with BAA and that external sodium would be necessary for this effect. During this investigation we discovered that external calcium acts to delay the onset of BAA-induced hemolysis.

2. Methods

2.1. Red blood cells

Rat erythrocytes were obtained from Fischer 344 males aged 9–11 weeks. Rats were maintained in the Baylor College of Medicine Vivarium and fed on a standard NIH diet for one week. Blood samples