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Age and dose sensitivities in the 2-butoxyethanol F344 rat model of hemolytic anemia and disseminated thrombosis

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Abstract

In hemolytic disorders, such as sickle cell disease and β-thalassemia, the mechanisms of thrombosis are poorly understood. Appropriate animal models would increase the understanding of the pathophysiology of thrombosis. We previously reported that rats exposed to 2-butoxyethanol (2-BE) developed hemolytic anemia and disseminated thrombosis resembling sickle cell disease and β-thalassemia. To characterize our model further, we investigated age- and dose-related differences in sensitivity to 2-BE. We exposed groups of 6- and 12-week-old F344 rats (5 animals/group) to 62.5, 125, and 250 mg/kg/day of 2-BE for up to 4 days. Blood was collected on days 2–4 for complete blood count and measurement of intracellular adhesion molecule-1 (ICAM-1). Histopathological evaluation was performed to find evidence of disseminated thrombosis. The maximum hemolytic response, resulting in decreased erythrocyte count and higher mean cell volume (MCV) occurred in the 12-week-old rats treated with the highest dose of 2-BE (250 mg/kg, $p<0.0001$). The highest increase in ICAM-1 levels occurred in the 12-week-old rats treated with 125 and 250 mg/kg 2-BE ($p<0.0001$). No intravascular thrombi were noted in the 6-week-old 2-BE-treated animals. The majority of intravascular thrombi occurred in the 12-week-old rats treated with 250 mg/kg 2-BE. Because our findings show age- and dose-related sensitivities, we suggest that 12-week-old rats and doses of 250 mg/kg be used in the 2-BE model.

Keywords: Animal models; 2-butoxyethanol; Hemolysis; Microvascular thrombosis; Sickle cell disease

Introduction

Thromboembolic manifestations in different organs constitute serious complications in human hemolytic disorders such as β-thalassemia and sickle cell disease (Barker and Wandersee, 1999; Borgna Pignatti et al., 1998; Eldor et al., 1993, 1999; Logothetis et al., 1972).

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We recently proposed that 2-butoxyethanol (2-BE)-exposed Fischer F344 rats develop hemolysis, thrombosis, and infarction resembling such abnormalities in human hemolytic disorders associated with thrombosis (Ezov et al., 2002; Koshkaryev et al., 2003; Lewis et al., 2006; Redlich et al., 2004).

Utilized in the manufacture of a wide range of domestic and industrial products, 2-BE is a major environmental chemical (Nyska et al., 1999b). As early as the 1940s, reports documented its induction of hematuria/hemoglobinuria in laboratory animals (Werner et al., 1943a–c). Its administration to male rats induces a time- and dose-dependent increase in hematocrit and mean cell volume (MCV), suggesting an early swelling of erythrocytes. Their subsequent hemolysis causes a time- and dose-dependent decrease in the number of circulating erythrocytes and hemoglobin concentration. These effects have been associated with an increase in the ratio of spleen weight to body weight as a result of splenic sequestration of damaged erythrocytes (Ghanayem et al., 1989).

Metabolic activation of 2-BE to form butoxyacetic acid (BAA) is a prerequisite to the development of hemotoxicity (Ghanayem, 1996, 1989; Ghanayem et al., 1987a), with hemolysis occurring more rapidly and severely in female rats (Ezov et al., 2002; Ghanayem et al., 2000). The reason for this gender difference is probably due to a slower production of sufficiently high levels of BAA in males (Ghanayem, 1989; Ghanayem et al., 1987b; Ghanayem and Sullivan, 1993).

Subchronic toxicity studies with 2-BE have demonstrated that, upon repeated administrations, the animals gradually become tolerant of its hematotoxic effects (Krasavage, 1986). Continued hemolytic insult results in gradual replacement of old and susceptible erythrocytes with young, more resistant populations (Carpenter et al., 1956; Ghanayem et al., 1992). Older rats are more susceptible to 2-BE-induced hematotoxicity than younger rats. This age-related difference in sensitivity may be related to a higher activation/detoxification index of 2-BE, with BAA exhibiting a longer half-life in older rats (Ghanayem et al., 1987a).

Exposure to 2-BE results in disseminated thrombosis, involving several tissues and organs in female rats, including the heart, eye, lung, brain, submucosa of the nasal cavity, pulp of the incisor teeth, liver, coccygeal vertebra, and femur (Ezov et al., 2002; Long et al., 2000; Nyska et al., 1999a, b; Redlich et al., 2004). Our experience indicates that certain tissues (coccygeal vertebrae, nasal submucosa, and incisor teeth) consistently develop a high incidence of thrombosis. In addition, infarction occurs in bones, liver, incisors, and eyes (Nyska et al., 1999b). Thrombosis coincides with the most severe episode of hemolysis (Ghanayem et al., 2000, 2001).

The precise cause of thrombosis is still under investigation. The administration of 2-BE does not affect the ability of erythrocytes to aggregate but markedly enhances their adherence to extracellular matrix; thus, enhanced adherence of erythrocytes to endothelial cells may be one of the mechanisms by which thrombosis and organ infarct are induced in 2-BE-treated rats (Koshkaryev et al., 2003). In agreement with this finding, treating rats with 2-BE results in increased expression of vascular cellular adhesion molecule-1 (VCAM-1) in the eyes; tissue expression of VCAM-1 correlates strongly with the presence and extent of thrombosis (Nyska et al., 2003). Furthermore, plasma levels of soluble ICAM-1 in rats treated with 2-BE are also increased; the peak levels coincide with the appearance of thrombosis (Lewis et al., 2006). These findings provide additional evidence that the thrombosis seen in the 2-BE-treated rats is due to increased adhesion of erythrocytes to the endothelium, similar to the proposed mechanism of thrombosis in sickle cell disease and β-thalassemia.

The association of hemolysis and thrombosis found in this model manifests similarities to that observed in human disorders, such as thalassemia and sickle cell disease. Furthermore, enhancement of the interaction between erythrocytes and endothelial cells has been demonstrated both in this animal model and human thrombotic diseases (Hovav et al., 1999; Koshkaryev et al., 2003; Yedgar et al., 1999). Thus, this rat model may be used as an effective, non-invasive tool for studying therapeutic agents that disrupt erythrocyte/endothelium interactions.

To characterize further our model of hemolysis and thrombosis, we investigated age- and dose-related differences in sensitivity to 2-BE, examining hemolytic parameters, soluble adhesion molecules, and the extent of thrombosis revealed histologically. Our objective was to determine the optimal age and dose levels for usage in this model. The manuscript also summarizes suggested mechanisms of disseminated thrombosis induced by administration of 2-BE. This model should be of particular relevance to deepen the understanding of the pathogenesis of human disorders in which thrombosis apparently results from enhanced erythrocyte/endothelial interactions rather than activation of the hemostatic system.

Materials and methods

Chemicals, animals, treatments, and experimental procedures

2-BE (CAS No. 111-76-2) of >99% purity was purchased from Sigma-Aldrich Chemical Co (Rehovot,