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Monocrotaline pyrrole-induced megalocytosis of lung and breast epithelial cells: Disruption of plasma membrane and Golgi dynamics and an enhanced unfolded protein response

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Abstract

The pyrrolizidine alkaloid monocrotaline (MCT) initiates pulmonary hypertension by inducing a "megalocytosis" phenotype in target pulmonary arterial endothelial, smooth muscle and Type II alveolar epithelial cells. In cultured endothelial cells, a single exposure to the pyrrolic derivative of monocrotaline (MCTP) results in large cells with enlarged endoplasmic reticulum (ER) and Golgi and increased vacuoles. However, these cells fail to enter mitosis. Largely based upon data from endothelial cells, we proposed earlier that a disruption of the trafficking and mitosis-sensor functions of the Golgi (the "Golgi blockade" hypothesis) may represent the subcellular mechanism leading to MCTP-induced megalocytosis. In the present study, we investigated the applicability of the Golgi blockade hypothesis to epithelial cells. MCTP induced marked megalocytosis in cultures of lung A549 and breast MCF-7 cells. This was associated with a change in the distribution of the *cis*-Golgi scaffolding protein GM130 from a discrete juxtanuclear localization to a circumnuclear distribution consistent with an anterograde block of GM130 trafficking to/through the Golgi. There was also a loss of plasma membrane caveolin-1 and E-cadherin, cortical actin together with a circumnuclear accumulation of clathrin heavy chain (CHC) and α-tubulin. Flotation analyses revealed losses/alterations in the association of caveolin-1, E-cadherin and CHC with raft microdomains. Moreover, megalocytosis was accompanied by an enhanced unfolded protein response (UPR) as evidenced by nuclear translocation of Ire1α and glucose regulated protein 58 (GRP58/ER-60/ERp57) and a circumnuclear accumulation of PERK kinase and protein disulfide isomerase (PDI). These data further support the hypothesis that an MCTP-induced Golgi blockade and enhanced UPR may represent the subcellular mechanism leading to enlargement of ER and Golgi and subsequent megalocytosis.
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Keywords: Pulmonary hypertension; Monocrotaline; Megalocytosis; Golgi blockade; Unfolded protein response (UPR); Endoplasmic reticulum stress (ER-stress)

Introduction

Pyrrolizidine plant alkaloids have been investigated extensively as agents which initiate diverse disease processes in the lung, liver and kidneys (reviewed in Davidson, 1935; Shah et al., 2005). As one example, monocrotaline (MCT) administration is now a well established and widely investigated model for studies of the pathogenesis of pulmonary hypertension (Lalich and Merkow, 1961; Rabinovitch, 2004; Shah et al., 2005). It is now recognized that the common underlying cellular mechanism of pyrrolizidine-induced disease is the development of a "megalocytosis" phenotype ("karyocytomegaly") in affected hepatic parenchymal, vascular endothelial, vascular smooth muscle and lung alveolar Type II epithelial cells (Afzelius and Schoental, 1967; Bull, 1955; Harris et al., 1942a; Harris et al., 1942b; Hsu et al., 1973; Lappin et al.,

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1998; Lappin and Roth, 1997; Mathew et al., 2004; Merkow and Kleinerman, 1966; Reindel and Roth, 1991; Rosenberg and Rabinovitch, 1988; Sugita et al., 1983; Thomas et al., 1996; Todorovich-Hunter et al., 1988; Wilson et al., 1998; Wilson et al., 2000; Wilson and Segall, 1990). This phenotype is characterized by development of markedly enlarged cells with enlarged nuclei and "proliferation" of intracellular organelles including enlarged Golgi, increased endoplasmic reticulum and vacuolation (Afzelius and Schoental, 1967; Allen et al., 1970; Merkow and Kleinerman, 1966; Rosenberg and Rabinovitch, 1988; Svoboda and Soga, 1966; Thomas et al., 1996; Todorovich-Hunter et al., 1988; Wilson and Segall, 1990). However, megalocytotic cells fail to enter mitosis and have been described as "growing but nondividing cells" (Afzelius and Schoental, 1967).

Because the half-life of the liver-produced bioactive pyrrolic derivative of MCT in aqueous buffers is only ~3 s (Mattocks and Jukes, 1990), and a single administration of MCT (which is cleared in less than 24 h) is effective in causing pulmonary hypertension (PH) 10–14 days later (Lalich and Merkow, 1961; Mathew et al., 2004; Merkow and Kleinerman, 1966), the pathogenesis of PH is in the nature of an "hit-and-run" mechanism. We reported recently that MCT induced a disruption of caveolin-1 (cav-1)/raft function in PAEC resulting in widespread alterations in cell signaling including stimulation of promitogenic STAT3 and ERK1/2 signaling (Mathew et al., 2004). In endothelial cells, there was an inverse relationship between loss of cav-1/rafts and stimulation of DNA synthesis (Mathew et al., 2004). Moreover, in such cells, the Golgi scaffolding protein GM130 was shifted from membranes with a heavy to a lighter density into fractions enriched for hypo-oligomeric cav-1 indicating dysfunctional cav-1 trafficking through the Golgi (Mathew et al., 2004; Shah et al., 2005). Immunofluorescence studies confirmed the trapping of cav-1 in a GM130-positive Golgi compartment (Shah et al., 2005). Additionally, megalocytotic MCTP-treated endothelial cells showed reduced entry into mitosis upon stimulation with 2-methoxyestradiol (2-ME), reduced 2-ME-induced Golgi fragmentation and a slowing of Golgi reassembly following nocodazole-induced fragmentation (Shah et al., 2005). These data suggested that a disruption of the trafficking and mitosis-sensor functions of the Golgi organelle (the "Golgi blockade" hypothesis) may represent the subcellular mechanism leading to MCTP-induced megalocytosis (Mathew et al., 2004; Shah et al., 2005).

In the present study, we investigated the applicability of the Golgi blockade hypothesis to lung epithelial cells in culture (A549 Type II-like alveolar cells). Moreover, in an attempt to evaluate the generality of the effects of MCTP on mammalian cells, we investigated the production of megalocytosis by MCTP and its mechanism in human breast epithelial cells (MCF-7). Parenthetically, the breast is an organ which has not previously been reported to be affected by MCT. Additionally, stimulated by the extensive prior electron micrographic reports of enlarged Golgi, increased endoplasmic reticulum and vacuolar accumulation in megalocytotic endothelial, smooth muscle and alveolar epithelial cells in lungs of MCTP-treated rats with pulmonary hypertension (Afzelius and Schoental, 1967; Allen et al., 1970; Merkow and Kleinerman, 1966; Rosenberg and Rabinovitch, 1988; Sugita et al., 1983; Svoboda and Soga, 1966; Thomas et al., 1996; Todorovich-Hunter et al., 1988; Wilson and Segall, 1990), we investigated whether an MCTP-induced Golgi blockade might include an enhancement of the unfolded protein response (UPR) [also called the endoplasmic stress (ER-stress) response].

Materials and methods

Cell culture, growth and fractionation. Human pulmonary Type II-like alveolar epithelial cell line A549 and the human breast carcinoma cell line MCF-7 were purchased from the American Type Culture Collection. Cultures of primary bovine PAEC in T-75 flasks were a gift from Dr. Susan Olson, New York Medical College, and were used between passage 4 and 20 (Mathew et al., 2004; Shah et al., 2002). Cultures of primary human pulmonary arterial smooth muscle cells (PASMC) were purchased from Cascade Biologics, Inc., Portland, OR. Growth of A549, MCF-7 and PAEC in T-75 flasks, 100 mm plastic Petri dishes or 6-well plates were carried out as previously reported (Mathew et al., 2004; Ndubuisi et al., 1999; Sehgal et al., 2002; Shah et al., 2002). PASMC cultures in T-75 flasks and 6-well plates were grown in Medium 231 supplemented with Smooth Muscle Growth Supplement (SMGS) containing basic fibroblast growth factor, epidermal growth factor and insulin as provided by Cascade Biologics.

For use in cell culture, MCTP was prepared from MCT (purchased from TransWorld Chemicals Inc., Rockville, MD) using the procedure of Mattocks et al. (1989). By mass analyses, approximately 30–50% of the input monocrotaline was converted to the pyrrolic derivative (data not shown). MCTP was stored in small aliquots in dimethylformamide (DMF) at −80 °C, diluted to the required concentration in DMF just before use and added directly to the cultures with gentle swirling. Control cultures received equivalent volume of DMF.

DNA synthesis. Respective cultures in 6-well plates were pulsed with BrdU (10 μM; Sigma-Aldrich, St. Louis, MO) for 20 min and labeling index evaluated as reported earlier (Shah et al., 2005).

Immunofluorescence studies. Respective cell cultures in 6-well plates were fixed using either of (a) a cold 4% paraformaldehyde-0.1% Triton X-100 solubilization protocol (Bryant et al., 2005) or (b) a warm 37 °C 3.7% formaldehyde-0.1% Triton solubilization protocol (Shah et al., 2005) or (c) a

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