Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 55 (2006) 279–281

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Preliminary Report

Apparent role of dynein in glucose-6-phosphate dehydrogenase trafficking in neutrophils from pregnant women

Ji-Biao Huang[a], Jimmy Espinoza[c,d], Roberto Romero[c,d], Howard R. Petty[a,b,*]

[a]Department of Ophthalmology and Visual Sciences, The University of Michigan Medical School, Ann Arbor, MI 48105, USA
[b]Department of Microbiology and Immunology, The University of Michigan Medical School, Ann Arbor, MI 48105, USA
[c]Perinatology Research Branch, National Institute of Child Health and Human Development, Bethesda, MD, USA
[d]Hutzel Hospital, Detroit, MI 48201, USA

Received 14 July 2005; accepted 27 September 2005

Abstract

To better understand the mechanisms of metabolic microcompartmentalization associated with neutrophil hexose monophosphate shunt activity during pregnancy, we have studied the intracellular trafficking of glucose-6-phosphate dehydrogenase (G6PDase). Microtubule motor proteins colocalize with G6PDase. Dynein inhibitors block G6PDase accumulation at the microtubule-organizing center in pregnancy cells. On this basis, we conclude that microtubule motor proteins participate in hexose monophosphate shunt enzyme transport within leukocytes.

1. Introduction

During pregnancy, reactive oxygen metabolite production by leukocytes is reduced because of diminished hexose monophosphate shunt (HMS) activity [1]. We have reported previously [1-3] that the HMS enzymes glucose-6-phosphate dehydrogenase (G6PDase), 6-phosphogluconate dehydrogenase (6PGDase), and transaldolase form a supramolecular complex within neutrophils that undergoes retrograde trafficking to a neutrophil's microtubule-organizing center (MTOC) during pregnancy. As glucose-6-phosphate is formed at the plasma membrane and used by peripheral glycolytic enzymes, its availability to the HMS, now located at the MTOC, is diminished, thereby reducing reactive oxygen metabolite production in neutrophils of pregnant women. Although the intracellular distribution of HMS enzymes is sensitive to microtubule inhibitors [1], the nature of their intracellular trafficking is unknown. One possibility is that these HMS complexes constitute cargo that is shuttled from place to place within cells on microtubules. Microtubules constitute a cellular cargo transport system that uses kinesin [4,5] and dynein [6], which are microtubule-activated adenosine triphosphatases that serve as force-generating motors. Dynein mediates retrograde transport [6], whereas many kinesin proteins direct anterograde movement of organelles along microtubules. However, the transport direction of any specific cargo depends upon additional factors, such as kinesin-dynein interactions. We hypothesize that the intracellular location of HMS depends upon microtubule motor protein transport.

2. Subjects and methods

Peripheral blood from nonpregnant and pregnant women was obtained with institutional review board approval after informed consent. Neutrophils were isolated from blood samples using Ficoll-Hypaque (Sigma, St Louis, MO) density gradient centrifugation. Neutrophil viability was more than 95% as assessed by trypan blue exclusion. Cells were suspended in Hanks balanced salt solution (HBSS; Life Tech, Grand Island, NY). Cells were fixed then observed as described [3]. Anti-G6PDase was purchased from Chemicon International (Temecula, CA). Rabbit anti-kinesin (KIF14) polyclonal antibody (Ab) (Bethyl Laboratories, Montgomery, TX) and goat antidynein (C-14)

* Corresponding author. Department of Ophthalmology and Visual Sciences, The University of Michigan Medical School, Ann Arbor, MI 48105, USA. Tel.: +1 734 647 0384; fax: +1 734 936 3815.
E-mail address: hpetty@umich.edu (H.R. Petty).

0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.09.002

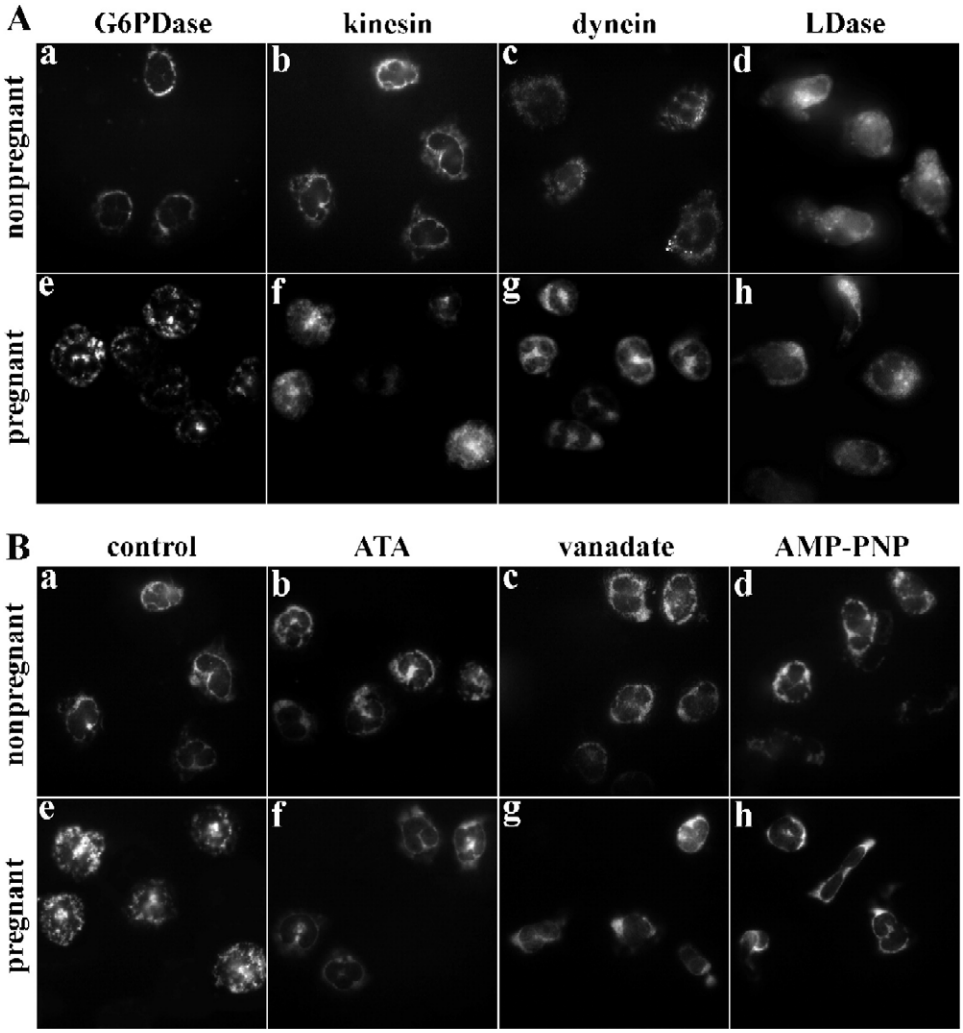

Fig. 1. Immunofluorescence labeling of G6PDase and motor proteins of neutrophils from nonpregnant donors and pregnant women. Different treatments are shown in each column, whereas different patients are illustrated in each row, which are representative of all studies for each patient group. Panel A, Localization of G6PDase, kinesin, and dynein in neutrophils from nonpregnant (a-c) and pregnant women (d-f). Permeabilized cells were labeled with anti–G6PDase–FITC (a, e), anti–kinesin-tetramethylrhodamine isothiocyanate (TRITC) (b, f), anti–dynein-FITC (c, g), or anti-LDase (d, h) (n = 4). Panel B, Effects of ATA (10 μmol/L), vanadate (10 μmol/L), and AMP-PNP (2 mmol/L) on G6PDase trafficking. Cells from nonpregnant donors (a-d) and pregnant women (e-h) were incubated with or without ATA (b, f), vanadate (c, g), or AMP-PNP (d, h) at 37°C for 2.5 hours, fixed, permeabilized, and labeled with G6PDase-FITC. The MTOC is unlabeled or poorly labeled in pregnancy cells during exposure to vanadate and AMP-PNP (original magnification ×760; n = 4). LDase indicates lactate dehydrogenase.

polyclonal Ab (Santa Cruz Biotechnology, Santa Cruz, CA) were used. Aurintricarboxylic acid (ATA), sodium vanadate, and 5′-adenylyl imidadiphosphate (AMP-PNP) were obtained from Sigma.

3. Results and discussion

Neutrophils from pregnant and nonpregnant women were stained separately with anti-G6PDase, antikinesin, and antidynein Abs. Fig. 1A shows the intracellular staining patterns of these reagents. In contrast to cells from nonpregnant women, substantial labeling of the MTOC was found for G6PDase, kinesin, and dynein in neutrophils from pregnant women. These molecules colocalize in cells and redistribute in parallel during pregnancy. However, this was not observed for lactate dehydrogenase (panels d and h). We next examined the effects of motor protein inhibitors. Neutrophils were incubated in phosphate-buffered saline with or without various inhibitors for 2.5 hours at 37°C in humidified 5% CO_2; no significant changes in cell viability were noted. The cells were then fixed and labeled with fluorescein isothiocyanate (FITC)–conjugated anti-G6PDase antibodies. As shown in Fig. 1B, G6PDase is localized in the peripheral areas of untreated neutrophils from nonpregnant donors (a), whereas it is centrally located in pregnancy neutrophils (e). When cells were treated with the kinesin inhibitor ATA (10 μmol/L) [7], no dramatic effects were observed. When vanadate

(10 μmol/L) [8,9] was used to block dynein trafficking, cells from nonpregnant donors demonstrated no remarkable changes in G6PDase localization. However, vanadate effectively blocked the central localization of G6PDase (g) in cells from pregnant women. The results suggest that G6PDase transport to MTOC in cells from pregnant women is dynein dependent. When we treated cells with AMP-PNP (2 mmol/L) (d and h) [9], which inhibits both kinesin and dynein, we found G6PDase to be predominantly found at the cell periphery for cells from nonpregnant and pregnant donors (d, h).

Here, we demonstrate that G6PDase trafficking depends upon microtubule motor proteins. Single-color fluorescence studies indicate that G6PDase, kinesin, and dynein have similar labeling patterns in cells from either pregnant or nonpregnant women. Double-labeling studies indicate that G6PDase and kinesin colocalize with one another in cells from both pregnant and nonpregnant women (data not shown). As previously reported [1-3], G6PDase, 6PGDase, and transaldolase, but not glycolytic enzymes, are enriched at the MTOC of neutrophils from pregnant women to down-regulate the HMS and oxidant production, which might damage the conceptus. The centrosomal trafficking of G6PDase in pregnancy cells can be manipulated by dynein inhibitors, which is consistent with its role in retrograde trafficking. As G6PDase, kinesin, and dynein trafficked together in the presence or absence of drugs for both cells from pregnant and nonpregnant women, we suggest that these proteins are part of one supramolecular complex. Similar cargo associations have been reported [10,11]. As kinesin and dynein are not specific motors for HMS enzymes, it is possible that other physiological processes are modulated similarly during pregnancy. Our findings that HMS enzyme complex transport is regulated by microtubule-based motors may lead to the discovery of new drugs controlling oxidant production and inflammatory processes through the regulation of motor activities.

Acknowledgment

This study was supported by grants from the National Institute of Allergy and Infectious Disease, the National Multiple Sclerosis Society, and the National Institute of Child Health and Human Development intramural program.

References

[1] Kindzelskii AL, Huang JB, Chaiworapongsa T, Fahmy RM, Kim YM, Romero R, et al. Pregnancy alters glucose-6-phosphate dehydrogenase trafficking, cell metabolism, and oxidant release of maternal neutrophils. J Clin Invest 2002;110:1801-11.

[2] Kindzelskii AL, Ueki T, Michibata H, Chaiworapongsa T, Romero R, Petty HR. 6-Phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase form a supramolecular complex in human neutrophils that undergoes retrograde trafficking during pregnancy. J Immunol 2004;172:6373-81.

[3] Huang JB, Espinoza J, Romero R, Petty HR. Transaldolase is part of a supramolecular complex containing glucose-6-phosphate dehydrogenase in human neutrophils that undergoes retrograde trafficking during pregnancy. Metabolism 2005;54:1027-33.

[4] Brady ST. A novel ATPase with properties expected for the fast axonal transport motor. Nature 1985;317:73-5.

[5] Vale RD, Schnapp BJ, Mitschison T, Steuer E, Reese TS, Sheetz MP. Different axoplasmic proteins generate movement in opposite directions along microtubules in vitro. Cell 1985;43:623-32.

[6] Paschal BM, Vallee RB. Retrograde transport by the microtubule associated protein MAP 1C. Nature 1987;330:181-3.

[7] Hopkins SC, Vale RD, Kuntz ID. Inhibitors of kinesin activity from structure-based computer screening. Biochemistry 2000;39:2805-14.

[8] Yamin MA, Tamm SL. ATP reactivation of the rotary axostyle in termite flagellates: effects of dynein ATPase inhibitors. J Cell Biol 1982;95:589-97.

[9] Hachiya NS, Watanabe K, Yamada M, Sakasegawa Y, Kaneko K. Anterograde and retrograde intracellular trafficking of fluorescent cellular prion protein. Biochem Biophys Res Commun 2004;315:802-7.

[10] Burkhardt JK, McIlvain Jr JM, Sheetz MP, Argon Y. Lytic granules from cytotoxic T cells exhibit kinesin-dependent motility on microtubules in vitro. J Cell Sci 1993;104:151-62.

[11] Vignal E, Blangy A, Martin M, Gauthier-Rouviere C, Fort P. Kinectin is a key effector of RhoG microtubule-dependent cellular activity. Mol Cell Biol 2001;21:8022-34.