

Nitric Oxide Production by Human Peripheral Blood Mononuclear Cells

P Toomtong,*^{MD}, J D Young,**^{BM, DM, FRCA}

Abstract

Introduction: There are conflicting data on the ability of human mononuclear cells to produce nitric oxide (NO). We investigated nitric oxide production from peripheral blood mononuclear cells (PBMs) by using a new sensitive fluorescent indicator. **Materials and Methods:** PBMs from healthy volunteers were collected, plated in 96-well microplates, and loaded with the fluorescent nitric oxide probe, 4,5-diaminofluorescein diacetate (DAF-2DA). Experiments were performed in normal control and endotoxin-stimulated PBMs, with and without exogenous L-arginine. The exogenous nitric oxide donor S-nitroso-N-acetyl-penicillamine (SNAP) was used as a positive control. Fluorescence intensity was measured with a fluorescence microplate reader. **Results:** Nitric oxide production by human PBMs can be demonstrated by the use of the fluorescent indicator, DAF-2DA, in both control and endotoxin-stimulated conditions. Nitric oxide production was independent of the concentration of exogenous L-arginine. The addition of endotoxin did not change nitric oxide production. PBMs treated with SNAP showed a concentration dependent increase in fluorescence. Nitric oxide production over 5 hours was constant and identical in both control and stimulated groups. **Conclusion:** This fluorescent indicator technique is useful for the study of NO production by human PBMs. Nitric oxide production by PBMs was independent of exogenous L-arginine concentration and was not affected by endotoxin.

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* Instructor

Department of Anesthesiology
Faculty of Medicine Siriraj Hospital
Mahidol University, Bangkok, Thailand

** Reader in Anaesthesia

Nuffield Department of Anaesthetics
Radcliffe Infirmary, Oxford, United Kingdom

Address for Reprints: Dr Patiparn Toomtong, Department of Anesthesiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700 Thailand.

E-mail: siptm@mahidol.ac.th