

## Short papers

# Ca<sup>2+</sup>-independent nitric oxide synthase activity in human lung after cardiopulmonary bypass

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## Abstract

**Background** – Because surgery involving cardiopulmonary bypass induces a systemic inflammatory response, the effect of cardiopulmonary bypass on nitric oxide (NO) generation was investigated in human lung tissue.

**Methods** – Nitric oxide synthase (NOS) activity was measured by the conversion of <sup>14</sup>C-L-arginine to <sup>14</sup>C-L-citrulline in tissue biopsy samples obtained before and after cardiopulmonary bypass.

**Results** – The Ca<sup>2+</sup>-independent production of NO found before cardiopulmonary bypass was extremely low (1.5 (0.5) pmol citrulline/mg/min), but was increased after the bypass operation (23.6 (11) pmol/mg/min).

**Conclusions** – Ca<sup>2+</sup>-independent NOS activity was detected in human lung after cardiopulmonary bypass. This finding may provide an important insight into the pathogenesis of the tissue damage and acute phase response observed after such surgery.

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**Keywords:** cardiopulmonary bypass, nitric oxide, nitric oxide synthase.

After open heart operations with cardiopulmonary bypass patients show symptoms resembling an acute phase response such as fever, complement activation, and synthesis of acute phase proteins.<sup>1</sup> Furthermore, increased concentrations of cytokines such as tumour necrosis factor (TNF), interleukin 1 (IL-1), and interleukin 6 (IL-6) have been detected, suggesting that they may be important mediators in this systemic inflammatory response. The participation of oxygen-derived free radicals is also likely.<sup>1,2</sup>

Nitric oxide (NO) synthesised from L-arginine by the constitutive and Ca<sup>2+</sup>-dependent low output pathway (cNOS) functions as an intracellular and intercellular signal for the stimulation of soluble guanylate cyclase and therefore plays a part in the regulation of cell function and communication.<sup>3</sup> On the other hand, NO is also synthesised by a cytokine inducible and Ca<sup>2+</sup>-independent high output

pathway (iNOS) and is an important cytotoxic effector molecule which accounts, at least in part, for the vasodilatation and tissue damage seen in endotoxic shock.<sup>4</sup>

Because several alterations observed in the lung after cardiopulmonary bypass are similar to those seen during haemorrhagic shock and endotoxaemia,<sup>5</sup> we have examined whether such alterations could be related to the L-arginine:NO pathway.

## Methods

All subjects gave their informed consent to the studies which had the approval of the Hermanos Ameijeiras Hospital ethics committee according to the National Medical Ethics Regulations.

Tissue samples from the middle lobe of the lung were taken before (n=3) and after (n=7) cardiopulmonary bypass from patients undergoing open heart operations. None of the patients had previous pulmonary disease. Indications for surgery included mitral (n=4) or aortic (n=3) valve dysfunction. Coronary artery disease was diagnosed in two patients and one had congenital heart disease. Before surgery blood tests showed no evidence of haematological or biochemical disorders and arterial oxygen tensions (PO<sub>2</sub>) were normal (10–13.2 kPa). After cardiopulmonary bypass a significant reduction in haemoglobin and altered blood clotting parameters were detected in all patients. Glucose levels were increased in three patients. The mean (SE) duration of the cardiopulmonary bypass operation in the seven patients was 122 (30) minutes.

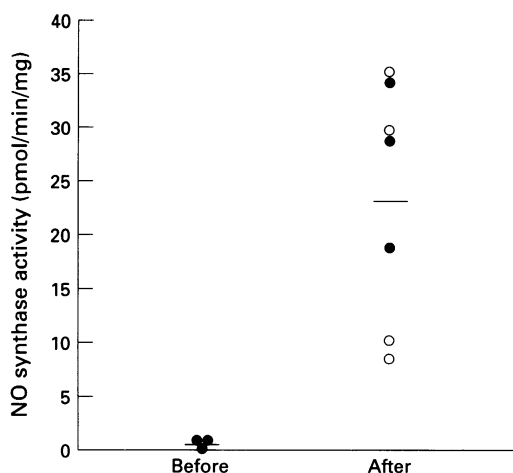
Tissue samples were washed twice with sucrose buffered solution (sucrose 320 mM, Hepes 10 mM, ethylenediaminetetraacetate (EDTA) 100 μM, pH 7.4) and homogenised in 1 ml ice cold homogenisation buffer (DL-dithiothreitol 1 mM, Tris 50 mM, sucrose 320 mM, EDTA 1 mM, soybean trypsin inhibitor 10 μg/ml, aprotinin 5 μg/ml, leupeptin 10 μg/ml, pH 7.0). The homogenate was then centrifuged at 100 000g for 20 minutes at 4°C. NOS activity in the supernatant (cytosolic) fractions was measured by the conversion of radiolabelled <sup>14</sup>C-L-arginine to <sup>14</sup>C-L-citrulline in the presence or absence of 1 mM ethylene glycol-bis (β-aminoethyl ether tetraacetic acid) (EGTA)

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$\text{Ca}^{2+}$ -independent nitric oxide synthase activity in cytosolic fractions from tissue biopsy samples of human lung before and after cardiopulmonary bypass. Closed circles represent paired data before and after cardiopulmonary bypass. Mean values for each group are indicated by bars and are statistically different ( $p < 0.05$ ).

or  $\text{N}^G$ -monomethyl-L-arginine (L-NMMA) as previously described.<sup>6</sup>

Statistical significance was assessed by the Student's  $t$  test. A  $p$  value of less than 0.05 was considered significant.

### Results

Lung tissue samples from patients after cardiopulmonary bypass showed significant  $\text{Ca}^{2+}$ -independent NOS activity (23.6 (11) pmol/min/mg) compared with that found before cardiopulmonary bypass (1.5 (0.5),  $p < 0.05$ , figure).

### Discussion

The cellular location of  $\text{Ca}^{2+}$ -independent NOS (iNOS) remains to be established, but during extracorporeal circulation there is a sequestration of polymorphonuclear cells within the alveolar vasculature<sup>7</sup> and human neutro-

phils have been shown to express iNOS.<sup>8</sup> As mentioned above, alveolar changes occurring after cardiopulmonary bypass are similar to those observed in endotoxaemia and haemorrhagic shock. Induction of the NO pathway has been reported in both pathological processes.<sup>3,4,9</sup>

Endothelial NO production (cNOS) has been implicated in maintaining low pulmonary vascular resistance in physiological circumstances.

Because both isoforms have recently been immunohistochemically localised in human lung,<sup>10</sup> the cNOS/iNOS balance may have important implications in vascular pulmonary homeostasis. It is therefore not clear at present whether the appearance of the  $\text{Ca}^{2+}$ -independent NOS activity after cardiopulmonary bypass will be damaging or protective. Although iNOS is implicated in tissue and cell injury, it is likely that this activity may modulate both pulmonary vascular hypertension and cardiogenic pulmonary oedema after cardiopulmonary bypass.

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