Inducible Nitric Oxide Synthase activity in human lung after cardiopulmonary bypass

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The identification of the enzyme iNOS in human lungs after cardiopulmonary bypass was the result of the experimental and clinical cooperation of two teams aiming to identify new pathways in the pathophysiology of postoperative cardiac surgery.[b]

The authors signing this paper were from two institutions: pharmacologists from the Centre of Pharmaceutical Chemistry and cardio-surgeons from the Hospital Hermanos Amejeiras in Havana. The aim of the study was to explore the inducible Nitric Oxide Synthase (iNOS) after cardiopulmonary bypass (CPB) in patients undergoing open heart operations [1]. Cardiopulmonary bypass refers to the extracorporeal circulation of the blood through a machine that takes the function of the heart and the lung during the open heart operations. Once the blood circulation in the heart and lung is restored, the ischemia and reperfusion trigger a cascade of inflammatory pathways affecting the postoperative outcome [2].

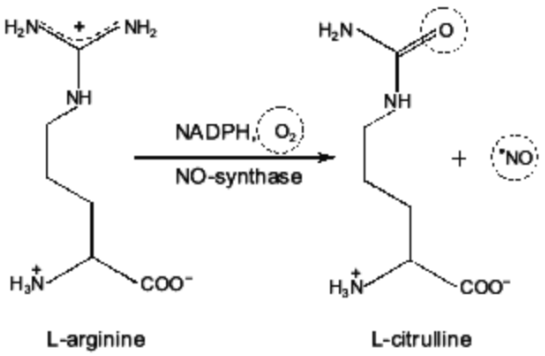

Figure 1: Reaction catalysed by the NOS

The Nitric Oxide Synthase catalyses the conversion of L-arginine into Nitric Oxide (NO) and L-citrulline (See Figure 1). There are three isoforms of the Nitric Oxide Synthase (NOS). Two are constitutive and calcium dependent: the endothelial (eNOS or NOS3) and the neuronal (nNOS or NOS1). The pulses of NO generated by these two isoforms are part of the signal transduction in the physiology of the vascular relaxation (eNOS) and in the neurotransmission (nNOS) mediated by the activation of the guanylate cyclase. The inducible isoform (iNOS, NOS2), however is involved in pathophysiology, expressed under inflammatory/infection settings, producing high output of harmful NO and it is calcium independent. The tissue damages reported after CPB [2] resemble those seen during experimental endotoxaemia [3] which, at least in part, are due to the detrimental excess of high output of NO on the vasculature.

Materials and Methods

All patients gave their informed consent to be part of the study, which had the approval of the Hermanos Amejeiras Hospital Ethics Committee, according to the National Medical Ethics Regulations. None of the patients had previous pulmonary diseases and there were no evidences of haematological or biochemical disorders previous to the operation. Tissue samples from middle lobe of the lung were taken before ($n = 3$) and after ($n = 7$) cardiopulmonary bypass from patients undergoing open heart operations to repair mitral or aortic valve dysfunction. The mean duration of the cardiopulmonary bypass was 122 ± 30 minutes. After the CPB a significant reduction in haemoglobin was detected in all patients, as well as altered blood clotting parameters. Tissue samples were immediately frozen on dry ice and kept at $-70°$C until processing them for the enzymatic activity of NOS. Chemical reagents were obtained from Sigma (St. Louis, MO) and radiochemicals from Amersham International (Aylesbury, GB) through the scientific collaboration with The Wellcome Research Laboratories, Kent. Tissue samples were washed in ice-cold sucrose buffer solution before homogenising them in an enzyme-preserving buffer as described before [4]. The homogenates were then centrifuged at $100,000\,g$ for 20 minutes at 4°C. The NOS activity was measured in the cytosolic fractions by the conversion of radio labelled ^{14}C-L-arginine to ^{14}C-L-citrulline. Triplicate aliquots of 0.018 ml of the same cytosolic fractions were added to 0.1 ml the enzymatic reaction buffer [5] containing cofactors and the proportion of cold-hot substrate of 0.024 mM L-arginine and ^{14}C-L-arginine (1.85 MBq/ml). Three tubes per sample were incubated for 20 minutes at 37°C having either water, or 1 mM L-NMMA (N^G-monomethyl-L-arginine, the NOS inhibitor), or 1 mM EGTA (Ca^{2+} chelator). Reaction was terminated by adding 1.5 ml of Na^+-form dowex-50 resin in order to chelate hot and cold L-arginine [5]. The Ca^{2+}-independent NOS activity (iNOS) in the cytosolic fraction was determined by the differential production of ^{14}C-L-citrulline formed in the presence and absence of the L-NMMA and EGTA,

expressed in pmol/min/mg protein.

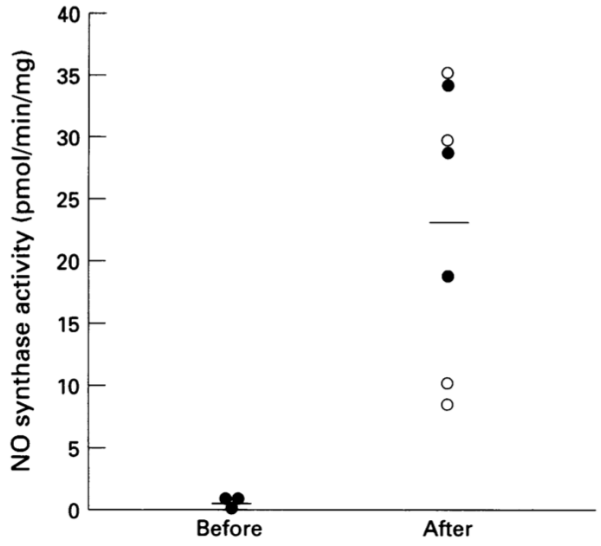

Figure 2: Ca^{2+}-independent NOS activity in cytosolic fractions of lung biopsies before and after CPB. Originally published in Thorax [1]

Findings, discussion and conclusion

Lung tissue samples exhibited significant higher activity of Ca^{2+}-independent NOS (23.6 pmol/min/mg) after CPB compared with that found before the initiation of the extracorporeal circulation (1.5 ± 0.5 pmol/min/mg), $p < 0.05$ (Figure 2). Although the cellular origin of the iNOS can not be precised in the current experimental setting, it is plausible to suggest that such difference before and after CPB in Ca^{2+}-independent NOS activity came from polymorphonuclear (PMN) cells within the alveolar vasculature, for two reasons: the neutrophils sequestration in alveolar circulation due to reduced flow when starting CPB [6] and their basal activity that has been found in human peripheral PMN cells [7]. Sair and Evans [8] discussing our results in the editorial section of this journal, have also referred the inflammatory role of neutrophils in the lungs after experimental CPB [9], addressing the time related cascade of inflammatory mediators. Indeed, our results can not differentiate if the increase of iNOS activity was due to more infiltrated PMN with basal and beneficial Ca^{2+}-independent NOS activity, or a pathological higher expression of iNOS in other lung tissues due to inflammatory mediators. Nevertheless, this findings may contribute to the understanding of the pathogenesis around the acute phase response observed after cardio-thoracic surgery.

The author acknowledge the contribution from Rene Delgado (IFAL, University of Havana) to this work and his subsequent input developing the research in Immunopharmacology in Cuba, and the posthumous recognition to Professor Noel Gonzalez, an outstanding researcher and the pioneer of Heart Transplant in Cuba [10] (`http://www.sld.cu/obituario/2016/01/17/profesor-julio-noel-gonzalez-jimenez`)

Notes

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b. Original version of this article is Ref. [1]

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