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Short-time non-enzymatic nitric oxide synthesis from L-arginine and hydrogen peroxide induced by shock waves treatment

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Abstract The evidence that nitric oxide (NO) production is possible by a non-enzymatic pathway has already been shown under restrictive experimental conditions. Here we show that NO can non-enzymatically be formed with short-time kinetics (min), by 'bombing' with shock waves a solution containing 1 mM hydrogen peroxide and 10 mM L-arginine. This procedure is widening its medical application with surprisingly positive effects in tissue regeneration and our finding could be one of the first steps for the understanding of the biochemical responsible for these therapeutical effects. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Shock waves; Non-enzymatic nitric oxide

1. Introduction

A shock wave (SW) [1] consists of an acoustic wave that generates a pressure impulse during 0.1–0.2 ms and is capable of determining a pressure gradient between +100 and −10 Mpa. Conveyed by an appropriate generator to a specific target area (focal area), the power created can be modulated in the range of 0.003–0.890 mJ/mm^2. In the last 15 years the clinic use of this technique has been significantly enlarged, especially for kidney and urinary calculus lithotripsy. Technological evolution in energy level control and in target area focusing has improved the clinical results, reducing at the same time undesired side-effects. Besides this 'primary' use, a few years ago a secondary anti-inflammatory effect in tendon and muscle tissues [2] and a complete recovery even in pseudoarthrosis pathologies were detected [2–5]. In particular, treatment of the tendon and muscle tissues was found to induce a long-time (1–4 months) tissue regeneration effect [5,6], besides a more immediate anthalgic and anti-inflammatory effect. This regenerative pathway seems to involve free radical production and revascularization events taking place in the SW-treated area [6].

Our idea is that one of the most important molecules involved in these therapeutic effects might be nitric oxide (NO). NO, normally produced in eukaryotes from L-arginine by different isoforms of NO synthase (NOS), exerts a potent and immediate vasodilatory action and modulates the subsequent angiogenesis [7,8]. Based on the evidence that the vasodilatory effect detectable in the area treated with SW is almost immediate, we decided to start our studies in an in vitro system, to verify first of all whether SW application could induce rapid and non-enzymatic formation of NO.

Information describing that NO can be produced without the catalytic activity of NOS has been already reported [9–12]. It includes both the non-enzymatic in vivo formation of NO due to the reaction of dietary/salivary nitrites with gastric acid [9], and the in vitro synthesis of nitrites in the solution containing L-arginine (10–20 mM) and hydrogen peroxide (10–50 mM) [12].

The present study was aimed at verifying if SW treatment elicits the non-enzymatic production of NO even under milder conditions than those previously described. Indeed, we performed the experiments at physiological pH in the presence of the amounts of H_2O_2 lower than those used by Nagase et al. [12] and in a shorter time (8 min) than that used in their system (up to 5 days) [12].

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Abbreviations: SW, shock wave; NOS, nitric oxide synthase; MGD, *N*-methyl-D-glucamine dithiocarbamate; DAN, 2,3-diaminonaphthalene

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