Regulation of Inducible nitric oxide synthase expression in IFN-γ-treated macrophages by Bio-Normalizer

HIROTSUGU KOBUCHI AND LESTER PACKER

Environmental Energy Technologies Division, Lawrence Berkeley National Laboratory, Membrane Bioenergetics Group, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3200 USA

Bio-Normalizer, a health food supplement prepared from *Carica papaya* has been reported to have therapeutic effects in a variety of human pathologies. We recently reported that it protected isolated rat hearts from either ischemia-reperfusion injury or from the effect of a peroxyl radical initiator in a supplementation study. However, despite accumulating reports on the practical advantages of Bio-Normalizer, little is known concerning the molecular mechanisms involved in its action. In this study, we investigated whether Bio-Normalizer could affect nitric oxide (NO) production, measured as accumulated nitrite in medium, and inducible nitric oxide synthase (iNOS) expression, capable of affecting a multitude of mechanisms in the immune response. The efficacy of Bio-Normalizer on NO production was investigated in a chemical and cellular system. Bio-Normalizer failed to interact with NO produced by sodium nitroprusside. Mouse RAW 264.7 macrophages did not produce NO constitutively, nor was NO production induced by treatment with Bio-Normalizer alone. However, a major increase of NO production from macrophages was observed after treatment with Bio-Normalizer plus interferon (IFN)-γ. This effect of Bio-Normalizer was dose-dependent. Such effects of Bio-Normalizer on NO production were not due to changes in the activity of iNOS. Reverse transcription-polymerase chain reaction analysis revealed that the increase in NO production corresponded to an increase in the accumulation of iNOS mRNA without any changes in mRNA stability. Furthermore, Bio-Normalizer augmented the IFN-γ-dependent expression of mRNA both of tumor necrosis factor-α and interleukin-1β which might also be implicated in the immune system. These results suggest that Bio-Normalizer itself does not provide a signal that triggers

induction of the NO pathway, but shows synergistic interaction with IFN-γ to induce NO synthesis. These properties of Bio-Normalizer may be in part responsible for its reported therapeutic activity against various diseases.