Thesis Title Effects of Nitric Oxide Synthase Inhibitors in N18 Neuroblastoma Cells

Name Panida Chutsrinopkun

Degree Master of Science (Pharmacology)

Thesis Supervisory Committee
Porntip Supavilai, Ph.D.
Molvibha Vongsakul, Ph.D.
Kittima Sriwatanakul, Ph.D.

Date of Graduation 21 August B.E. 2540 (1997)

Abstract

Experiments were performed to verify whether N18 neuroblastoma cells (N18 cells) in culture medium without serum produced nitric oxide (NO) and whether they were sensitive to the nitric oxide synthase (NOS) inhibitors. NO is synthesized *in vivo* from L-arginine and oxygen by enzyme NOS and plays a major role in several diverse physiological and pathological functions including regulation of vascular tone, neurotransmission and mediation of immune response. Recent studies have shown that excessive NO plays a key role in the etiology of neurodegenerative diseases such as Alzheimer's disease and stroke. In this study, NO formation was determined from the NO breakdown product, nitrite, by measuring spectrometrically the purple colour developed after it had been reacted with sulfanilamide and N-1-

napthylenediamine dihydrochloride under acidic condition (Griess method).

N18 cells when cultured in medium without serum exhibited time-dependent production of nitrite. The induction of NO in N18 cells was found to be increased more than 10-fold at 48 hr after serum withdrawal.

NOS inhibitors including N^{ω}-nitro-L-arginine methyl ester (L-NAME), N^{ω}-nitro-D-arginine methyl ester (D-NAME), N^{ω}-methyl-L-arginine (L-NMA), N^{ω}-methyl-D-arginine (D-NMA), aminoguanidine (AG), and N^{ω}-nitro-L-arginine (L-NNA) at the concentrations of 0.1, 1, 10, 100, and 1000 μM were used to evaluate for their inhibitory effects on nitrite production. It was found that low concentrations (0.1-10 μM) of these NOS inhibitors did not produce significant inhibition on the nitrite production, whereas at higher concentrations (100 and 1000 μM) of optically active L-NAME and L-NMA but not L-NNA significantly inhibited the nitrite production ($P<0.05$). However, racemic AG at higher concentrations (100 and 1000 μM) were found to significantly inhibit the nitrite production ($P<0.05$). On the other hand, the inactive enantiomer of NOS inhibitors, D-NAME and D-NMA, did not inhibit nitrite productions.

These data thus demonstrated that deprivation of serum in culture medium of N18 cells resulted in the induction of nitrite production which was attenuated by L-NAME and iNOS inhibitors such as L-NMA and AG, but not by cNOS inhibitor (L-NNA) and the inactive enantiomers (D-NAME and D-NMA).