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Inhibitory effect of alternariol on nitric oxide synthase in different parts of rat brain

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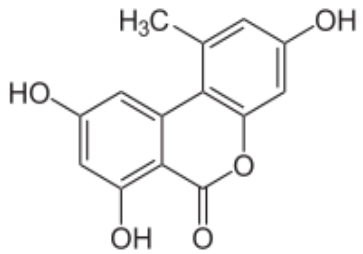
Abstract

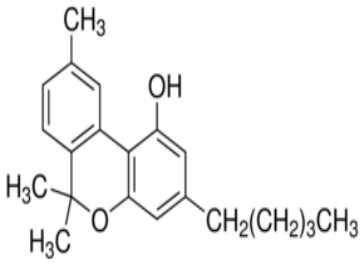
Alternariol (3,4,5 trihydroxy 6-methyl dibenzoxy-a –pyrone) is a metabolite result of various strains of alternariatenuis organism. Its structure has closeness to cannabinol subordinates. The impact of the alternariol on nitric oxide snythase (NOS) extracted from distinct parts of rat brain; particularly: frontal cortex, basal ganglia, cerebellum, pons, medulla oblongata was considered. Kinetic studies were done to decide the sort of inhibition of NOS and the inhibitor dissociation constants (K_I) by alternariol. The outcomes showed that alternariol inhibited NOS of the cortex, medulla oblongata and cerebellum more than that of alternate parts of the brain. These parts are in-charge of observation, tactile, psychic exercises and reflex focuses of breath. The inhibition of these enzymes increased with increasing the dose of alternariol added to the examined blend, i.e., the inhibition was dose dependent and of the competitive type. The estimations of K_I for alternariol –NOS complexes shifted from 1.8 to 5.6 mM. The distinction in the level of inhibition of the extracts of these brain parts could be ascribed to the slight contrast in the structure, i.e. course of action of their amino acids (isozyme phenomenon) and to their particular gene loci.

Keywords: Alternariol, Nitric oxide synthase, Brain parts, Inhibition.

Introduction

Alternariolis, a metabolite created by distinctive strains of alternariatenuis.[1] Its structure was built up as 3,4,5 trihydroxy 6-methyl dibenzoxy - □ - pyrone C14 H10 O5 (I). It has striking likeness to cannabinol subordinates (II).





Common events of Alternaria toxins have been accounted for in different natural products, handled organic product items, for example, squeezed apple, tomato items, wheat and different grains, sunflower seeds and pecans.[2-3] Pre-cancerous changes were observed in the esophageal mucosa of Alternariol fed mice for 10months.[4]

Nitric oxide (NO) is an administrative organic substance and an essential intracellular errand person that goes about as a particular middle person of different neuropathological disorders.[5]NO is incorporated by nitric oxide synthase (NOS), and increased in an assortment of tumor cells. NO controls various cell reactions by S-nitrosylation.[6] Nitric oxide synthase (NOS) was initially recognized and depicted in 1989. The development of NO by NOS in vascular endothelial cells opened up what can be viewed as another territory of organic research.

Brain NOS is a constitutive compound, the capacity of which is to deliver NO on interest for various neurophysiological exercises.[7] NOS intercedes the development of the neurotransmitter NO in addition to citrulline from L-arginine. NO plays an important role as a neurotransmitter in the nervous system, as a vasodilator in the cardiovascular system and as a cytotoxin in the host defence mechanism of macrophages.[8]

The present review was led to research the inhibitory impact of alternariol on NOS extracted from entire and five sections of rat brain, to be specific; frontal cortex, basal ganglia, cerebellum, pons and medulla oblongata. Compound kinetic studies were done to decide the sort of inhibition and enzyme-inhibitor dissociation constant (K_I) of NOS by alternariol, and to know which of these parts was repressed by alternariol more than different parts.

Material and Methods

Alternariol was prepared by growing a strain of alternariatenuis, Catalogue number S.M.108, on Czepek-Doxmedium using either glucose or molasses, as a carbon source.[1]The metabolite was extracted from the dried defatted mycellian with ether, then purified by repeated crystallization from dioxane giving colorless needles m.p. 350° (decomp).

Chemicals: Naphthyl ethelenediamine dihydrochloride, Sigma (Deisonhofen Germany), Sulfanylamide, Sigma

Deisonhofen Germany, Phosphoric acid (H_3Po_4): Deisonhofen Germany and Buffer: Phosphate (KH_2PO_4 and Na_2HPO_4) 0.1 M, pH 8.0 BDH.

Animals: Thirty male albino rats (125-150 gram body weight) age 2 months were used in the experiments. Rats were supplied from Medical Research Institute Animal House, Alexandria University (Egypt) and were housed in group cages (five in each) and given free access of food and tap water (ad libitum). The brains of these rats were used as a source for NOS. The rats were profoundly anesthetized and slaughtered by beheading after an overnight fast, with free access of water. Each brain weighed about 1.6 gm.

Assay of Nitric oxide synthase (NOS) activity: Brain Nitric oxide synthase (NOS) is a constitutive compound and the capacity of which is to create nitric oxide (NO):

$$\text{L-Arginine} \xrightarrow{\text{NOS}} \text{L-citrulline} + \text{NO}$$

NOS was controlled by evaluating nitrite (NO2) (being the most stable metabolite). It is utilized as a file for NOS.[9-10]

Because of the transient and unpredictable nature of NO which makes it precarious, continually oxidized to the steady forms nitrite (NO2) and nitrate (NO3), the most advantageous discovery technique for NO was to be measured as far as NO2 which can be identified by photometric strategy utilizing Griess reagent[9]at 546 nm. Griess reagent consists of one part of naphthyl ethelenediamine dihydrochloride (NED) in distilled water and one part of 1% sulfanilamide in 5% concentrated phosphoric acid (H_3PO_4). The two parts being mixed together within 12 hours of use and kept chilled. Each part may be stored separately refrigerated up to 2 months. The mixture of the two parts incubate with a NO_2-continaing sample (ratio 1:1) to form purple azo dye and its absorbance is measured at a wave length of 546 nm.[9,10]

To decide NO_2 in brain tissue; 150 µl brain homogenate containing 20mg tissue were blended with 1.5 ml of Griess reagent and the blend incubated for 5 min at room temperature. The color formed was measured at absorbance 546 nm. Nitrite was computed from NO_2 standard curve.

For the assurance of the binding constant (K_B), bimolecular rate constant (ka) and the inhibition rate (k_i),[11]alternariol was added to the previously mentioned blend in the following concentrations: 140, 280, or 420 or 560 µM while keeping the substrate at a steady fixation (166 µM), then the catalyst was examined after various interims (0, 15, 30, 45 and 60 sec).

The experimental design and interpretation of the results were based on the following equation:[11]

$$\frac{[I].\Delta t}{2.3\Delta \log v} = \frac{[I]}{k_i} + \frac{1}{k_a}$$

where [I] is the inhibitor fixation , and (Δ t/2.3 Δ log v) is the first order rate constant at steady [I].

For the assurance of the kind of inhibition and the enzyme-inhibitor dissociation constant (K_I), the substrate fixation was differed: 111, 166, 222 and 333 µM while the inhibitor (alternariol) was kept constant for every investigation: 140, 280 or 560µM.The inhibitor and substrate were added all the while simultaneously to the previously mentioned blend.

Results

Graphical representation of the inhibition of NOS by alternariol at various concentrations (140, 280, 420 or 560 µM) got by plotting log v (the speed of reaction) against time (t) gave straight lines (Fig. 1a). The slopes were processed and gave the esteem 2.3Δ log v/Δt. These results were utilized to develop the chart of [I].Δt/2.3Δ log v plotted against [I] (Fig 1b).

The slope of the straight line gave $1/k_i$(k_i is the rate of inhibition), the catch on the [I] pivot gave K_B (binding constant) and the capture on the ordinate gave $1/k_a$(k_a is the bimolecular rate constant).The estimations of k_i, K_B and k_a are given in Table 1.

As regards the kind of inhibition of NOS by alternariol; Figure 2 demonstrates that the double reciprocal curves of 1/v (v is the speed of the reaction) versus 1/[S] (S is the substrate concentration in µmol/L), keeping the inhibitor [I] constant for every analysis and changing the substrate concentration as mentioned by Dixon and Webb.[12] The slopereplots(inset of Fig.2) demonstrate that alternariol is a linear competitive inhibitor (after Cleland nomenclature).[13]

The subsequent estimations ofK_I (enzyme - inhibitor dissociation constant), K_m (Michaelis' constant) and K_I/K_m (affinity constant) for the distinctive parts under analysis, are recorded in Table 1.

Table 1: Kinetic constants characterizing the inhibition of NOS of whole and different parts of rat brain by alternariol. Values represent mean of 3 repeated experiments. S.D. was 10%

Part of the brain	k_i[(a)] (min^{-1})	K_B[(b)] (mM)	k_a [(c)] ($\mu M.min)^{-1}$	K_I[(d)] (mM)	K_m[(e)] (µM)	K_I/K_m[(f)]
Whole brain	3.25	1.1	3.0	3.5	325	10.7
Basal ganglia	1.7	0.8	2.2	1.8	333	5.4
Frontal cortex	5.4	0.4	14	5.6	290	19.3
Medulla oblongata	5.6	0.8	7.0	5	400	12.5

Pons	1.75	0.4	4.5	4.5	460	9.8
Cerebellum	3.67	0.6	6.1	4.7	415	11.3

(a) Inhibition constant (d) enzyme-inhibitor dissociation constant
(b) Binding constant (e) Michaelis' constant
(c) Bimolecular rate constant (f) Affinity constant

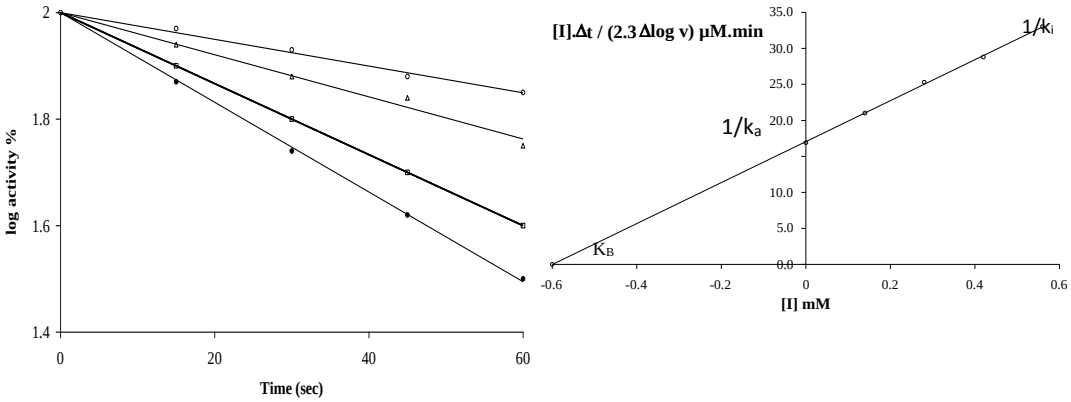
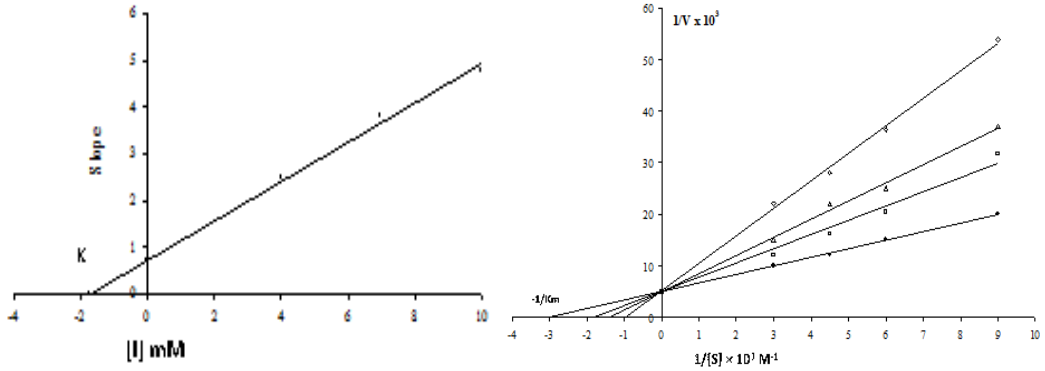


Fig. 1(a): The time course of inhibition of *cerebellum* Fig 1(b): Main plot of the data of the inhibition of *NOS* by alternariol in vitro at constant substrate cerebellum NOS by alternariol. The slope of the concentration (0.3mM), concentrations of straight line gave 1/k_i (k_i: rate of inhibition), the alternariol: 140(●), 280 (□) 420 (△), 560 (○) µM. intercept on [I] axis gave K_B (binding constant) and the intercept on the ordinate gave 1/ka(ka: bimolecular rate constant)



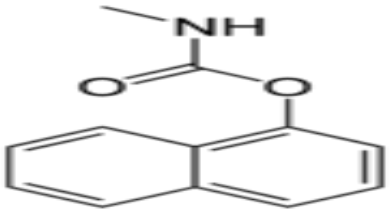
Inset: Cleland replot of the slopes obtained from Fig. 2 against the inhibitor concentration [I].
Fig. 2: Competitive inhibition obtained from Line weaver-Burk plot of 1/V versus 1/S, under the effect of alternariol on activity of *basal ganglia NOS.* (●) Control, (□) 4.0, (△) 7.0 & (○) 10 mmol/L

Discussion

The preliminary experiments showed considerable difference in NOS activity in the various parts of rat brain. The highest activity was detected in the medulla oblongata, frontal cortex and cerebellum, lower in basal ganglia and pons. The high NOS activity in the cerebellum is in agreement with the results described by other authors[14] who found that NOS activity was significantly decreased by 75% in the cerebellum. Decreased NOS activity in the nervous tissue was associated with decreased motor activities and spontaneous behavior of rats.

In the present work, the in vitro study of the effect of alternariolon NOS activity showed that alternariol had an inhibitory power on NOS of the cerebellum, pons and medulla oblongata more than that on the other parts which was in agreement with other authors[15] who found that the V_{max} and K_m values of the enzyme in cortex and cerebellum of rats were higher than in other brain parts. These studies show differential distribution and higher activity of NOS in rat brain regions and spinal cord compared to mouse tissues.

Alternariol possessed higher affinity and binding to the enzyme extracts of medulla oblongata (responsible for neural control on heart rate, respiration and blood pressure) than to the extracts of the other parts. This indicated that it resembled serine[16] that was introduced as a NOS inhibitor which was time and dose-dependent. It also resembles carbaryl (sevin)(a carbamate analogue of eserine which was introduced as a cholinesterase and NOS inhibitor).[17]



Carbaryl (Sevin)

Alternariol was introduced in this work as a NOS inhibitor as it was previously postulated[18]that NOS inhibitor agents play crucial roles in neurodegeneration and neuropathic pain, as it is known that NO levels rise after neurological insults causing the previously – mentioned malfunctions.

Alternariol was previously introduced as having a dual inhibitory effect on cholinesterase (ChE) and monoamine oxidase (MAO).[19]

Finally; it is note-worthy to mention that the difference in the rate of NOS inhibition by alternariolin different parts of rat brain could be due to:

a. The inhibition of NOS in the various regions of the brain is dependent on the concentration or the form of the enzyme i.e., isoenzyme phenomenon.[20]
b. The high effect of alternariolon NOS activity may be due to the solubility of this compound in the lipid layer in the brain exactly as eserine behaved and also affecting the parts containing white matter (e.g. medulla oblongata) more than the parts containing gray matter, as previously proved by Osman *et al.*[21]
c. The difference in isozyme inhibition could be due to distinctly different gene loci[22] and the amino acid sequence[23] leading to substrate specificity of the isozymes.
d. The difference in inhibition could be due to NOS content in each part of the brain.

So, it is recommended that further studies are to be done to produce medications from alternariol as a triple inhibitory agent on ChE, MAO and NOS to overcome neurodegenerative disorders and accompanying pain.

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