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Synthesis, characterization and in vitro antioxidant activity of (1e, 4e)-1,5- bis(4- hydroxyl-3-methoxyphenyl) penta- 1,4-dien-3-one

Mazin Nadhim Mousa

Pharmaceutical Chemistry Department, Pharmacy College, Basrah University, Basrah, Iraq

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ABSTRACT

The aim of this study was to synthesis and evaluation of in vitro antioxidant activity of (1E, 4E)-1,5- bis(4- hydroxyl-3-methoxyphenyl)penta- 1,4-dien-3-one (divanillic acetone DVA) in nitrite-induced hemoglobin (Hb) oxidation. DVA was prepared by aldol condensation of vanillic aldehyde and acetone. Tow concentrations of DVA were added at time 0 and 5 minutes intervals of Hb oxidation in erythrocytes lysate, and formation of methemoglobin (MetHb) was monitored spectrophotometrically. The results showed that DVA successfully attenuates Hb oxidation after addition of sodium nitrite; this protective effect was found to be not related to the catalytic stage of Hb oxidation, though such effect was reported to be more prominent when DVA was administered before nitrite. In conclusion, DVA can effectively, in concentration-dependent pattern, attenuate sodium nitrite-induced Hb oxidation.

Key words: Aldol condensation, nitrite-induced methemoglobin, vanillic aldehyde

INTRODUCTION

Reactive oxygen species (ROS) are produced in the cells by cellular metabolism and other exogenous environmental agents. They are generated by a process known as redox cycling and are catalysed by transition metals, such as Fe2+ and Cu2+ (1). Overproduction of ROS can damage cellular biomolecules like nucleic acids, proteins, lipids, carbohydrates, proteins and enzymes, resulting in several diseases. Living systems have specific pathways to overcome the adverse affects of various damages. However, sometimes these repair mechanisms fail to keep pace with such deleterious effects (2).

In recent years, antioxidants have been subjected to many epidemiological studies that have related their consumption to a reduction in the incidence of oxidative damage related diseases (3). They are potent scavengers of ROS like superoxide anions and hydroxyl radicals .They are also able to reduce ferric ions to ferrous state. Their antioxidant property is further shown by its capacity to inhibit lipid peroxidation in rat brain homogenate(4). Many compounds (like Phenolic compounds) posses biological properties such as: antioxidant, antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-arthrosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities (5.) Therefore, much attention has been focused on the use of antioxidants for improvement of human health

The oxidation of hemoglobin to methemoglobin by nitrite has been widely studied (6) The formation of methemoglobin occurs in two stages. The first is a slow stage, and the second is a rapid autocatalytic stage . Superoxide has been implicated in the autocatalytic stage of the oxidation .which is inhibited by superoxide dismutase.(4)

As the reducing agents are potent scavengers of ROS, the present work was made to evaluate the ability of DVA for its potential in inhibiting nitrite-induced oxidation of hemoglobin as a reference for their antioxidant activity.
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***Corresponding author.**
Mazin Nadhim Mousa
Pharmaceutical Chemistry Department, Pharmacy College, Basrah University, Basrah, Iraq

MATERIAL AND METHOD

Synthesis of (DVA) (7)
To a solution of 10 mL of 3 *M* sodium hydroxide , 16 mL of 95% ethanol and 3 g (10 mmole) of vanillic aldehyde, add 0.58 g (20 mmole) of acetone and shake the mixture , a yellow precipitate product forms. Continue to shake for the next 10 min. Remove the liquid from the beaker and add 30 mL of water and shake it vigorously. Remove the wash liquid and wash the crystals twice more with 15-mL portions of water and then filtrate by vacuum , dry and recrystallized from a mixture of ethanol and ether (1:1). DVA obtained as a yellow crystals having m.p. 390-393°C. The yield was about 69% .

IR (KBr)u:
3226.7 (OH stretching),(Ar-C-H stretching embedded), 2854.5 (C-H stretching), 1668.5 (C=O stretching),1640.9 (C=C stretching), 1591.1, 1512 (Ar.C=C stretching), (C-O stretching), 1370.2(C-H bending), 1126.3(C-O stretching) 860, (Ar. C-H out of plane) .

^{1}HNMR
(DMSO): δ 7.82 (2H), δ 7.16 (2H), δ 7.03 (2H), δ 6.99 (2H), δ 6.79 (2H), δ 5.35 (2H), δ 3.83 (6H),**$C_{19}H_{18}O_5$ (326.3):** C, 69.93; H, 5.56; O, 24.51. Found: 69.81; H, 5.52; O, 24.59.

Nitrite-induced methemoglobin in hemolysate
Blood samples were obtained from healthy individuals by vein-puncture, and kept in Ethylene diamine tetraacetic acid (EDTA) containing tubes; then centrifuged at 2500 rpm for 10 minutes to remove plasma and the buffy coat of white cells. The erythrocytes were washed thrice with Phosphate Buffer Saline (PBS, pH 7.4) and lysed by suspending in 20 volumes of 20mM Phosphate Buffer (PB, pH 7.4) to yield the required hemolysate concentration of 1:20 (8).

The reaction was initiated by the addition of sodium nitrite (1 ml, 0.6 mM) to 1.5 ml of freshly prepared solution of hemolysate and the formation of methemoglobin was measured by monitoring absorbance at 631 nm using a Shimadzu Graphicord UV 240 Spectrophotometer.

DVA was added (1 ml, 200µM and 100µM) just before or after 5 minutes from the addition of nitrite. Control experiments were conducted without DVA and all experiments were in triplicate and were repeated many times. (4)

RESULTS

Nitrite causes a rapid oxidation of hemoglobin to methemoglobin. In the presence of DVA, the oxidation process was delayed in a dose-dependent manner (figure I). The time required to convert 50% of the available hemoglobin to methemoglobin was 8 min with nitrite alone , whereas with DVA the time was increased (table 1).

Table(1): t½ of MetHb formation with different concentrations and at different time intervals

Time course of addition of DVA		(t½)(min)
standard		8
Time 0	100µM	>100
	200µM	>100
Time 5	100µM	28
	200µM	22

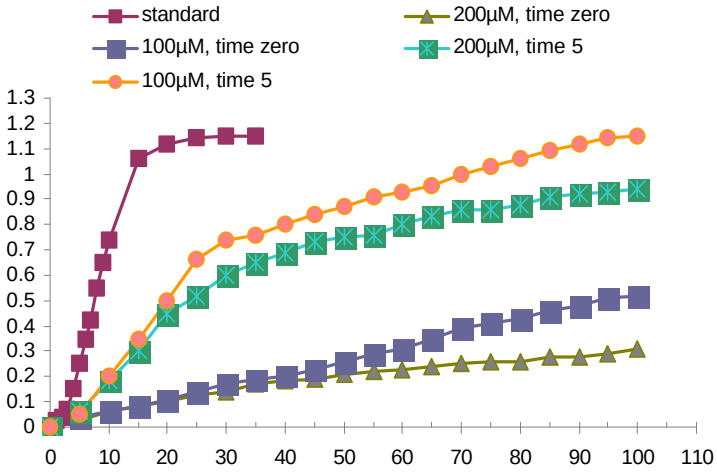

Fig.(1): Effect of DVA on the time course of nitrite oxidation of hemoglobin

Fig. 1 describes the effect of DVA on the time course of nitrite oxidation of hemoglobin. Without DVA, the time-course of oxidation shows a characteristic pattern of slow initial transformation followed by a rapid autocatalytic process. When DVA was added along with nitrite, i.e. at 0 min, the formation of methemoglobin was inhibited to a great extent.

Addition of DVA after nitrite addition i.e. (during autocatalytic phase) decreases absorbance of light and methemoglobin formation to a lesser extent.

DISCUSSION

The present study has shown that DVA can protect hemoglobin from oxidation by sodium nitrite in hemolysate. However, it did not to reverse the effect of nitrite if added at the later stage. It is well established that oxidation of hemoglobin takes place in two stages. There is a slow initial stage followed by a rapid autocatalytic stage, which carries the reaction to completion (6). The DVA is able to prevent the onset of the autocatalytic stage. Since superoxide is implicated in the autocatalytic stage and the fact that vanilline is a potent scavenger of free radicals (9) and protects DNA and mitochondrial membrane against oxidative stress in vitro (10) suggests that the protective action of DVA is by scavenging superoxide generated during the oxidation. Superoxide dismutase also inhibits the onset of the autocatalytic stage (6). Direct interaction between nitrite and DVA as a reason for protection is ruled out because the concentration of DVA causing protection is very low (=200 µM) compared to the nitrite concentration (0.6 mM). Although DVA can reduce ferric ions to the ferrous state, it fails to reverse the oxidation of hemoglobin, suggesting that protection is not due to reduction of methemoglobin to hemoglobin. Many antioxidants like ascorbic acid, uric acid, 3-ribosyl uric acid, and glutathione protect hemoglobin from oxidation by nitrite. These antioxidants also inhibit the onset of the autocatalytic stage of nitrite (11). Thus, the effect of DVA may be similar to these antioxidants in protecting hemoglobin from nitrite ions.

The antioxidant activity comes from the resonance stabilized hydroxyl groups. DVA conjugated system is expected to be easily oxidized in a manner similar to Benzalacetone.(12).

The more the double bond, the easier it will be oxidized. Therefore, it is assumed that DVA will show antioxidant activity because the resultant radical is stabilized by the conjugated system.

CONCLUSION:

DVA has an obvious antioxidant activity. It protective activity is greater when it is added before the oxidation stress. This suggest that it has a prophylactic antioxidant activity from the oxidizing agents.

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