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LIPID PEROXIDATION IN LIVER : HYDROXY DIMETHYL CARBAZOLE A NEW POTENT INHIBITOR

Claude Malvy, Claude Paoletti, Andrew J.F. Searle* and Robin L.Willson*.

LA 147 CNRS - Groupe INSERM U140 - Institut Gustave-Roussy
94800 - VILLEJUIF

* Biochemistry Department, Brunel University, Uxbridge-Middlesex, U.K.

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SUMMARY : The results of our structure-activity relationship study on the anticancer drug 9-hydroxyellipticine for its antioxydant properties show that 6-hydroxy-1,4-dimethyl carbazole has a remarkable inhibitory action in several *in vitro* peroxidative systems. The effectiveness of this carbazole is one order of magnitude larger than that of promethazine in the iron ascorbate or cysteine initiated systems.

INTRODUCTION : The process of lipid peroxidation has been suggested to play a major role in the progression of a wide range of pathological disturbances. An extensive literature concerning the induction of peroxidation, both *in vivo* and *in vitro* exists (1,8). Several compounds, including a number of natural and artificial antioxidants, e.g.vitamin E, propyl gallate and promethazine, have been shown to inhibit lipid peroxidation to varying extents. Generally, however, the concentrations of these compounds which inhibit rat liver microsomal lipid peroxidation systems by 50 % (ED_{50}) have been found to be greater than 1 µM.

During a recent investigation into the mechanisms of action of some cancer chemotherapy drugs of the ellipticine series (9-11) we observed, that 9-hydroxy ellipticine (9-OHE) reduced the toxicity of NADPH activated rat liver microsomes towards Walker cells grown *in vitro* (12).A paper (6)suggested to us that this effect could be due to some antioxidant properties of 9-OHE and a structure activity relationship study was started. Quite unexpectedly this has now led to the identification of a compound closely related to 9-OHE, 6-hydroxy, 1,4 dimethyl carbazole (HDC, Figure 1)which has a remarkable inhibitory action on several *in vitro* peroxidative systems greater than or equal to the activity of any other compound of which we are aware.

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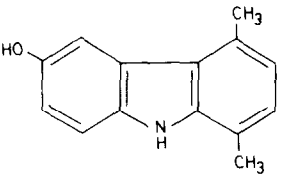

FIG. 1 : Structure of 6 hydroxy, 1,4 dimethyl carbazole (HDC)

MATERIAL AND METHODS

HDC was synthesised by Dr. E. LESCOT according to previously published methods(13).Its inhibitory action has been examined in the following stimulated lipid peroxidation systems :

1) Ferrous sulphate/cysteine(14,15).
Peroxidation of 1.5ml of a 1.5 mg/ml microsomal suspension in a 1:2 mixture of 0.1 M Tris buffer (pH 7.4) : 0.15 M KCl was initiated by the addition of cysteine and ferrous sulphate to final concentrations of 500 µM and 5 µM respectively. Samples were incubated at 37°C and peroxidation stopped after 30 ' by the addition of 3 ml of ice-cold 10 % w/v trichloroacetic acid. Following centrifugation triplicate 2 ml samples of the supernatant fluid were removed and added to 2 ml of 0.67 % w/v thiobarbituric acid (TBA), incubated for 15 ' in a boiling water bath, cooled and the absorbance measured at 535 nm in a 1 cm cuvette. Control absorbance values, a measure of uninhibited peroxidation, were in the range A = 0.83 ± 0.07. Inhibitors of interest were added to the microsome suspension prior to the addition of cysteine or ferrous sulphate.

2) Ferrous sulphate/adenosine diphosphate/reduced pyridine nucleotide (4)
A method similar to that for the ferrous/cysteine system was used with the exception that NADPH (final concentration 120 µM) and ferrous sulphate and ADP (final concentrations 5 µM and 400 µM respectively) were added instead of cysteine and ferrous sulphate. Control absorbance values were in the range A = 0.67 ± 0.10.

3) Ferrous sulphate/ascorbic acid (5).
A similar method was again used except that ascorbic acid (final concentration 500 µM)was added to start the peroxidation and the incubation time was only 15 '. Control absorbance values were in the range A = 1.16 ± 0.05.

4) Cumene hydroperoxide (16-17)
A similar method was again used except that cumene hydroperoxide (final concentration 100 µM) was added to initiate the peroxidation. Control absorbance values were in the range A = 0.31 ± 0.07.

5) Carbon tetrachloride
The method described by Slater and Sawyer (7) was used with a microsomal suspension concentration of 2.25 mg protein/ml. Control absorbance values were in the range A = 0.24 ± 0.14.

RESULTS AND DISCUSSION : The extents of lipid peroxidation in the absence and presence of inhibitors were measured over a range of concentrations. Percentage inhibition of peroxidation versus drug concentration plots were determined for HDC, and for the well-documented protective agents propyl gallate (PG) and promethazine (PZ)(Fig.2.)ED_{50} values derived from such plots are shown in Table 1. Promethazine is one of the most

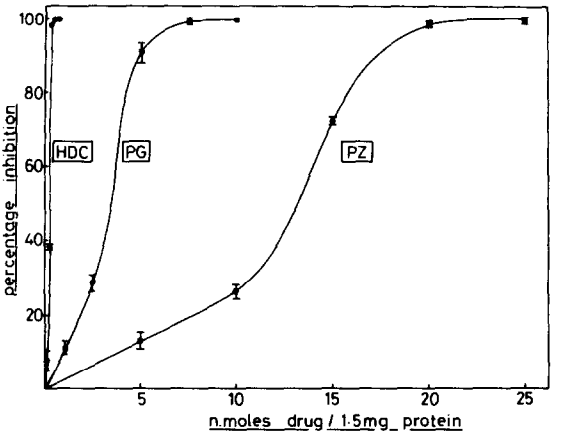

FIG. 2 : Inhibition of lipid peroxidation in rat liver microsomes.

effective compounds known to us in preventing lipid peroxidation due to carbon tetrachloride and Fe^{II}/ADP/NADPH(18)and has correspondingly received wide attention. It can be clearly seen that HDC is an order of magnitude more effective than promethazine in the iron ascorbate or cysteine initiated systems, and in all systems considerably more effective than propyl gallate.

The fact that HDC works at such low concentrations is intriguing particularly as unlike 9-OHE it is non-toxic toward L 1210 cells even at concentrations of 100 μM. It seems likely that some mechanism, such as the intimate involvement of the drug with the site of peroxidative initiation, operates in addition to free radical scavenging.

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Table 1 ED_{50} values for inhibition of lipid peroxidation

	Fe^{II} / Ascorbate	Fe^{II} / Cysteine	Fe^{II} / ADP NADPH	CCl_4 / NADPH	Cumene hydroperoxide
HDC	0.26	0.31	0.30	0.20	12.5
PZ	13.1	8.2	0.74	0.19	14.9
PG	3.3	4.4	4.5	1.6	46.7

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