Tetrahydroprotobererines inhibit lipid peroxidation and scavenge hydroxyl free radicals

JIN Xi-Lu1 SHAO Yue2 WANG Ming-Jie2 CHEN Li-Juan1 JIN Guo-Zhang3
1 Shanghai Institute of Materia Medica Chinese Academy of Science 2 Department of Pharmacology School of Pharmacy Shanghai Medical University Shanghai 200031 China

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ABSTRACT

AIM To study the effects of tetrahydroprotobererines THPB on rat liver and brain lipid peroxidation LPO and oxygen free radicals generation. METHODS The malondialdehyde MDA levels in rat brain and liver homogenates induction of MDA by Fe2+ Vit C in mitochondria OH generation by Fenton reaction and O2- generation by pyrogallol oxidation were observed in vitro. RESULTS 1 THPB lowered the MDA contents in the liver homogenate and mitochondria and the IC50 values of l-THPB-18 and l-stepholidine SPD in the liver mitochondria were 3.1 and 12.7 μmol L-1 respectively. SPD decreased the MDA contents in the brain homogenate and mitochondria with IC50 values of 102 and 35.0 μmol L-1 respectively. 2 THPB scavenged OH- and the IC50 values of l-THPB-18 and SPD were 0.21 and 3.8 μmol L-1 respectively but no effect on O2- was observed. CONCLUSION THPB could reduce the MDA contents and scavenge OH- and THPB-18 was the most potent amongst them.

INTRODUCTION

We have found that some dihydroxy-tetrahydroprotobererine analogues dihydroxy-THPB such as l-stepholindin SPD THPB-18 and scoulerine possess an intrinsic activity to D1 receptors12 and have a D1 agonistic action in the 6-OHDA-lesioned rats a Parkinson disease PD animal model 2-4. Furthermore SPD has been used to treat tardive dyskinesia and alleviate PD syndrome in preliminary clinical trials 5-7. According to the current insight PD is a neurogenerative disease of DA neurons in the substantia nigra based on the oxygen free radicals and lipid peroxidation LPO induced by the oxidative stress 7-9. The new effective remedy that could stop or slow down this degeneration can be a strategical drug which both directly activates DA receptors particularly D1 and protects the surviving DA neurons in the substantia nigra 10-12. Although this viewpoint has been well understood the perfect drug remains elusive. The present work studied the anti-oxidative effects of THPB particularly SPD and THPB-18 on LPO and oxygen free radicals generation.

MATERIAL AND METHODS

Animals and Materials Sprague-Dawley rats male Grade II certificate No 005 weighing 200 – 300 g were purchased from Shanghai Experimental Animal Center Chinese Academy of Sciences. Eleven THPB and 2 protobererines PB Fig 1 supplied by Shanghai Institute of Materia Medica were dissolved in sulphuric acid 0.1 mol L-1 and adjusted with NaOH 0.1 mol L-1 to pH 4 – 5. Other reagents were AR.

Measurement of MDA in rat liver and brain homogenate The contents of MDA in the liver or brain homogenate were measured with the thiobarbituric acid TBA reaction 12. In brief the liver and brain were homogenized with Tris-KCl buffer Tris 0.1 mol L-1 KCl 1 mol L-1 pH 7.4. The supernatants were incubated with THPB at 37 °C and then with TBA and kept in boiling water bath. After cooling the optical density OD value was measured at 535 nm 752 C spectrophotometer. MDA content was expressed as nmol g-1 wet tissue.

Measurement of MDA initiated by Fe2+ Vit
C in liver and brain mitochondria  Rat liver and brain were homogenized with 10 % w/v ice-cold Tris-KCl buffer then the supernatant was centrifuged at 18 000 x g 4 ℃ for 15 min HITACHI 25-PR520. The mitochondrial suspension 0.5 mL containing 1 mg protein was incubated at 37 ℃ for 60 min in the presence of FeSO₄ 5 μmol L⁻¹ Vit C 10 μmol L⁻¹ and the test compounds then 2 mL 0.1 mol L⁻¹ HCl and 1 mL 0.67 % TBA were added suspension mixture was then heated for 15 min in a boiling water bath and extracted with 4 mL BuOH after cooling. The OD value of the BuOH phase was measured at 535 nm. The MDA content was expressed as μmol g⁻¹ protein.

Measurement of hydroxyl radicals  OH− generated in Fe²⁺-EDTA and H₂O₂ system in vitro The suspension mixture 3.5 mL containing benzoic acid 2.187 mmol L⁻¹ FeSO₄ 21 μmol L⁻¹ edetic acid-2Na 64 μmol L⁻¹ K₂HPO₄-KH₂PO₄ buffer 109 mmol L⁻¹ pH 7.4 H₂O₂ 32 μmol L⁻¹ and the test compound was incubated for 5 h at 25 ℃. The fluorescence intensity of OH radicals generated as a result was measured 300/408 nm HITACHI 650-10S fluorospectrophotometer.

Measurement of superoxide anion O₂⁻ resulting from pyrogallol autoxidation  The effects of THPBs on scavenging O₂⁻ was measured by determining rate of pyrogallol autoxidation.

Statistical analysis  The inhibitory concentration 50 % IC₅₀ and its 95 % confidence limits were calculated by logit’s method. Statistical analysis was performed using unpaired t test.

RESULTS

Effect of SPD on MDA content  SPD decreased the MDA contents in rat liver and brain homogenate in a dose-dependent manner Fig 2. At the concentrations from 1.875 - 300 μmol L⁻¹ the inhibitory rate IR in the liver and brain homogenate were 4 ± 7 % td 83 ± 4 % and 18 ± 5 % td 80 ± 6 % respectively and its IC₅₀ values 95 % confidence limits were 18.5 ± 15.5 - 22.0 % and 102 ± 94 - 110 μmol L⁻¹ respectively.

SPD could also inhibit the MDA induced by Fe²⁺-Vit C in rat brain mitochondria Fig 3. At the concentrations form 7.5 - 240 μmol L⁻¹ IR were 24 ± 5 % td 92 ± 14 % with IC₅₀ value of 35.0 21.7 - 56.7 μmol L⁻¹. These results indicate that SPD possesses anti-LPO activity.

Effect of THPB on MDA content of rat liver homogenate  Among the tested 11 THPB and 2 PB l-THPB-18 d-THPB-18 l-THPB-19 PB H-143 and SPD all lowered the MDA level at 5 μmol L⁻¹ n = 5 P < 0.05 vs control with IR of 68 % 56 % 56 % 56 % 56 % and 28 % respectively. The anti-LPO effect of l-THPB-18 was more potent than that of SPD and other THPB. At 10 μmol L⁻¹ l-THPB could lower the MDA content P < 0.01 vs control with an IR of 89 % but palmitine dehydrogenated l-THPB had no obvious effect on it.

Effect of THPB on Fe²⁺-Vit C initiated MDA content in rat liver mitochondria  Fe²⁺-Vit C...
polyphenols 0.625 - 5 μg·mL⁻¹ all inhibited the MDA levels dose-dependently \( r = 0.9186 - 0.9973 \) \( P < 0.01 \) or \( P < 0.05 \). Among them \( l \)-THPB-18 was most potent \( n \) while SPD was the least Tab 1. The \( IC_{50} \) of tea polyphenols was 1.49 ± 0.20 μg·mL⁻¹ \( n = 4 \) experiments in duplicate. \( ^{\circ}P < 0.01 vs l \)-THPB-18.

### Effects of THPB on generation of hydroxyl radical \( OH \) \( \cdot \) and superoxide anion \( O_2^- \)

\( l \)-THPB-18 0.01 - 3 μmol L⁻¹ \( d \)-THPB-18 0.1 - 3 μmol L⁻¹ \( l \)-THPB-19 0.1 - 2 μmol L⁻¹ \( THPB-1 1 - 20 μmol L⁻¹ \) scoulerine 0.03 - 30 μmol L⁻¹ \( H-25 0.1 - 30 μmol L⁻¹ \) H-143 0.3 - 60 μmol L⁻¹ \( and SPD 0.3 - 60 μmol L⁻¹ \) all could significantly scavenge \( OH \) in a dose-dependent manner. The rank order of the inhibitory potency of THPB was \( l \)-THPB-18 \( d \)-THPB-18 \( l \)-THPB-19 > scoulerine > SPD > H-25 H-143 \( THPB-H \) Tab 1. The \( IC_{50} \) of \( l \)-THPB-19 was 0.23 ± 0.09 μmol L⁻¹ \( n = 3 \). However none of them had any significant effect on pyrogallol autoxidation rates even with the higher doses \( 100 - 640 μmol L⁻¹ \) which indicated that THPB could not scavenge \( O_2^- \).

### DISCUSSION

The MDA level was used as an autoxidation index of LPO in tissues is used to screen the antioxidants. The present results show that some THPB can remarkably lower the MDA levels which indicate that they possess the antioxidant effects. In addition THPB can scavenge \( OH \) radicals in vitro. Among them the most potent one is THPB-18 which has a halogen substituted group at \( C_{12} \) position suggesting that the halogen group is impor-
tant for enhancing the pharmacological effects of THPB analogs.234

THPB can scavenge OH radical but has no effect on O$_2^-$.
This property of THPB is to that of bromocript-
ine.234 Whether THPB has the scavenging effects in other O$_2^-$ generating systems such as the xan-
theine-xanthine oxidase system remains to be further
studied.

The oxygen free radicals especially OH$^-$ induced in DA oxidative stress is very important in the neurode-
generation of DA neurons of the substantia nigra in PFC234. The present results show that THPB such as
THPB-18 and SPD possess anti-oxidative pharmacological
dproperties. Furthermore recent in vitro and in vivo res-
ults have demonstrated that SPD and THPB-18 could
protect DA neurons from the damage induced by selective
DA neuron toxin MPTP and 1-methyl-4-phenyl pyridini-
im ion$^+$ unpublished data. Recently SPD has been preliminarily used to treat the PD patients in the
later stage and has alleviated the syndrome by combina-
tion with the lowest dose of bromocriptine.234 Thus it
can be inferred that THPB can be potentially beneficial
by preventing further DA neuro degeneration in PD.

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