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用电子自旋共振自旋捕集技术研究吗丙嗪对活性氧自由基的清除作用[1]

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Scavenging effects of probimane on active oxygen free radicals by electron spin resonance[1]

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ABSTRACT Probimane, *dl*-1,2-bis (4-morpholine-methyl-3, 5-dioxopiperazin-1-yl) propane, is a new antitumor agent synthesized by Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The scavenging effects of probimane on active oxygen radicals produced in 3 different systems were studied with the ESR spin trapping methods.

In Fenton's reaction, probimane remarkably scavenged hydroxyl radicals (·OH) and the rate of scavenging ·OH by probimane 0.05 mmol/L was 47%, compared to 5% by vitamin E (V_E) and 30% by ascorbic acid (AA). In irradiation riboflavin system, in which superoxide ($O_2^{\cdot-}$) was produced, the agent also had the scavenging effects on $O_2^{\cdot-}$. The rate of scavenging $O_2^{\cdot-}$ by probimane 0.05 mmol/L was 13%, higher than that by V_E (7%) but lower than that by AA (90%). In cell system where the active oxygen radicals were produced during the respiratory burst of human neutrophils (Neu) stimulated by TPA (tetradecanoylphorbol acetate), probimane exhibited a dose-dependent scavenging action on the radicals. The rate of the radical scavenging by probimane 0.05 mmol/L was 37%, much higher than that by V_E (9%) but lower than that by AA (68%). Probimane had no effect on the rate of oxygen consumption by human Neu, measured with spin probe oxymetry.

KEY WORDS probimane; electron spin resonance; vitamin E; ascorbic acid; tetradecanoylphorbol acetate; neutrophils; free radicals

摘要 用ESR自旋捕集技术研究发现，吗丙嗪可明显清除Fenton反应产生的·OH，在0.03 mmol/L时，其清除率为30%，强于抗坏血酸(ascorbic acid, AA)和维生素E(V_E)。该药对光照核黄素反应产生的$O_2^{\cdot-}$的清除作用较弱，浓度达0.1 mmol/L时，其清除率仅为19%，强于V_E，弱于AA。吗丙嗪也能清除由TPA刺激引起人多形核白细胞呼吸暴发时产生的活性氧自由基。

关键词 吗丙嗪；电子自旋共振；维生素E；抗坏血酸；佛波醇；嗜中性白细胞；自由基

吗丙嗪(probimane, AT-2153)是由中国科学院上海药物所合成的新型抗肿瘤药物，对多种实验肿瘤有明显抑制作用，并在低剂量范围内可选择性抑制小鼠的体液免疫反应[1]。我们实验室又发现吗丙嗪可对抗阿霉素的心脏毒性。由于阿霉素的心脏毒性与活性氧自由基有关[2,3]，本实验利用电子自旋共振(electron spin resonance, ESR)自旋捕集技术，观察了

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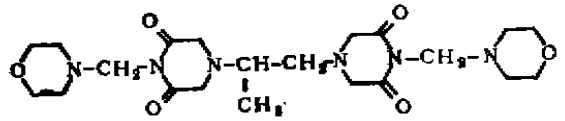
吗丙嗪对 Fenton 反应、光照核黄素反应和促癌剂(TPA)刺激引起的人多形核白细胞呼吸暴发 3 个实验模型中所产生的活性氧自由基的清除作用，以探讨吗丙嗪抗肿瘤和对抗阿霉素心脏毒性的机理。



Probimane (AT-2153)
dl-1,2-bis (4-morpholine-methyl-3,5-dioxopiperazin-1-yl) propane

MATERIALS AND METHODS

吗丙嗪由中国科学院上海药物研究所提供。5，5-Dimethyl-1-pyrroline-1-oxide (DMPO) Sigma 产品，经活性炭纯化其 ESR 波谱中无杂质信号存在。3-Carbamoyl-2，2,5，5-tetramethyl-3-pyrroline-1-yloxy (CTPO) Sigma 产品，先用少量乙醇溶解，再用磷酸缓冲液(0.05 mol/L，pH 7.4) 稀释至适当浓度备用。促癌剂 tetradecanoylphorbol acetate (TPA，Sigma) 溶解于少量丙酮后置 −20℃ 保存，临用前用磷酸缓冲液(0.05 mol/L，pH 7.4)稀释至所需浓度。核黄素为上海化学试剂采购供应站试剂厂产品，临用前用磷酸缓冲液(0.05 mol/L，pH 7.4)稀释至所需浓度，避光。其他均为国产 AR 级试剂。

人多形核白细胞 (human neutrophils，Neu)的分离提纯[4] 在 4℃下从新鲜抗凝健康人血中分离出 Neu，将其悬浮于磷酸缓冲液(0.05 mol/L，pH 7.4)备用。

Fenton 反应[4] 将 1% H_2O_2，硫酸亚铁铵 100 μmol/L，DMPO 0.1 mol/L 和不同浓度的药物(对照组用等容量的磷酸缓冲液 0.05 mol/L，pH 7.4 代替之) 混匀后 2 min，放入 ESR 谐振腔测试。

光照核黄素反应[4] 将含核黄素 0.3 mol/L，EDTA 5 mmol/L，DMPO 0.1 mol/L 和不同浓度的药物混匀后，经氙灯照射 0.5 min (功率 1 kw 光距 70 cm)后，立即测试 ESR 信号。

Neu 呼吸暴发实验[4] 将 diethylenetriaminepentacetic acid (DETAPAC) 0.1 mmol/L，Neu 1.5×10^7/ml，TPA 100 ng/ml 和不同浓度药物的混合物置 37℃ 水浴 2 min 后，加入 DMPO 0.1 mol/L，混匀后立即测试 ESR 信号。

用 Varian E-109 型 ESR 波谱仪测试ESR. 测试条件为：功率 15 mW，X 波段，100 kHz 高频调制，调制幅度 1 mT，扫描速度 25 mT/min，时间常数 0.128 s，温度 25℃。

***Neu* 的氧消耗测定**[4,5] 将含 TPA 100 ng/ml，Neu 2.5×10^7/ml，CTPO 1 mmol/L 和吗丙嗪 0.05 mmol/L 混匀后密封于石英毛细管中置 37℃ 水浴 1 min，分别于混匀后 2，7，12，17 min 测试，ESR 仪器条件同前，但调制幅度 0.05 mT，功率 1 mW，扫描速度 0.37 mT/min。

RESULTS AND DISCUSSION

吗丙嗪对 Fenton 反应产生的羟基自由基 ·OH 的清除作用 Fenton 反应是产生纯 ·OH 的经典反应[6]。Fig 1 中，A，B 分别是在 Fenton 反应体系中不加和加入吗丙嗪所得的自旋捕集剂 DMPO 与 ·OH 形成的 DMPO-OH 的 ESR 谱。用各谱线的第二组峰对峰高度 h 表示 ESR 信号的相对强度 (Fig 1 A)。吗丙嗪对 ·OH 的清除作用以清除率 E 表示：

$$E=(h_0-h_x)/h_0\times100\% \qquad [1]$$

h_0 为对照样品 DMPO-OH 的第二组的峰对峰高度的均值，h_x 为加药样品 DMPO-OH 的第二组的峰对峰高度的均值。

结果表明，吗丙嗪可清除 Fenton 反应生成的 ·OH。其浓度为 0.03 mmol/L 时，ESR 信号显著减小，清除率达 30%，浓度愈大清除作用愈强(Fig 2)。

吗丙嗪对光照核黄素体系产生的超氧阴离子自由基 $O_2^{\cdot-}$ 的清除作用 光照核黄素体系

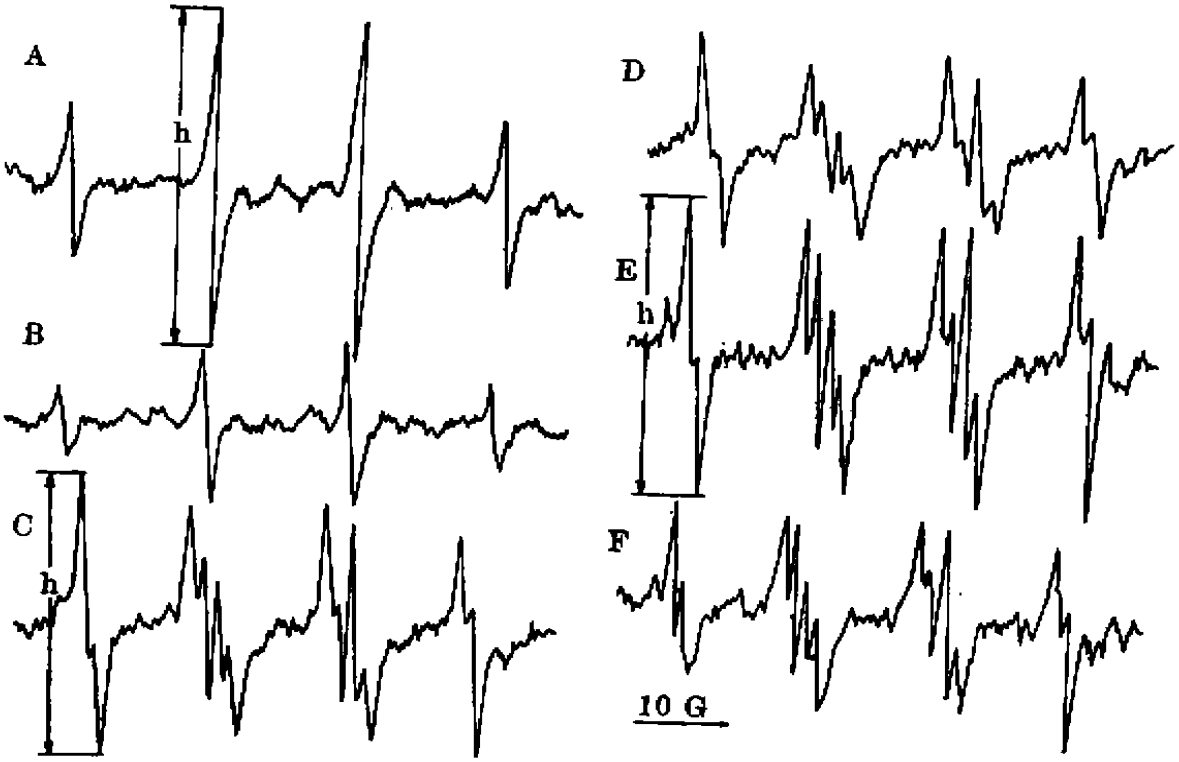


Fig 1. Electron spin resonance (ESR) spectra of (A, B) DMPO spin trapped ·OH in Fenton's reaction, (C, D) DMPO spin trapped $O_2^{\cdot}$ in irradiation of riboflavin, (E, F) DMPO spin trapped the active oxygen radicals in the respiratory burst of TPA-stimulated human neutrophils (A, C, E) without probimane; (B, D, F) with probimane.

可产生$O_2^{\cdot}$[4]。Fig 1 中，C，D 分别为光照核黄素体系中不加和加入吗丙嗪所得的 DMPO 与$O_2^{\cdot}$形成的加合物 DMPO-OOH 的 ESR 波谱。用 DMPO-OOH 的 ESR 波谱的第一组峰中幅度最大两峰的峰对峰高度 h 表示 ESR 信号的相对强度(Fig 1 C)。按[1]式计算对$O_2^{\cdot}$的清除率。结果发现，吗丙嗪也能清除$O_2^{\cdot}$，但是作用较弱。当浓度达 0.1 mmol/L 时，才明显清除$O_2^{\cdot}$(Fig 2)，清除率为 19%。

吗丙嗪对 TPA 刺激引起的 Neu 呼吸暴发时产生的活性氧自由基的清除作用 Neu 呼吸暴发时可产生大量的$O_2^{\cdot}$和 ·OH[7,8]。Fig 1 中，E，F 分别为反应中不加和加入吗丙嗪所得的活性氧自由基 DMPO 加合物的 ESR 波谱。虽然，这种谱线由 DMPO-OOH 和 DMPO-OH 两种自旋加合物的波谱混合组成，为了粗略定

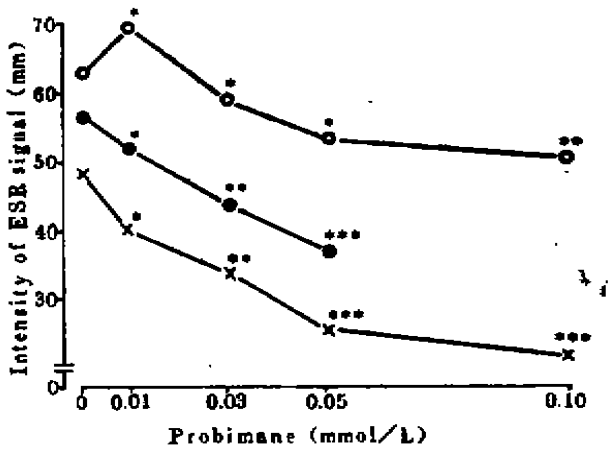


Fig 2. The dose-dependent scavenging effects of probimane on the $O_2^{\cdot}$ in irradiation of riboflavin (○), the ·OH in Fenton's reaction (●) and the active oxygen radicals produced by TPA-stimulated human neutrophils (×) measured by ESR. *P>0.05, **P<0.05, ***P<0.01 *vs* control.

量计算实验结果，我们以 ESR 波谱中第一组波峰内幅度最大的两峰的峰尖间距 h 表示 ESR 信号的相对强度(Fig 1 E)。按[1]式计算清除率 E。

吗丙嗪可使 TPA 刺激引起的 Neu 呼吸暴发时产生的活性氧自由基的 DMPO 加合物 ESR 信号明显减弱，其作用随浓度增加而增强 (Fig 2)。

吗丙嗪对 Neu 氧消耗的影响 必须在确定吗丙嗪不影响 Neu 正常呼吸暴发活性的条件下，才能认为吗丙嗪减弱 Neu 呼吸暴发试验中活性氧自由基 DMPO 加合物的 ESR 信号是由于该药清除活性氧自由基的直接结果。用自旋探针 CTPO 氧测定法观察 Neu 受 TPA 刺激后产生呼吸暴发时的氧消耗可做为观察 Neu 呼吸活性的一项指标[4]。实验证明，吗丙嗪 0.05 mmol/L 不影响 TPA 刺激引起的 Neu 呼吸暴发时氧消耗速率，表明在 Neu 呼吸暴发试验中，吗丙嗪使活性氧自由基 DMPO 加合物的 ESR 信号减弱，不是通过直接或间接抑制 Neu 对氧的利用造成的，而是清除了活性氧自由基的结果。

吗丙嗪、V_E 和 AA 清除活性氧自由基作用的比较 由 Tab 1 可见，在 0.05 mmol/L 时，吗丙嗪对 Fenton 反应产生的 ·OH 的清除作用强于 V_E 和 AA，对光照核黄素反应产生的 $O_2^{\cdot}$ 的清除作用强于 V_E，明显弱于 AA。

Tab 1. **Comparative study of the scavenging effects (%) of probimane, vitamin E (V_E) and ascorbic acid (AA) at the concentration of 0.05 mmol/L on the active oxygen radicals in 3 different systems.** $^*P>0.05$, $^{***}P<0.01$ *vs* control. $^{\dagger\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.01$ *vs* probimane.

Drug	Fenton's Reaction	Irradiation of riboflavin	Respiratory burst of neutrophils
Control	0	0	0
Probimane	47^{***}	13^{*}	37^{***}
V_E	$5^{*\dagger\dagger\dagger}$	$7^{*\dagger\dagger\dagger}$	$9^{*\dagger\dagger\dagger}$
AA	$30^{***\dagger\dagger\dagger}$	$90^{***\dagger\dagger\dagger}$	$68^{***\dagger\dagger}$

在 Neu 呼吸暴发试验中，虽然，吗丙嗪对活性氧自由基的清除作用强于 V_E，但弱于 AA。在以上 3 体系中，V_E 的活性氧自由基清除作用都弱于吗丙嗪，这可能与 V_E 在水溶液中呈悬浮状态有关。

大量实验证明[2,3,8]，阿霉素心脏毒性与活性氧自由基有关。阿霉素的蒽醌核能可逆地转变成半醌自由基，后者将电子传递给 O_2，使之还原为 $O_2^{\cdot}$，进而产生 ·OH，通过脂质过氧化反应损伤细胞，尤其是心肌细胞。本实验发现吗丙嗪可以清除活性氧自由基，尤其是 ·OH，这可能是吗丙嗪对抗阿霉素心脏毒性的原因之一。但是，由于心肌细胞中超氧化物歧化酶含量较低[10]，阿霉素的心脏毒性可能主要由 $O_2^{\cdot}$ 直接引起。因此，吗丙嗪对抗阿霉素心脏毒性的作用除通过清除活性氧自由基外，可能还有其他作用机理。

致谢 本所 ESR 技术组提供技术帮助，侯京武同志参加部分实验工作。

REFERENCES

1 Zhang TM, Wang MY, Wang QD. Antineoplastic action and toxicity of probimane and its effect on immunologic function in mice. *Acta Pharmacol Sin* 1987; 8 : 369

2 Goodman J, Hochstein P. Generation of free radicals and lipid peroxidation by redox cycling of adriamycin and daunomycin. *Biochem Biophys Res Commun* 1977; 77 : 797

3 Myers CE, McGuire WP, Liss RH, Ifrim I, Grotzinger K, Young RC. Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor response. *Science* 1977; 197 : 165

4 Zhao BL, Li XJ, He RG, Cheng SJ, Xin WJ. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys* 1989; 14 : 175

5 Lai CS, Hopwood LE, Hyde JS, Lukiewicz S. ESR studies of $O_2^{\cdot}$ uptake by Chinese hamster ovary cells during the cell cycle. *Proc Natl Acad Sci USA* 1982; 79 : 1166

6 Piette LH, Baxley LH, Grover TA, Harwood PJ. A comparative kinetic study of the initiation of lipid peroxidation with ·OH radicals and ferrous ion. In : Bors W, Saran M, Tait

D, eds. *Oxygen radicals in chemistry and biology*. Berlin: de Gruyter 1984 : 137-45
7 Bratigan BE, Rosen GM, Chai Y, Cohen MS. Do human neutrophils make hydroxyl radical? *J Biol Chem* 1986; 261 : 4426
8 Bannister JV, Bannister WH. Production of oxygen-centred radicals by neutrophils and macrophages as studied by electron spin resonance (ESR). *Environ Health Perspect* 1985; 64 : 37
9 Bachur NR, Gordon SL, Gee MV. Anthracycline antibiotic augmentation of microsomal electron transport and free radical formation. *Mol Pharmacol* 1977; 13 : 901
10 Doroshow JH, Locker GY, Myers CE. Enzymatic defenses of the mouse heart against reactive oxygen metabolites. *J Clin Invest* 1980; 65 : 128

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月桂氮䓬酮对小鼠皮肤和肉瘤180细胞膜超微结构的影响

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Influence of laurocapram on ultrastructures of mouse skin and sarcoma 180 membrane

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ABSTRACT Laurocapram (Lau), 1-dodecylhexahydro-2 *H*-azepin-2-one, (azone) is a new percutaneous penetration enhancer. However, the mechanism of its action for absorption promoter of other agents is still unknown. In this paper the effect of Lau on ultrastructures of skin surface and tumor cell membrane were studies. Lau (2 %) suspensiod was applied to abdominal skin of ICR/JCL, C 57 BL mice or one side of abdominal skin of nude mouse with drug and other side with the vehicle solvent once daily for 2-3 d. The skin was excised at 4 h after the final medication for examination under scanning electron microscope (SEM). The results showed the numerous small infolding lines which divided the skin surface into small areas with vesiculation and peeled the epidermal surface to form a few minor holes. The cuticles of the hair shaft dropped off and became thinner. Numerous desquamated cells around the orifice of the hair were fractured, detached and widened.

Sarcoma 180 cells were incubated with Lau 25 μg/ml at 37℃ for 4 h. The microvilli of some cells dropped off and the size of villi became thinner and shorter. The top of some villi of the cells appeared occasionally thick to make the profile as a bat. The surface of numerous naked cells became rugged and rough and showed many black minor holes in the area of denuded cell membrane or dropped microvilli. More

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