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咯萘啶和磺胺多辛/乙胺嘧啶对感染伯氏疟原虫小鼠的合并用药作用

施晓华、邵葆若、叶秀玉 (中国预防医学科学院寄生虫病研究所[1]，上海 200025，中国)

Combined action of pyronaridine and sulfadoxine/pyrimethamine against *Plasmodium berghei* ANKA strain in mice

SHI Xiao-Hua, SHAO Bao-Ruo, YE Xiu-Yu
(*Institute of Parasitic Diseases*[1], *Chinese Academy of Preventive Medicine, Shanghai 200025, China*)

ABSTRACT Pyronaridine, a highly effective antimalarial drug, was synthesized and developed by this institute. In order to test whether the joint blood schizontocidal action of pyronaridine (PND) and 2:1 mixture of sulfadoxine/pyrimethamine (SP) resulted in a potentiation or an additive effect, groups of *P berghei* ANKA strain-infected mice were treated with various single oral doses of PND and SP. Thin blood smears were made after 72 h and the parasitemia-negative rates were calculated. The ED_{50} values obtained were plotted in isobolograms. An additive action of this triple combination was demonstrated.

Mice were inoculated with *P berghei* ANKA strain-infected erythrocytes 3 and 2 d after the mice were given a single oral dose of PND 10 mg/kg alone, or combined with SP 3 mg/kg. Thin blood smears made on d 3, 5, 8 and 10 revealed that the parasitemia-positive rates and the duration of residual blood schizontocidal action of PND used alone was similar to that of PND used in combination ($P>0.05$).

Three groups of mice carrying *P berghei* ANKA strain gametocytes were administered orally with pyrimethamine 0.1 mg/kg, SP 0.3 mg/kg and SP 0.3 mg/kg plus PND 0.5 mg/kg, respectively. *Anopheles stephensi* were then fed on the mice 2 h after the medication. There resulted no significant differences of gametocytocidal and sporontocidal effects among the 3 groups, since the oocyst-positive rate and the gland-positive rate were similar in these groups.

KEY WORDS malaria; *Plasmodium berghei*; antimalarials; pyronaridine; sulfadoxine; pyrimethamine; drug combinations

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[1] WHO Collabrating Centre for Malaria, Schistosomiasis and Filariasis

提要 感染伯氏疟原虫 ANKA 株小鼠 1 次 ig 药物后 72 h 血检，求原虫血症转阴率及 ED_{50}，用等效应图解法分析咯萘啶(PND)与磺胺多辛/乙胺嘧啶(SP)合并为相加作用。PND，SP 或 3 药合并于小鼠接种前 3 d 1 次 ig，未见 3 药合并影响 PND 的持效作用。小鼠 1 次 ig 药物后 2 h 感染斯氏按蚊，合并用药对配子体及其孢子增殖的抑制作用与乙胺嘧啶相仿。

关键词 疟疾；伯氏疟原虫；抗疟药；咯萘啶；磺胺多辛；乙胺嘧啶；合并用药

咯萘啶(pyronaridine, PND)与磺胺多辛(sulfadoxine, S)、乙胺嘧啶(pyrimethamine, P)合用能明显延缓伯氏疟原虫对 PND 产生抗性[1]，对小鼠的急性毒性呈相加作用(湛崇清等，个人通讯)。3 药合并在抗氯喹恶性疟流行地区治疗恶性疟患者，较单用 PND 减少1/2以上的用量又明显降低了复燃率[2,3]，且疗效优于 SP[4]。鉴于目前恶性疟原虫抗药性问题仍较严重，因此进一步开展 3 药合并应用的研究及研制复方片，无论对延缓抗药性还是对提高治愈率均有实际意义。本文目的是进一步了解 3 药合并的作用特征以及对 PND 的持效作用和 P 的抑制配子体及其孢子增殖作用的影响，从而为扩大临床研究和研制复方片提供基本的实验资料。

MATERIALS AND METHODS

伯氏疟原虫 ANKA 株(*Plasmodium berghei* ANKA strain)，1981 年从英国伦敦卫生与热带医学院医学原虫学系引进，1985 年转引自第二军医大学，按常规在实验室经交替蚊传与血传保种。小鼠为本所动物室繁殖的昆明杂交品系，体重 20±2 g，♀♂各半，给药前禁食 12 h。

斯氏按蚊 (*Anopheles stephensi*, Hor strain), 1973 年从英国引进，由本所媒介生物与防治研究室保种并提供。实验选用羽化后 3–5 d 的成蚊，饲养于 21±1℃ 相对湿度80±1% 的室内，每天喂饲 5–10% 葡萄糖水，光照时间 12±2 h，实验前禁食 12 h。

咯萘啶(PND)由杭州第一制药厂生产，为磷酸盐，其基质含量为 57%，临用前用蒸馏水配制。磺胺多辛(S)由上海第二制药厂生产，乙胺嘧啶(P)由上海中西药厂生产，均用 1% 西黄蓍胶配成混悬液。SP 是由 S 和 P 按 2:1 的比例在实验前 24 h 内混合而成。所有药物均按基质含量计算，1 次 ig 给药，对照组不予任何处理。

药物合并应用的 ED_{50} 测定和合并作用特征的评价方法 于 d_0 每鼠 ip 感染疟原虫的 rbc 5×10^6个，d_{2-5} 按体重分组，每组 10 只小鼠，♀♂各半。以各药单用时 d_6 测得的 ED_{50} 为最大初始剂量，依次设 5 个剂量组，将两药的 5 个剂量分别两两配伍，于 d_3 同时 1 次 ig 药物。药前取尾血制成薄血膜，甲醇固定，Giemsa 染色，计算 rbc 感染率(EIR)，以 2.5×10^4 个红细胞未发现原虫为阴性，d_0 以同法取血染色镜检，计算给药后各剂量组小鼠的原虫血症转阴率，用 Finney 法求 ED_{50}，并用等效应图解法[5]分析药物合并作用的特征。

药物合并时的持效作用的观察 小鼠每组 10 只，于接种前 3，2 d (d_{-3}, d_{-2})分别分组 1 次 ig 药物，d_0 接种小鼠，方法同前。d_3, d_6, d_8, d_{10} 按前述方法取血镜检，计算 EIR，分别求各组小鼠的原虫血症阳性率。

药物合并影响配子体及其孢子增殖的观察 小鼠 d_0 接种，d_{3-5} 血检(方法均同前)，选出配子体密度>$5/10^4$ rbc 的小鼠，每组 3 只，d_4 分组 1 次 ig 药物，ig 前后对各供血鼠血检，在光镜下观察配子体形态、数量，计算 EIR 和配子体率。给药后 2 h 将各组小鼠分别感染一笼斯氏按蚊(♀蚊约 300 只)，蚊吸血 45 min 后将吸血蚊分笼饲养。血餐后 11–12 d 解剖蚊胃，计算卵囊阳性率；17–18 d 解剖唾液腺，计算子孢子阳性率。

RESULTS

PND 合并 SP 对感染伯氏疟原虫 ANKA 株小鼠的疗效 结果见 Tab 1。药物配伍组小鼠的原虫血症转阴率普遍高于同样剂量的单用药组，接近 100% 转阴剂量时，这种差异更为明显。由此求得的药物配伍组的 ED_{50} 亦普遍

Tab 1. Suppressive effects (parasitemia-negative rate, %) of drug combination for a single oarl dose against *Plasmodium berghei* ANKA strain in mice 72 h after ig dosing. $n=10$.

Pyronaridine (mg/kg)	Sulfadoxine/Pyrimethamine (S:P=2:1) (mg/kg)										ED_{50}(mg/kg) (95% confidence limits)
	0	0.11	0.14	0.20	0.25	0.34	0.45	0.60	0.80	1.07	
0	0					0	10	20	40	80	0.82(0.67–1.00)
0.71							0				
0.95				0	0	0	20	70			0.54(0.47–0.62)
1.27			10	0	0	10	50				0.43(0.34–0.56)
1.69	10		10	20	20	30	44.4				0.55(0.23–1.34)
2.25	20		30	50	40	50	90				0.24(0.16–0.35)
3.00	60	80	60	70	55.6	70					——
4.00	80		70								
5.00	90										
ED_{50}(mg/kg) (95% confidence limits)	2.85 (2.46–3.30)		2.91 (2.28–3.68)	2.36 (1.96–2.86)	2.66 (2.01–3.52)	2.27 (1.83–2.86)	1.44 (1.20–1.73)				

低于单用药组。作等效应图，可见配伍组的等效应点多分布在等效线的95%可信区间内(Fig 1)，PND与SP的合并作用特征为相加作用。

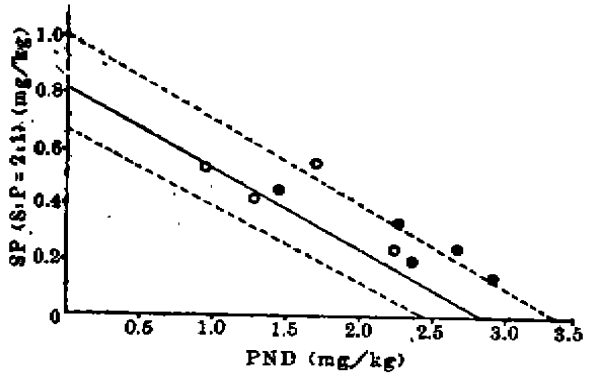

Fig 1. Isobologram showing additive action of pyronaridine (PND) and sulfadoxine/pyrimethamine (SP) against *P berghei* ANKA strain in mice. (○) ED_{50} of SP in combination with various doses of PND. (●) ED_{50} of PND in combination with various doses of SP.

PND, SP或3药合并时的持效作用 以3倍于ED_{50} (3.24 mg/kg)剂量的PND 10 mg/kg于d_{-3} ig小鼠，有部分小鼠接种原虫后不出现原虫血症。PND 10 mg/kg合并SP 3 mg/kg对小鼠亦有保护作用，且原虫血症阳性率稍低于单用PND组(Fig 2)，但无显著性差异($P>0.05$)。以4倍于ED_{50} (0.74 mg/kg)的SP 3 mg/kg ig小鼠，对小鼠无保护作用，d_3血检原虫血症全部阳性，与对照组一致。小鼠于d_{-2} ig给药的结果与上述结果相仿。

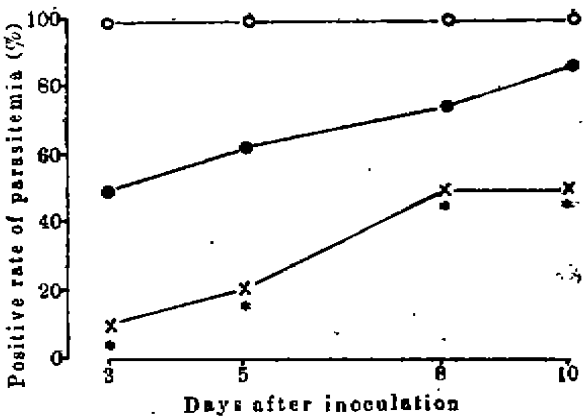

Fig 2. Residual blood schizontocidal action of 2:1 mixture of sulfadoxine and pyrimethamine (SP) 3 mg/kg (○), PND 10 mg/kg alonee (●) and PND plus SP (×). Drugs were dosed ig 3 d before inoculation of *P berghei* ANKA strain in mice. $n=10$. $^*P>0.05$ *vs* PND.

PND, P和SP单用或合并时对配子体及其在蚊内发育的影响 由Tab 2可见，无论SP还是SP与PND合并，均能抑制配子体在蚊体内的发育，卵囊阳性率和子孢子阳性率均明显低于对照组($P<0.05$)，与单用P组相仿($P>0.05$)，SP组与PND+SP组之间亦未见有显著差异($P>0.05$)。小鼠于ig前后血检观察配子体形态、数量，未见明显变化。

DISCUSSION

在本实验所选用的剂量范围及实验条件下，PND与SP合并对鼠疟原虫红内期的疗效

Tab 2. Effects of a single oral dose of pyrimethamine used alone or in combination with sulfadoxine and pyronaridine on sporogony of *P berghei* ANKA strain in *Anophele stephensi*. $^*P>0.05$, $^{**}P<0.05$.

Drug (ig)	Dose (mg/kg)	Gametocytes before dosing (♀:♂/10^4 rbc)	Gut positive (%) (Positive/dissected mosquitoes)	Gland positive (%) (Positive/dissected mosquitoes)
Control	0	9.0 : 8.0	95 (18/19)	45 (9/20)
Pyrimethamine (P)	0.1	4.0 : 6.7	50 (10/20)**	25 (5/20)**
Sulfadoxine (S)	0.2	3.7 : 6.7	80 (16/20)*	50 (10/20)*
SP (S:P = 2:1)	0.3	1.4 : 3.7	54 (12/22)**	10 (2/20)**
Pyronaridine + SP	0.5 + 0.3	9.0 : 10.0	50 (11/22)**	25 (5/20)**

为相加作用，PND 的持效作用和 P 对配子体及其孢子增殖的抑制作用均未因合并用药受到影响，因而支持在已有实验的基础上进一步开展 3 药合并应用的研究以及进一步对 3 药复方片研制的可行性加以探讨。国外新近研制的复方片 Fansimef 由甲氟喹，S 和 P 3 药组成，该 3 药合并虽亦为相加作用，但因 Fansimef 能延缓鼠疟原虫对甲氟喹产生抗性，故已广泛用于疟疾治疗。世界卫生组织根据目前的疟疾形势及专家学者的建议也多次倡导联合使用抗疟药，因此，PND/S/P 3 药合并应用值得研究。

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阿苯达唑及其代谢物在人体内的药物动力学

关天颖、宫相义、孙伟之、刘淑珍、郑连舫[1]、李明珪[1]
(吉林农业大学药理教研室，[1]囊虫病研究所，长春 130118，中国)

Pharmacokinetics of albendazole and its metabolites in human body

GUAN Tian-Ying, GONG Xiang-Yi, SUN Wei-Zhi, LIU Shu-Zhen, ZHENG Lian-Fang[1], LI Ming-Gui[1] (*Division of Pharmacology and [1]Institute of Cysticercosis, Jilin Agricultural University, Changchun 130118, China*)

ABSTRACT High-performance liquid chromatography method was used to determine blood sulfoxide and sulfone, two metabolites of albendazole. Methanol containing 0.4% acetic acid was employed as mobile phase. Wavelength of 290 nm was chosen for uv-detector and mebendazole was used as internal standard. After a single dosage of albendazole 25 mg/kg was taken orally by healthy volunteers and neurocysticercosis patients, sulfoxide and sulfone were found in blood at 20 and 40 min respectively. The blood peak level appeared at 6 h on an average. The metabolites remained as long as 58-72 h in humanblood.

KEY WORDS high pressure liquid chromatography; carbamates; albendazole; sulfoxides; sulfones; pharmacokinetics; cysticercosis

摘要 用反相高效液相色谱法测定血中阿苯达唑的代谢物阿苯达唑亚砜和砜。以含 0.4% 乙酸的甲醇为流动相，uv 检测器波长为 290 nm，以甲苯咪唑为内标物，脑囊虫患者同健康自愿者单剂量 po 阿苯达唑 25 mg/kg 后，20 min 血中出现阿苯达唑亚砜，40 min 血中出现阿苯达唑砜，平均 6 h 达峰，在人血内可维持 58-72 h。

关键词 高压液相色谱法；氨甲酸酯类；阿苯达唑；亚砜类；砜类；药物动力学；囊尾蚴病

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阿苯达唑(albendazole，丙硫苯咪唑)为一