

# 利用实验动物模型研究中草药 内用治疗银屑病的作用

大连医学院皮肤病学教研室 黄 政 林熙然

**内容提要** 作者利用小鼠阴道上皮和鼠尾鳞片表皮两个实验动物模型,研究了三种临床上报告内用治疗银屑病有效的中草药有效成份的作用机理,见到喜树碱能显著地抑制上皮细胞有丝分裂并促进表皮颗粒层形成,肿节风浸膏主要是促进表皮颗粒层形成,而苦参素则只有抑制上皮细胞有丝分裂的作用。利用这两个实验模型对喜树碱、肿节风浸膏和苦参素的疗效及毒性,以氮甲喋呤为对照进行评价,结果与临床观察大致相符。

近年来我国皮肤科工作者在应用中草药治疗银屑病方面做了大量工作,报告了许多在临床上见效的方剂和药物。为了进一步探讨有效中草药治疗银屑病的作用机理,并发现治疗银屑病的有效新药,这些都需要借助实验动物模型。

迄今为止,银屑病还没有可以实际应用的动物疾病模型,但已有若干种可用于药物研究的实验动物模型,这些模型模拟了银屑病的主要病理生理特点。Bonder和Van Scott所提出的小鼠阴道上皮模型<sup>(1,2)</sup>与Jarrett和Spearman所提出的鼠尾鳞片表皮模型<sup>(3)</sup>即是这些模型中的两种。银屑病表皮的病理生理主要特点包括增生过快和分化不全两个方面。雌激素周期的小鼠阴道上皮增生活跃,有丝分裂增多,细胞转换加快,模拟了银屑病表皮增生过快的特点。而鼠尾鳞片表皮的角化缺少颗粒层形成的过程,模拟了银屑病表皮角化不全的特点。我们设想将前述两种模型结合起来应用,可能更接近于银屑病的实际条件。

为了探讨实验动物模型在研究中草药内用治疗银屑病方面的应用,我们首先观察了三种临床报告有效的中草药的有效成份和氮甲喋呤腹腔注射对小鼠阴道上皮增殖和鼠尾鳞片表皮角化的影响。

## 材料与方法

### 一、实验材料

1. 动物:本院动物室提供的小白鼠,体重约20~30g,小鼠阴道实验用雌性小白鼠。鼠尾鳞片模型,使用小白鼠性别不拘。

2. 药物及剂量:(1)喜树碱(南京第三制药厂)12.5mg/kg体重;(2)苦参素(贵阳制药厂)100mg/

kg体重;(3)肿节风浸膏(江西贵溪制药厂)100mg/kg体重;(4)氮甲喋呤1mg/kg体重,作为阳性对照。一般实验用药剂量为临床用量的5~10倍。各种药物都用生理盐水稀释成一定浓度,使腹腔注射0.1ml/10g体重时,恰好符合前述要求之实验剂量。此外还设生理盐水对照组,每次按0.1ml/10g体重腹腔注射生理盐水,作为阴性对照。

### 二、实验方法

1. 小鼠阴道模型实验:先给小鼠腹腔注射乙烯雌酚,每次0.2mg,每日一次,连续注射三天,使所有小鼠处于雌激素期。第4天起分组腹腔注射前述药物,每日一次,连续三天。为了避免细胞有丝分裂的日夜节律对数据的影响,最后一次药物注射统一规定在上午8时,上午9时腹腔注射秋水仙碱2mg/kg体重,秋水仙碱使纺锤体无法形成,使有丝分裂停止于M期的中期而便于计数。下午2时杀死小鼠,取阴道标本以10%福尔马林固定,石蜡包埋,HE染色,用光学显微镜观察组织学所见,确定其为雌激素期阴道上皮,然后观察有丝分裂,计数300个基底细胞中的有丝分裂数,折算出每100个基底细胞的有丝分裂数,称为有丝分裂指数。将实验组和对照组的有丝分裂指数作比较,进行统计学处理。

2. 鼠尾鳞片模型实验:按规定剂量分组给小鼠腹腔注射药物,隔日一次,连注8次。然后杀死小鼠,取尾部距尾根约2cm处背面皮肤一长条,用10%福尔马林固定,石蜡包埋,HE染色。在光学显微镜下对每个标本逐个观察鳞片的表皮有无颗粒层形成,凡是一个鳞片有连接成行的颗粒层细胞者,称为有颗粒层的鳞片。计数每100个鳞片中有颗粒层的鳞片数,进行比较,并作统计学处理。

## 实验结果

实验结果见附表。

附表 腹腔注射苦参素、肿节风浸膏、喜树碱及氮甲喋呤对小鼠阴道上皮有丝分裂及鼠尾鳞片颗粒层形成的影响

组 别	小鼠阴道上皮有丝分裂指数			鼠尾每 100 个鳞片颗粒层形成数		
	动物数	M $\pm$ SD	P 值	动物数	M $\pm$ SD	P 值
生理盐水	31	26.43 $\pm$ 3.96		8	6.68 $\pm$ 4.96	
苦参素	11	11.03 $\pm$ 2.41	< 0.01	8	6.59 $\pm$ 2.71	> 0.5
肿节风浸膏	13	14.43 $\pm$ 5.22	< 0.01	9	14.02 $\pm$ 4.42	< 0.1 > 0.05
喜树碱	15	7.5 $\pm$ 3.93	< 0.01	7	13.83 $\pm$ 8.70	< 0.1 > 0.05
氮甲喋呤	13	8.21 $\pm$ 3.92	< 0.01	8	18.23 $\pm$ 6.47	< 0.02 > 0.01

## 讨 论

本实验所用的三种中草药有效成份中,喜树碱<sup>(4)</sup>和肿节风浸膏<sup>(5)</sup>都有报告用于银屑病的内用治疗。苦参素为氧化苦参碱和氧化槐果碱的混合物,是中药广豆根(*Sophora subprostrata* Chun et T. Chen)的主要有效成份,而广豆根内用治疗银屑病已有报告<sup>(6,7)</sup>。

药物如能抑制小鼠阴道上皮的有丝分裂,说明其亦可能抑制银屑病表皮的增生,因此可能具有治疗作用。但是对细胞有丝分裂的抑制,也可能成为对细胞的毒性作用,因而也可能说明药物的毒性作用。药物如能促进鼠尾鳞片表皮生成颗粒层,说明其可能改变表皮的角化过程,从角化不全恢复到正常角化,因此可能对银屑病具有治疗作用。而与毒性作用无关。

从实验结果看,氮甲喋呤、喜树碱、苦参素和肿节风浸膏都有显著的抑制细胞有丝分裂的作用,其中以氮甲喋呤和喜树碱的抑制作用最强,苦参素次之,肿节风浸膏最弱。因此推测氮甲喋呤与喜树碱可能对银屑病表皮的增生有较强的抑制作用,但亦可能有较大的毒性。从这些药物对鼠尾鳞片的颗粒层形成作用来看,氮甲喋呤具有显著的颗粒层形成作用,喜树碱和肿节风浸膏仅有可疑的作用,而苦参素则毫无颗粒层形成作用。因此综合两个模型的实验结果来看,氮甲喋呤应对银屑病有较强的治疗作用,而喜树碱则次之,肿节风浸膏和苦参素又次之。同时,氮甲喋呤和喜树碱应有较大毒性作用,苦参素的治疗作用应与毒性作用接近,而肿节风浸膏的毒性最小。

从临床观察来看<sup>(4~8)</sup>,氮甲喋呤和喜树碱都有明显毒性作用,但亦有明显治疗作用,而氮甲喋呤的疗效最佳。肿节风浸膏和广豆根对银屑病的治疗作用

都较差,但肿节风浸膏的毒性作用很小,广豆根的副作用虽不及氮甲喋呤和喜树碱大,但亦较为明显,且其治疗剂量与毒性剂量甚为接近<sup>(6)</sup>。

比较四种药物的动物实验和临床观察结果,大体是相符的。因此,将小鼠阴道和鼠尾鳞片两种实验动物模型结合起来利用,可以在一定程度上说明药物对银屑病的治疗作用,作用机理和毒性反应,该方法可能是研究内用治疗银屑病的中草药的一种可行手段和途径。

## 参 考 文 献

1. Bonder RH, Van scott EJ. Use of mouse vaginal and rectal epithelium to screen antimitotic effect of systemically administered drugs. *Cancer Res.* 1971; 31: 851.
2. Van Scott EJ, Bonder RH. Intravaginal and intrarectal screening of antimitotic drugs for topical effectiveness. *J Invest Dermatol.* 1971; 56:132.
3. Jarrett A. Comparative and experimental keratinization, in: Jarrett A eds, *The physiology and pathophysiology of the skin.* London: Academic Press 1973:123.
4. 四川省皮肤病防治研究所牛皮癣防治研究小组. 喜树碱治疗银屑病 33 例疗效观察报告. *四川中草药通讯* 1973; 2:7.
5. 谢品辉. “肿节风”治疗银屑病 30 例初步小结. *皮肤病防治研究通讯* 1977; 6(4):196.
6. 南京地区皮肤科协作组. 中药菝葜、山豆根、丹参治疗银屑病 65 例小结. *皮肤病防治研究通讯* 1975; 4(1):42.
7. 遵义医学院附院皮肤科中草药室. 广豆根治疗银屑病 58 例观察报告. *皮肤病防治研究通讯* 1979; 8(1):31.
8. 第三军医大学第二附属医院皮肤科. 七十年代银屑病的治疗. *重庆医药* 1976; 5:112.

### Study on the Biological Active Ingredients of *Astragalus Membranaceus*

Hou Yunde (侯云德)\*, Song Daijun (宋代军)\*\*, Fu Fongyong (傅丰永)\*\*\*, Liu Bingyang (刘秉阳)\*\*\*\*, et al

\*Institute of Virology, Beijing \*\*Shanghai Institute of Hypertension, Shanghai

\*\*\* Institute of Pharmacology, Beijing \*\*\*\* Institute of Epidemiology, Beijing

Antibacterial, antiviral and immunoregulatory activities of ten fractions extracted from *Astragalus membranaceus* (Am) were compared in vitro and in vivo. Results obtained indicate that the multiple biological activities of Am are not due to one and the same fraction but different fractions. Am-2 containing polysaccharides is shown to be able to stimulate 19S antibody response; Am-4 and 8 containing aminoacids and alkaloids possess antiviral, antibody-stimulating actions and can prolong cell life-span in vitro; Am-6 containing flavonoids has antiviral and antibacterial actions; Am-7 containing glucosides can prolong cell life-span in vitro, stimulate humoral immune response and inhibit virus multiplication in vitro

(Original article on page 420)

### A Study of Pharmacodynamic Effects of Calculus Felleus Humaneus

Zhao Shizheng (赵士正)\*, Xue Chengyi (薛澄一), et al

\*Research Group of Pharmacology, Staff Medical College of Wuxi

Pharmacology Laboratory, No 2 Peoples's Hospital of Wuxi

Based on Li Shizhen's description of the bile as a drug in his "Compendium of Materia Medica", we conducted experiments to study the pharmacodynamic effects of human gallstone (Calculus Felleus Humaneus, CFH) obtained in the operation room, Calculus Bovis from Australia and factitious Calculus Bovis on the nerve centres of mice, including: (1) effects on the active movements of mice; (2) hypnogenic synergism with pentobarbital; (3) anticonvulsive effect against pentylenetetrazol; (4) anticonvulsive effect against electricity; (5) effect on vaccinum typho-paratyphosum fever; (6) analgesic effect (by van der Wende's experiment and Woolfe-Macdonald's experiment).

As a result of the experiments it was found that with intraperitoneal administration of CFH (400mg/kg) the spontaneous activity of mice could be obviously restrained ( $P < 0.01$ ), the period of hypnotism caused by pentobarbital (20mg/kg) could be prolonged ( $P < 0.05$ ), the spasmodic convulsion caused by pentylenetetrazol (50mg/kg) could be counteracted ( $P < 0.01$ ) and twist reflex caused by potassium antimony tartrate (0.02%, 0.2ml/10g) could also be abolished ( $P < 0.01$ ), but the time of Woolfe-Macdonald's reaction could not be lengthened. Oral administration of CFH (60mg/kg) for five days can effectively prevent electric convulsion. With a dose of 100mg/kg, it can lower the high body temperature of rabbits induced by vaccinum typho-paratyphosum (0.8ml/kg). These results showed that CFH had sedative, anticonvulsive, antifebrile and analgesic effects on the animal nerve centres. It had some pharmacodynamic effects similar to those of Calculus Bovis and factitious Calculus Bovis. It may have some practical value in therapeutics in the future.

(Original article on page 425)

### Animal Models Used to Study the Therapeutic Effect and Mechanism of Chinese Herbal Drugs in the Treatment of Psoriasis

Huang Tian (黄 旼), Lin Xiran (林熙然)

Department of Dermatology, Dalian Medical College, Dalian

Using mouse vaginal epithelium and mouse tail-scale epidermis as experimental animal models, the authors studied the therapeutic mechanism of 3 kinds of Chinese herbal drugs reported to be clinically effective in systemic therapy of psoriasis. It was observed that intraperitoneal injection of camptothecine significantly reduced the mitosis of mouse vaginal epithelium and promoted the formation of a granular layer in the mouse tail-scale epidermis, suggesting that the therapeutic mechanism of the drugs may be on the one hand suppressing-hyperplasia of the psoriatic epidermis, and, on the other, turning parakeratosis into orthokeratinization. The main effect of Sarcandra glaber N. extract is to promote the formation of a granular layer. Oxymatrine-oxysophocarpine mixture can only reduce the mitosis of epithelial cells. Methotrexate, used as a positive control, reduces the mitosis of epithelial cells in a degree similar to camptothecine, but its promotive effect on the formation of a granular layer is stronger than camptothecine.

Considering that decrease of mitosis not only accounts for the therapeutic effect of a drug, but also represents its toxicity, an experimental assessment on the therapeutic and toxic effects of the above-mentioned drugs can be made as the following: (1) methotrexate possesses a significant therapeutic effect, while its toxicity is obvious; (2) camptothecine is a little less effective than methotrexate, but it still shows a significant toxicity; (3) Sarcandra glaber N. extract is less effective than camptothecine, but it shows no obvious toxicity; (4) Oxymatrine-oxysophocarpine mixture is also less effective than camptothecine, and its therapeutic effect may be associated with its toxicity. This experimental assessment appears to be considerably correspondent with the reported clinical observations.

(Original article on page 428)