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【论著】

In Vitro Effect of Cyclosporine A on Juvenile *Schistosoma mansoni* Labeled by AF18 (5-N-octadecanoyl aminofluorescin)

LIU Jian-fa[1], John R Kusel[2]

【Abstract】 **Objective** To explore the *in vitro* effect of cyclosporine A on the tegument of juvenile *Schistosoma mansoni* labeled by AF18 and investigate the effect of cyclosporine A on schistosomula surface membrane fluidity. **Methods** Preparation of transformed schistosomula, adding cyclosporine A into tubes containing schistosomula and labeling of transformed schistosomula with AF18, then observe schistosomula under fluorescence microscope. **Results** Schistosomula of different groups labeled by AF 18 were damaged by cyclosporine A *in vitro*. **Conclusion** Cyclosporine A increases the uptake of AF18 by schistsomula *in vitro* which is dose-dependent, and decreases the parasite surface membrane fluidity.

【Key words】 *Schistosoma mansoni*; Cyclosporine A; Schistosomula; AF18

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环孢素 A 体外抗 AF18 标记的曼氏血吸虫童虫的研究

柳建发, **John R Kusel**

(宁波大学医学院,宁波 315211)

【摘要】 **目的** 探讨环孢素 A 体外抗 AF18 标记的曼氏血吸虫童虫的作用以及虫体表膜流动性。 **方法** 制备童虫,环孢素 A 体外作用,AF18 标记,荧光显微镜观察。 **结果** 童虫损伤或死亡,虫体逐渐从绿色变成黄色,虫体表面损伤。 **结论** 环孢素 A 增加童虫 AF18 的含量,减低童虫表膜流动性。

【关键词】 曼氏血吸虫;环孢素 A; 童虫; AF18

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Cyclosporine A (CsA) is a metabolite isolated from the culture broths of the fungal species "*Tricoderma polysporum*, *Cylindrocarpon lucidium*, *Tolypocladium inflatum*" with a molecular weight of 1 203, and suppresses specifically the immune response, it is a neutral, hydrophobic cyclic peptides composed of 11 amino acid residues, it was discovered almost simultaneously that cyclosporine A has remarkable anti-parasitic effect. The anti-schistosomal activity of cyclosporine A was reported by Millcrship J J[1], and its actions against cestodes and other trematodes were also demonstrated by Millership JJ[2], but at the moment, the action mode of CsA against parasites is still unknown. CsA inhibits parasite growth *in vitro* as well as *in vivo*; this activity is almost certainly not an indirect result of immunosuppression. This study is to explore the action mode of CsA against schistosomula of *Schistosoma mansoni in vitro* labeled by AF18.

MATERIALS AND METHODS

1 Preparation of cyclosporine A

CsA was dissolved into 75% ethanol, and divided into concentrations as follows: 1μg/ml of CsA; 5 μg/ml of CsA; 10μg/ml of CsA; 15μg/ml of CsA; 20μg/ml of CsA; 25μg/ml of CsA as control.

AF18, 5-N-octadecanoyl aminofluorescin (Molecular probes Europe BV, PoortGe bonw, the Netherlands) was dissolved in bulk ethanol at a stock solution of 10 mg/ml. A staining solution of 5 μg/ml was used in experiments by diluting the stock in GMEM (1:2 000 dilution, Glasgow Minimum Essential Medium).

2 Incubation with AF18

Schistosomula were transferred to three 15 ml cell culture tubes. One tube containing 1 ml GMEM and 15 mol/l MβCD (Methyl-β-Cyclodextrin). One containing 1% formaldehyde in medium while the control contained only the GMEM. The formaldehyde was used as a positive control to fix the lipids and restrict their movement in the membrane. After this time the AF18 probe was added to both tubes and returned to the incubator for 15 min.

8 μl of a Carbachol (Sigma-Aldrich Co., Poole, UK) stock solution of 10 mg/ml in GMEM was added to 100 μl of schistosomula suspended in GMEM on a microscope slide. It was used to stop the worms' moving *in vitro*.

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1 Medical School of Ningbo University, Ningbo 315211, China 2 Davidson Building, Institute of Biochemistry and Molecular Biology, University of Glasgow, Glasgow G12 8QQ

3 Preparation of schistosomula

Two *Schistosoma mansoni* strains were used in this project. They were the "Glasgow" strain which has been maintained in the Institute of Biochemistry and Molecular Biology, University of Glasgow. The life cycle of the two strains were maintained in laboratory mice and *Biomphalaria glabrata*. The snails were induced to shed cercariae by exposing them to bright light for 2 hrs. The cercariae were transformed to universal tubes and suspended in 5 μl GMEM. The cercariae were mechanically transformed using the Colley and Wikel method[3], by vigorously passaging them through a sterile 21 G blunt ended needle attached to a 10 ml sterile syringe ten times. This was done to break off their tails and transform them into schistosomula. The universal tube with schistosomula and a final volume of 10 ml fresh GMEM was then placed in a 37 ℃ incubator for 2 hrs to allow them to develop their double membrane.

4 Procedures of experiment

Take fresh schistosomula, place them in culture tubes with 10 ml medium, 500 μl penicillin/streptomycin and 250 μl FCS. Take schistosomula from 6 wells into 7 tubes, add CsA into tubes and the groups were established as follows: 1 μg/ml of CsA; 5 μg/ml of CsA; 10 μg/ml of CsA; 15 μg/ml of CsA; 20 μg/ml of CsA; 25 μg/ml of CsA as control, then incubated for 2 hrs. Add AF18 to tubes with 10 μl/ml, wash schistosomula naturally and slightly, and add cabarchal 10 μl/ml into each tube to paralyse the worms, then add one drop of FCS into each tube. Make a square on slide with silicone grease, and coverslip the worms, and observe carefully under fluorescent microscope and take photographs.

RESULTS

When the worms were damaged, the intensity of fluorescence is strong. The figures showed microphotographs of schistosomula of *Schistosoma mansoni* which were incubated with cyclosporine A and AF18 lipid probe *in vitro*. In the control group, the colour is green, the gut was also labeled, no damage was found on the surface membrane of the schistosomula. In the group with 1 μg/ml of CsA, much fluorescences of worms were showed; at 5 μg/ml of CsA group, more fluorescences of worms and worms slightly damaged; at 10 μg/ml of CsA group, schistosomula damaged, the colour was slightly yellow; at 15 μg/ml CsA group, schistosomula seriously damaged, the colour was yellow; at 20 μg/ml of CsA group, most fluorescences of worms damaged, there was very strong yellow colour; at 25 μl/ml of CsA group, schistosomula were dead with strongest fluorescences on the surface membrane (Fig. 1).

DISCUSSION

The cyclosporines are a family of structurally related cyclic endecapeptides composed of predominantly hydrophilic L and D amino acids[4]. CsA partitions into phospholipid bi-layers by virtue of its extremely hydrophilic character. Insertion of CsA into the lymphocyte membrane may increase membrane surface area and fluidity, leading to an upcoupling of electrochemical membrane hyperpolarization, in a manner similar to that of lipid-soluble anesthetics. About the action mode of CsA *in vitro*, it was related with cyclophilins[5].

CsA are mediated primarily by binding to cyclophilins. The resulting CsA-CyP complex inhibits the calcium regulated protein phosphatase activity of calcineurin and down-regulates signal transduction events. Cyclophilin is peptidyl-prolyl cis-trans isomerase. CsA is a potent inhibitor of infection transmitted by the human pathogenic parasites by binding cyclophilin. The enzyme activity of those cyclophilins was inhibited by CsA, the complex inhibits a calcium activated protein phophatase, calcineurin, and may exert toxic to schistosomula *in vitro*[6]. So, we put forward some of hypotheses: ① AF18 has high affinity for the complex of CsA and cyclophilin. ② After CsA action, there was an increase in protein concentration in the surface membrane. This decreases the membrane fluidity. ③ CsA has membrane-rigidifying effect which reduces membrane fluidity of schistosomula. Further study on action mode of CsA against schistosomula is still a great challenge.

There is a strong possibility that the lipid domains in the schistosome outer membrane could posses the same characteristics as lipid rafts in the mammalian cell membrane, therefore the CsA would disrupt the lipid rafts[5]. In this paper we describe the uptake of the strongly fluorescent dye AF18 by the schistosomula *in vitro*[7]. This new fluorescent technique using AF18 to label the schistosomula maybe applicable to other platyhelminths.

Resorufin excretion from adult schistosomes was remarkably perturbed with classical P-gp modulators including calcium channel blockers, calmodulin antagonists and CsA. CsA at 20 μg/ml concentration seriously damaged the surface of schistosomula. Great intrinsic cytotoxicity of CsA was noticed limiting its ability to be used with AF18[8]. From our results, it is speculated that an increased specificity in binding to cyclophilines might result in damage of schistosomula. Probenecid at any concentration used in this study did not damage schistosomula[9].

The more the CsA in incubation with AF18, the more damage and yellow demonstrated by AF18. It is probable that the labeling of schistosomula by AF18 reported here is due to the activity of schistosomes transport protein SMDR2. This means that worms sensitive to CsA and other schistosomicides may appear not only in genetically selected parasites, but maybe induced in parasites by a variety of non-schistomicides interacting with a P-gp homologue of schistosomes. Future work will be needed to establish whether the flame cells and surface membrane fluidity of schistosomula is one of the mechanisms of drug attack in platyhelminths[10]. CsA and praziquantel may be synergistically attack schistosomes *in vivo* and *in vitro*[11], further work should be undertaken. (Color pictures are on Page I)

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【病例报告】

皮肤蝇蛆病一例报告

贺骥, 崔玉宝, 朱玉霞

中图分类号:R757.9 文献标识码:D

患儿,男,9 岁,安徽籍。因下颌部皮下肿块疼痛 2 d 入院。主诉:两个多月前左侧小腿无明显诱因下出现红肿疼痛,此后肿块先后移至左腿内侧、背部、颈部、下颌部,肿块局部表现有红、肿、热、痛,患儿自觉皮肤虫爬感和皮下游走性疼痛、酸痛等,同时伴有轻度发热、头晕、头痛,近3~4 d 肿块颈部明显肿胀。体检:T 37 ℃,P 94 次/min,左小腿肿胀,触及数个硬结。下颌部肿胀处见鲜红色斑块,边缘清晰,质稍硬。实验室检查:Hb 125 g/L, WBC 14 × 10^9/L,中性粒细胞 47%,淋巴细胞 22%,嗜酸粒细胞 31%。某医院以大剂量抗生素处理,1 d 后肿胀稍减。住院 3 d 后,即 11 月 5 日下颌部肿块中央隆起处出现水疱,继而有白色小虫爬出。10% 甲醛固定小虫后观察,虫体长约 8~11 mm,口钩发达,其前端尖细不分叉,后端有一对气门,其上有许多小孔,口钩后方有向后的尖齿 2 个。鉴定为纹皮蝇(*Hypoderma lineatum*)第 I 龄幼虫。虫体爬出后患者症状消失,于 17 日痊愈出院,随访半年无复发。

讨论:蝇类幼虫俗称蛆,其在人体皮下移行或皮内其它部位寄生刺激人体组织或器官,以及其分泌物、排泄物等化学性刺激,均可危害人体。临床症状主要为游走性皮下肿块,有时出现匍行疹、疖肿。对蝇蛆病患者可对症治疗,皮肤肿块上如有小孔,可直接挤出幼虫,也有用白糖或蜂蜜引诱皮下蝇蛆爬出。幼虫排出后,患者症状消失。抗生素的使用对细菌继发感染有效,但对蝇蛆无作用。

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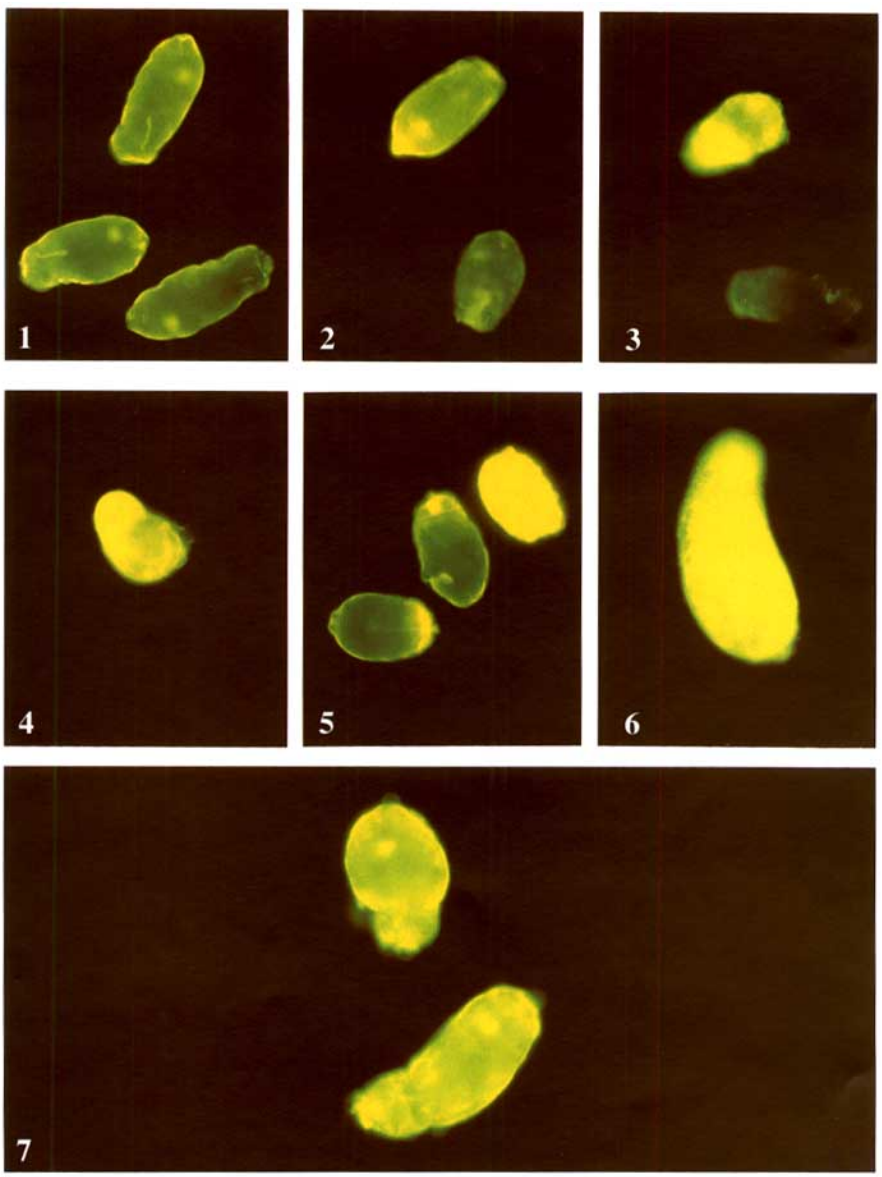
作者单位: 安徽理工大学医学院,淮南 232001

环孢素A体外抗AF18标记的曼氏血吸虫童虫的研究

In vitro effect of cyclosporine A on juvenile *Schistosoma mansoni* labeled by AF18 (5-N-octadecanoy 1 aminofluoresin)

(正文见第328页　For text, see P.328)



1 Control group colour is green,no schistosomula damaged(× 400)　2 At 1 μ g/ml of CsA group, much fluorescences of worms(× 400)　3 At 5 μ g/ml of CsA group, more fluorescences of worms(× 400)　4 At 10 μ g/ml of CsA group, schistosomula damaged(× 400)　5 At 15 μ g/ml of CsA group, schistosomula seriously damaged(× 400)　6 At 20 μ g/ml of CsA group, most fluorescences of worms(× 400)　7 At 25 μ g/ml of CsA group, schistosomula were dead with strongest fluorescences(× 400)

Fig.1　Ultraviolet microscopical observation on the *in vitro* effect of cyclosporine A on juvenile *S.manoni*