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In Vitro Evaluation of Fasciolicidal Effect of Emetine Hydrochloride on Newly Excysted Flukes of Japanese *Fasciola* sp.

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Abstract

Fasciolicidal effect of emetine hydrochloride was examined by *in vitro* cultivation of newly excysted flukes of Japanese *Fasciola* sp.. The 50% lethal dose after 5 days of cultivation ($LD_{50}/_{5\ days}$) was adopted for evaluation of the fasciolicidal effect. The $LD_{50}/_{5\ days}$ of the drug was 8.1 μg/m*l*. The method employed in the present study is considered efficient for screening new possible anthelmintics against *Fasciola* sp..

Introduction

The effect of anthelmintics on the motility and survival of *Fasciola hepatica* has been studied *in vitro* for years[1-4, 8, 9]. In the earlier stage of those studies, the analysis did not take long because the cultivation method of *Fasciola* flukes was not yet fully developed. A successful method for cultivation of newly excysted flukes of *Fasciola hepatica* was reported by SMITH and CLEGG[10]. Subsequently, IBARRA and JENKINS[5] reported on the *in vitro* screen for new fasciolicidal agents using the SMITH and CLEGG cultivation method. We reported previously that newly excysted flukes of Japanese *Fasciola* sp. could be maintained *in vitro* for a longer period of time[6]. In the present study, the fasciolicidal effect of emetine hydrochloride on newly excysted flukes was examined *in vitro* to establish a screening method for new possible anthelmintics against *Fasciola* sp..

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Materials and Methods

Metacercariae of *Fasciola* sp. were obtained by experimental infection of *Lymnaea ollula* with miracidia hatched from eggs collected from the gallbladders of naturally infected cattle. Excystment of metacercariae was performed by a method described previously[7].

Basal medium used for cultivation of newly excysted flukes was RPMI 1640(Nissui Pharmaceutical Co., Tokyo) supplemented with 50% fetal calf serum, 200 units/m*l* of penicillin G, 200 μg/m*l* of streptomycin sulfate and an appropriate amount of 7.5% $NaHCO_3$ for pH adjustment of the medium to approx. 7.4 . Emetine hydrochloride (Nakarai Tesque, Inc., Kyoto) was diluted with the culture medium for test concentrations. Excysted juvenile flukes were maintained for a maximum of 24 hr with the culture medium before use, and transferred to a 70−mm dia. Petri dish, which contained 5 m*l* of the test medium including emetine hydrochloride. Then, the flukes were cultured for 14 days at 37℃ in an atmosphere

Table 1. Fasciolicidal effect of emetine hydrochloride on newly excysted flukes of *Fasciola* sp.

Drug concentration (μg/ml)	No.of flukes tested	Total number of dead flukes on day													$LD_{50}/_{5\ days}$ (μg/ml) [a]
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
20	15	0	2	15	15	15	15	15	15	15	15	15	15	15	15	
10	14	0	0	1	7	9	14	14	14	14	14	14	14	14	14	8.1
5	12	0	0	1	2	2	7	8	10	10	10	10	10	10	10	
2.5	15	0	0	0	0	0	6	10	15	15	15	15	15	15	15	

a) The 50% lethal dose after 5 days of cultivation ($LD_{50}/_{5\ days}$) was calculated by the BEHRENS-KÄRBER formula.

containing 5% CO_2. Replacement of the test medium was not carried out. Death of the flukes was judged by complete movement ceasement microscopically observed.

Results and Discussion

Fasciolicidal effect of emetine hydrochloride was examined by *in vitro* cultivation of newly excysted flukes of *Fasciola* sp. (Table 1). All flukes died within 3 and 6 days at 20 and 10 μg/ml of emetine hydrochloride, respectively. At 5 μg/ml, all flukes survived for 2 days and a few survived for 14 days. At 2.5 μg/ml, all flukes survived for 5 days but died within 8 days.

On the basis of drug concentrations tested and their relations to fasciolicidal effect, the 50% lethal dose after 5 days of cultivation ($LD_{50}/_{5\ days}$) could be adopted tentatively for evaluation of fasciolicidal effect of emetine hydrochloride (Table 1). The $LD_{50}/_{5\ days}$ was 8.1 μg/ml, which was calculated by the BEHRENS-KÄRBER formula.

In vitro screen for new possible anthelmintics may be more efficatious than the *in vivo* test due to the following: accuracy in interpretation of the results, and consequently, more quantitative dose-effect analysis is expected; and it is clear that for which stage of the growth of *Fasciola* flukes a drug is effective. In the present study, the fasciolicidal effect of emetine hydrochloride on the newly excysted flukes of *Fasciola* sp. could be evaluated *in vitro* and expressed quantitatively by the $LD_{50}/_{5\ days}$. Although data are not shown, three commercial fasciolicides were also examined *in vitro*. Values of the $LD_{50}/_{5\ days}$ of the drugs were 114.3, 101.9 and 51.3 μg/ml as expressed by the concentration of the main agents of nitroxynil, bromofenofos and oxyclozanide, respectively. The method employed in the present study could be efficient for screening new possible anthelmintics against *Fasciola* sp..

Effect of anthelmintics on flukes of other stages of growth than the newly excysted flukes was not able to be examined in the present study because the cultivation method of those flukes is still under development. Establishment of the cultivation method for all stages of growth of *Fasciola* sp. will provide a more better *in vitro* screen for new fasciolicides.

In the present study, the $LD_{50}/_{5\ days}$ was used as a parameter of the fasciolicidal effect. The new drug should be previously tested at concentrations of 1, 10, and 100 μg/ml, and retested at a range of several steps of concentration so that a LD_{50} value can be calculated. To obtain a LD_{50} value of a drug

is simultaneously to obtain an effective concentration range (0 －100% lethal dose) , which may lead to an accurate evaluation of the fasciolicidal effect of the drug.

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In vitro における塩酸エメチンの幼肝蛭殺滅効果の判定

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要　約

日本産脱嚢幼肝蛭に対する塩酸エメチンの殺滅効果を人工培養法を用いて検討した。培養5日までの50％虫体致死濃度（LD_{50} / $_{5\ days}$)を指標として殺滅効果を判定した。塩酸エメチンにおけるLD_{50} / $_{5\ days}$値は8.1 μg/mlであった。本法は *in vitro* における肝蛭駆除剤の効果判定法として有用と考えられる。