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CHALONE-LIKE INHIBITION OF DNA SYNTHESIS IN HELMINTHS

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Abstract. Chalone-like inhibitory activity was found in extracts from tissues of *Taenia crassiceps* cysticercus and tissues of adult *Ascaris suum*. The inhibitory effects were partially species non-specific: The extracts from *T. crassiceps* larvae inhibit both *T. crassiceps* and *Mesocestoides corti* larvae DNA synthesis. Extracts from *A. suum*, however, inhibit only *T. crassiceps*. Besides them, the inhibitory factor from mouse brain inhibits both *T. crassiceps* and *M. corti* DNA synthesis. When the inhibitory factor from *A. suum* was applied 4 times during 24-hr culture (at 6-hr intervals), the inhibitory effect was decreased.

The aqueous extract of mammalian tisues contains a factor regulating DNA synthesis by negative feed-back mechanism. The existence of this factor was hypothetically predicted by Shafer (1913) and he proposed to term it "chalone". Recently chalones have been isolated from more than 20 mammalian tissues including cancer tissues (Balázs and Blazsek 1979). They have a lot of characteristical features, the most important being the tissue specificity and the species non-specificity (Attalah et al. 1975, Mathé et al. 1973). The chalone effect appeared to be reversible and non-toxic even in a high concentration. The affected cells, as soon as the inhibitory effect is exhausted, are able to continue the cell cycle (Sassier and Bergeron 1977). The efficient dose of highly purified chalones was estimated at about 1 pg/ml of culture in vitro and 1 μg/kg in vivo (Rytömaa and Toivonen 1979). The mechanism of chalone action and the mechanism mediating the tissue specificity are only little known. Nakai (1967) and Nakai and Gergély (1980) found the inhibitory effect of chalone to DNA polymerase alpha and beta.

An effort to use chalones in practice and above all in the therapy seems to be rather complicated. Insuficient purity of chalone preparations, presence of "anti-chalone" in chalone extracts (Bjerkness and Iversen 1974, Houck et al. 1973) and mechanism of action are the causes of so far little success in the therapy usage.

In animal models the inhibition of graft-versus-host reaction (Kiger et al. 1973), prolonged survival of the skin allografts (Houck 1978) and retardation of malignancy (Rytömaa and Kiviniemi 1969) were accomplished. Rytömaa et al. (1976) first used the commercial preparation "Myelostat" containing the granulocyte chalone for the treatment of acute and chronic myeloid leukemia of man and they achieved an actual regression in 6 cases and a complete cure in 1 case.

The aim of our work presented here was to find if the helminth tissues contain the chalone-like activity.

MATERIAL AND METHODS

DNA synthesis was measured in vitro in larval stages of cestodes *Taenia crassiceps* (strain KBS) and *Mesocestoides corti*.

Chalone preparation. The extracts were prepared by the methods of Kiger et al. (1973) and Kuramitsu et al. (1982) from *T. crassiceps* larvae, adults of *Ascaris suum* and from the mouse brain. All tissues were homogenized in distilled water until the cell-free extract was gained. The homo-

genate was contrifuged at 5000 g for 30 min. The supernatant was precipitated by a slow addition of 95 % ethanol up to a final concentration of ethanol - 42 %. The precipitate was sedimented at 5000 g for 30 min and the supernatant (containing chalones) from *T. crassiceps* was marked T42, from *A. suum* A42 and from the mouse brain B42. The supernatants were divided into two parts and was evaporated from one part under vacuum. The aqueous solution was freeze-dried and kept at —18 °C. The second part of the supernatants was further precipitated by ethanol up to a final concentration of ethanol - 70 %. The precipitate (containing chalones) was sedimented at 10 000 g for 30 min, then redissolved in distilled water and centrifuged again under the same conditions. The insoluble material was removed and the supernatant (containing chalones) was freezedried and stored at —18 °C. The extracts obtained were marked T70 (from *T. crassiceps*), A70 (from *A. suum*) and B70 (from mouse brain).

Larvae culture. The larvae were cultivated in RPMI 1640 culture medium (GIBCO) supplemented with FCS (5 %), L-glutamine (2mM), 2-mercaptoethanol (10^{-5} M), HEPES (25mM), penicillin (100 IU/ml) and streptomycin (100 μg/ml).

Chalone assay. 10 larvae of *T. crassiceps* (about 2 mm in diameter) and/or 20 larvae of *M. corti* were incubated in 1 ml of culture medium at 37 °C. The experimental group was cultivated in the medium containing 1 mg of chalone extracts per ml of medium, and the control group was cultivated in the medium alone. Three types of experiments were performed.

1. The larvae of *T. crassiceps* were cultivated for 24 hr and chalones were added 4 times at 6-hr intervals. ^{3}H-thymidine had been added 5 hr before the cultivation was terminated (1 μCi, i.e. 37 KBq/ml of medium).
2. The larvae of *M. corti* were cultivated for 6 hr. Chalones and ^{3}H-thymidine were added simultaneously at the beginning of cultivation.
3. The larvae of *T. crassiceps* were cultivated for 6 hr. Chalones and ^{3}H-thymidine were added as in exp. 2.

In experiments 1 and 2, the cultivation was terminated by addition of a cold saline. The larvae were washed 3 times by cold saline and then lysed in 1 M KOH at 50 °C. The proteins and DNA labelled by ^{3}H-thymidine were precipitated by addition of 10 % PCA (perchloric acid). The precipitate was sedimented and washed 3 times by 5 % PCA. Thereafter DNA was extracted from the precipitate by 1 ml 5 % PCA at 90 °C for 15 min (Schmidt and Thannhauser 1945). Equal parts (0.4 ml) of DNA extracts were mixed with 5 ml of scintillation liquid SLD 41 (SPOLANA) and the activity was measured in Beckman LS 7000 liquid scintillation counter.

In the experiment of the 3rd type, the cultivation was terminated by harvesting through the Synpor filter. The larvae were washed with 20 ml of cold saline, 20 ml of 5 % PCA and 10 ml of methanol. The filters with larvae were placed in scintillation vials with 5 ml of scintillation liquid SLD 41 and the activity was measured as above.

RESULTS AND DISCUSSION

The results of our work prove the presumption about the presence of chalone-like activity in helminth tissues. It cannot be exactly said whether the factor is a chalone. Only the reversible action as one of the typical features of chalones was observed (see below). For this reason the term "chalone-like" is used.

The significant inhibition of DNA synthesis by means of the extracts of helminth tissues prepared in the same way as chalones of mammalian tissues shows that the regulating mechanism of DNA synthesis in helminths will be similar to that in mammals. Although this effect was predicted 60 years ago (Shafer 1913) and described 20 years ago (Bullough 1962), our knowledge of it is very poor, and surprisingly enough, most studies have been concerned with the mammalian tissues (Balázs and Blazsek 1979).

We prepared for the first time the chalone-like extracts from helminth tissues. As we prepared the extracts of *T. crassiceps* and *A. suum* from the whole bodies, no tissue specificity was observed. On the other hand, a partial species non-specificity was found: the *A. suum* extract did not affect the DNA synthesis of *M. corti* larvae (Table 2), whereas the *T. crassiceps* extract inhibited both *T. crassiceps* and *M. corti* larvae (Tables 1, 2, 3). Besides that we found the inhibitory influence of mouse brain extract on both *T. crassiceps* and *M. corti* larvae. It could mean, with regard

Table 1. Influence of chalones on DNA synthesis of *Taenia crassiceps* larvae during 24 hr culture

Type of chalone	No. of exp.	Incorporation of ^{3}H-thymidine (cpm) [c]	% of inhibition
—	3	4,023.4 ± 29.1	—
A 42	3	3,013.8 ± 36.6	25.1 ($p < 0.01$)[b]
A 70	3	2,543.4 + 13.4	36.8 ($p < 0.01$)
A 70[a]	3	4,345.1 ± 198.0	0
T 42	3	3,034.2 ± 51.1	24.6 ($p < 0.01$)
B 42	2	2,515.9 ± 30.2	34.5 ($p < 0.01$)

a — two times precipitated extract, b — statistically significant differences were determined by F and Student's t-test, c — cpm — counts per minute

Table 2. Influence of chalones on DNA of *Mesocestoides corti* in 6 hr culture

Type of chalone	No. of exp.	Incorporation of ^{3}H-thymidine (cpm)	% of inhibition
—	2	699.8 ± 12.4	—
A 42	2	906.1 ± 3.5	—
A 70	2	773.2 ± 24.1	—
A 70[a]	2	1,139.1 ± 9.8	—
T 42	2	260.7 ± 3.2	62.7 ($p < 0.01$)
B 42	2	354.3 ± 3.3	49.4 ($p < 0.01$)

Table 3. Influence of chalone on DNA synthesis of *Taenia crassiceps* in 6 hr culture

Type of chalone	No. of exp.	Incorporation of ^{3}H-thymidine (cpm)	% of inhibition
—	4	8,298.0 ± 3,029.4	—
A 42	4	2,561.4 ± 1,496.9	69.1 ($p < 0.05$)
A 70	4	2,283.2 ± 1,085.5	72.5 ($p < 0.01$)
T 42	4	4,611.7 ± 2,152.1	44.4 n.s.

n.s. — not significant

to the presence of a nervous tissue in the larval organism, not only the species non-specificity, but also the phyllum non-specificity of some tissue extracts.

The strong inhibition (more than 50 %) of DNA synthesis in cultures lasting 6 hr (Tables 2,3) and the weak (only 20—30 %) inhibition of 24-hr cultures (Table 1) may be caused by the presence of the so-called "anti-chalone" in the extracts or by its synthesis during the chalone action (Houck et al. 1973, Bjerkness and Iversen 1974).

By means of partially purified extracts (A70) a higher inhibition than by A42 has been accomplished. Twice precipitated extract by 70 % ethanol (A70[a]) caused a disappearance of inhibitory activity (Tables 1, 2).

In the experiments of the 3rd type we tried to harvest the cultures by means of the filtration through Synpor filters. The higher variation of data in this case (Table 3) may be caused by quenching of beta radiation of ^{3}H-thymidine in the rela-

tively large amount of *T. crassiceps* body. It seems that this method is not suitable for the extraction of DNA as described by Schmidt and Thannhauser (1945).

Our results presented here and our finding of chalone-like activity in Protozoa (Kudrna and Prokopič 1985) and bacteria, as well as the inhibitory effect of bacterial chalone-like inhibitors exerted on mice lymphocytes (Kudrna and Matha—unpublished results), suggest that the negative feed-back mechanism of DNA synthesis regulation mediated by chalones could be common for procaryotic and eucaryotic cells. In this case the practical usage could be very interesting.

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ЧЕЛОНОПОДОБНАЯ ИНГИБИЦИЯ СИНТЕЗА ДНК В ГЕЛЬМИНТАХ

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Резюме. Челоноподобное ингибирующее действие обнаружено в экстрактах из тканей цистицерка *Taenia crassiceps* и половозрелых экземпляров *Ascaris suum*. Ингибирующее действие оказалось быть частично видонеспецифичным: экстракты из личинок *T. crassiceps* ингибируют синтез ДНК как у *T. crassiceps*, так и у *Mesocestoides corti*. Однако экстракты из *A. suum* ингибируют только *T. crassiceps*. Ингибирующий фактор из мозга мыши ингибирует синтез ДНК как у *T. crassiceps*, так и у *M. corti*. Если ингибирующий фактор из *A. suum* применяли 4 раза в течение суток (в интервалах 6 ч), то ингибирующее действие понизилось.

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