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Cell proliferation kinetics of mouse ascites tumour AISM after treatment with chalone containing tumour extract (CCTE) in vitro

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It has been shown that a chalone containing tumour extract (CCTE) obtained from AISM tumour depressed the proliferative activity of the same tumour at in vitro conditions. CCTE affects both the mitotic index and labelled index by application of 250 μg/ml. The data obtained show that the decrease of proliferative activity is a result of temporary block of tumour cells in G_1 and G_2 phases of the mitosis cycle. The duration of G_1-block is about 2 hours while G_2-block continues 2-7 h.

Key words: chalone, tumour, cell culture, proliferative activity.

Attempts to purify the inhibitors of cell proliferation (chalones) date back to the late 1960s. The identification of mitosis inhibitors of granulocytes [4], epidermal epithelium [3], intestinal epitelium [7] and liver [6] gave no immediate results. Now it becomes known that the mitosis inhibitors are small peptides and they are similar in chemical structure, suggesting a larger family of regulatory peptides [5].

In a previous work [8] it has been established that the proliferative activity of mouse ascites tumour AISM changes rhythmically during twenty four hours. Later we isolated a mitosis inhibitor (chalone) from the same tumour and showed that its activity also changes during a twenty four hour span [1]. The encapsulation of the chalone into liposomes evoked a significant prolongation of inhibitory effect on the tumour cell division [2].

In the work presented here we followed up the effect of chalone containing tumour extract (CCTE) obtained from AISM tumour on the cell proliferation activity of the same during a 24-hours span at in vitro conditions.

Material and methods

The mouse ascites tumour AISM is maintained by i.p. transplantation of 10^6 tumour cells on BALB/c mice every seven days. Explanted tumour cells from seven-days-old AISM tumour were used for preparation of a suspension cell culture. The composition of culture media was: 20% bovine serum (NIEM – Sofia), 56% RPMI 1630 with Hepes (GIBCO), 24% S-MEM Eagle (GIBCO) and antibiotics (Penicilin 10000 E/ml, Streptomycin 100 μg/ml, Nistatin 20 E/ml).

The culture media was distributed in glass vials by 1,5 ml/vial. The initial concentration of tumour cells was 6.10^5/ml. The glass vials were termostated in Elpan water bath shaker setted at T 37° C, speed 120 c.p.m., amplitude 6. Four experimental and four control samples were presented for each experimental point.

Two hours after starting of culture growth 250 μg/ml CCTE was added to the experimental samples, dissolved in advance in 0,5 ml sodium saline. Saline only in the same quantity was added to the control samples. The effect of CCTE on proliferative activity of AISM was followed up after 30 minutes, 1, 2, 4, 6, 8, 10, 20, 22 and 24 hours. Thirty minutes before sampling 1 μCi*/ml Methyl-^{3}H thymidine (Chemapol, sp. ac. 925 Gbq) and 4 μg/ml Colchicin (Fluka) were added to the respective control and experimental samples. The smears were fixed with concentrated Methanol, dyed with Schiff's reagent and covered with ILFORD K2 photosensitive emulsion. The smears were analysed under microscope (oc. 12,5; ob. 100) after development. The mitotic index (MI) was defined after scoring of metaphases on 1000 tumour cells while the labelled index (LI) after scoring of labelled nuclei on 1000 tumour cells. The nuclei with more than five grains were accepted as labelled.

The chalone containing tumour extract (CCTE) was obtained from AISM tumour according method described earlier in detail [2].

All data are statistically processed by using of Student-Fisher test.

Results and discussion

The data concerning effect of CCTE on AISM cell proliferation are presented in Table 1. It is shown that 30 min after treatment with CCTE the MI decreases up to 49% below the control level, one hour later up to 52% and two hours after treatment up to 45% ($P < 0,05$). We observed also a complete recovery of MI to the control level at the 4th and 6th hour after treatment and a rise of the MI up to 75% over the control level 8 hours after treatment. Later it has been established a second decrease of MI up to 32% below the control level which was of short duration and took place 10 hours post treatment. During the rest investigation span no differences between the MI of the control and the experimental groups were established.

The observation of CCTE effect on the DNA-synthesis showed a decrease of the amount of DNA-synthesizing cells up to 36% ($P < 0,05$) four hours after treatment. However, two hours later the LI exceeds the control level by 40% ($P < 0,05$). During the rest investigation span no significant differences between the LI of control group and the LI of experimental group were established.

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* $1 Ci = 3,7.10^{10}$ Bq.

Table 1. Influence of chalone containing tumour extract (CCTE) obtained from mouse ascites tumour – AISM on cell proliferation of the same tumour after single application of 250 μg/ml in vitro

Time of exposition	Control group		Experimental group	
	MI, ‰	LI, ‰	MI, ‰	LI, ‰
30 min	64,7±3,2	316,7± 5,2	33,0±2,6 $P<0,001$	311,7± 4,4 $P>0,05$
1 h	57,7±2,2	313,7±12,3	26,0±1,7 $P<0,001$	330,3± 9,4 $P>0,05$
2 h	55,3±2,0	301,3± 9,7	30,3±0,9 $P<0,001$	308,3± 9,1 $P>0,05$
4 h	52,3±5,0	271,0± 6,7	55,3±2,2 $P>0,05$	172,6±11,3 $P<0,01$
6 h	39,3±1,8	254,3± 6,8	36,3±2,9 $P>0,05$	356,3±26,2 $P<0,05$
8 h	38,3±1,2	215,0± 7,6	67,0±3,0 $P<0,001$	255,3±11,3 $P>0,05$
10 h	34,3±2,7	226,7± 9,3	23,3±1,3 $P=0,05$	231,0± 9,7 $P>0,05$
20 h	43,0±1,2	143,0±11,9	42,3±2,0 $P>0,05$	122,3± 8,1 $P>0,05$
22 h	38,7±1,4	145,0± 3,6	37,7±1,5 $P>0,05$	156,3± 5,5 $P>0,05$
24 h	29,7±1,2	106,0± 7,0	29,3±2,3 $P>0,05$	108,3± 8,8 $P>0,05$

The analysis of the experimental data shows that CCTE acts almost immediately on the tumour proliferation. Most apparently it is demonstrated by the amount of devided cells. A lowering of MI by 49% below the control level is measured 30 min post treatment. The MI remains stable below the control level during the first two hours after treatment with CCTE. We asuume that the tumour cells are delayed in G_2-phase of the mitotic cycle and the minimal duration of G_2-block is two hours. Later the tumour cells gradually get free from G_2-block, enter in mitosis and as a result the MI reaches the control level at the 4th and the 6th hour after treatment. The increase of MI up to 75% over the control level observed at the 8th hour post treatment probably due to a more sinchroniously entering in mitosis of the tumour cell population that has been arrested in G_2-phase to this moment. Thus formed by CCTE the depot of G_2-arrested tumour cells has gradually empty and we supposed that the maximum duration of G_2-block is about 6-7 hours.

As it was mentioned above, a second decrease of MI up to 32% below the control level was established 10 hours post treatment. We tend to propose that it is a result of a short duration decrease of LI that takes place at the 4th hour after CCTE treatment. The LI decrease shows that a part of tumour cell population is arrested in G_1-phase of mitotic cycle due to CCTE influence. Such delay of tumour cells in G_1-phase could possibly result in: first – a decrease of cell amount entering in synthetic phase of mitotic cycle that was demonstrated by the LI decrease four hours after treatment; and second – a later influence on the MI as a consequence of the decrease cell amount passed through S-phase of the mitotic cycle. The blocking of tumour cells in G_1-phase is of short duration (about 2 hours) and complete reversibly as the LI increases up to 40% over control level two hours later.

The obtained results indicate that CCTE blocks temporary and reversibly the tumour cells in G_1- and G_2-phases of the mitotic cycle. The G_1-block has a duration about two hours while G_2-block continues from two to seven hours. The release of arrested tumour cells allows more synchronous entering in S- and M-phases of the mitotic cycle, which is manifested by the increase of LI and MI over the control level respectively.

References

1. Bogoeva, M., B. Botev, M. Krusteva. Availability and dynamics of tissue-specific inhibitor of cell division in ascites form of tumour ISM. – Comp. Rend. Acad. Bulg. Sci., 39, 1986, No 4, 131-134.
2. Bogoeva, M., E. Gabev, B. Botev. The effect of liposome-entrapped specific cell growth inhibitor (chalone) on tumour cell proliferation. – J. Microencapsulation, 5, 1988, No 1, 59-64.
3. Elgjo, K., K. L. Reichelt. Purification and characterization of a mitosis inhibiting epidermal peptide. – Cell. Biol. Int. Rep., 8, 1984, No 5, 379-382.
4. Paukovits, W. R., O. D. Laerum. Isolation and synthesis of a hemoregulatory peptide. – Z. Naturforsch. C., 37, 1982, 1297-1300.
5. Paukovits, W. R., K. Elgjo, O. D. Laerum. Pentapeptide growth inhibitors. – In: Handbook of Experimental Pharmacology. Vol. 95/II. Peptide Growth Factors and Their Receptors. Berlin, Springer Verlag, 1990.
6. Reichelt, K. L., J. E. Paulsen, K. Elgjo. Isolation of a growth and mitosis inhibitory peptide from mouse liver. – Virchows Archiv, B, Cell Pathol., 59, 1990, 137-142.
7. Skraastad, O., T. Fossil, P. D. Edminson, K. L. Reichelt. Purification and characterisation of a mitosis inhibitory tripeptide from mouse interstinal extracts. – Epithelia, 1, 1987, No 2, 107-119.
8. Богоева, М., М. Кристева, К. Цанова. Суточная динамика пролиферации в асцитной форме ISM. – Compt. Rend. Acad. Bulg. Sc., 39, 1986, No 11, 153-155.