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ANNA JAROSZ, MAŁGORZATA SKÓRSKA, JOLANTA RZYMOWSKA *, JANINA KOCHMAŃSKA-RDEST and ELŻBIETA MALARCZYK

EFFECT OF THE EXTRACTS FROM FUNGUS *INONOTUS OBLIQUUS* ON CATALASE LEVEL IN HeLa AND *NOCARDIA* CELLS

*Biochemistry Department and Cell Biology Department * University of M. Curie-Skłodowska, pl. M. Curie-Skłodowskiej 3; 20-033 Lublin, Poland*

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Growth medium of *Inonotus obliquus* exerts antimitotic effect on HeLa cells mostly in M, G_1 and G_2 phases increasing at the same time catalase activity. This effect was not observed in prokaryotic *Nocardia*. Significant antimitotic effect of mycelium was not associated with stimulation of catalase activity in HeLa cells.

Depression of liver catalase is a well known symptom of cancer in animals [1]. Because of the postulated anticancer properties of naturally growing birch arboreal fungi [2] we have examined the effect of *Inonotus obliquus* on growth and catalase activity of HeLa cells. For comparison purposes prokaryotic *Nocardia* was included in the experiments.

MATERIALS AND METHODS

The fungus *Inonotus obliquus* was grown in the Lindeberg medium according to Trojanowski & Leonowicz [3]. After two weeks the medium was separated from mycelium, condensed by evaporation and dialysed on Sephadex G-25 Medium. The column was washed with distilled water and the eluate containing low molecular compounds was extracted with the acidified ethyl ether. Mycelia were crumbled in the blade homogenizer and boiled with double volume of distilled water for half an hour. All the above mentioned preparations were introduced to the cultures of HeLa [4] or *Nocardia autotrophica* DSM 43100 and PCM 2186 [5] in the amount of 1.5 mg% per dry mass of mycelium.

Catalase activity was determined according to Pifferi *et al.* [6] as modified by Nowak [7], the absorbance of the Ti (IV): H_2O_2 complex was measured at 415 nm.

Mitotic index in all phases of growth was calculated following staining with hematoxyline and eosine.

RESULTS AND DISCUSSION

As can be seen from Fig. 1 all preparations from *Inonotus obliquus* decrease mitotic index of HeLa cells. The effect was exerted by both mycelium and

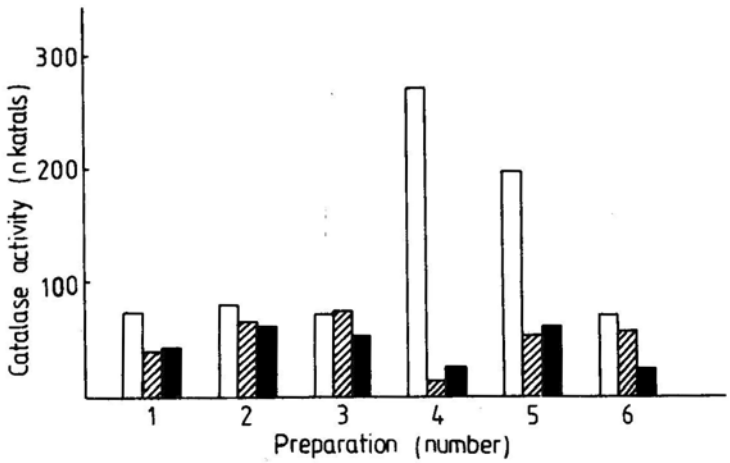

Fig. 1. Changes in the catalase activity in HeLa cells grown in the presence of different preparations from *Inonotus obliquus*. 1, Intact HeLa cells; 2, growth medium condensed five times; 3, dialyzate (high molecular fraction); 4, ether phase of eluate (low molecular fraction); 5, water phase (low molecular fraction); 6, water extract of mycelium. □, 24 h; ▨ 48 h and ■, 72 h

growth medium (Table 1). Low molecular water and ether fractions from Sephadex G-25 chromatography reduced mitotic index stronger than the condensed initial growth medium.

The effect was observed mainly during metaphase. Growth medium of the fungus blocks mitosis in M or G_2 phase, while mycelium resulted in lengthening of M, G_1 and G_2 phases.

Significant impairment of chromosome in metaphase (M) and lysis of some cells were observed; numerous autophagal vacuoles as well as large vacuoles appear on cytoplasm which causes evident impairment of cellular membranes.

These differences in cytological response to the fungal preparations from growth medium are consistent with the results of catalase determinations. It is noteworthy that the compounds of low molecular weight are responsible for the stimulatory effect. Extraction of the water eluate with ether doubled this effect. The nature of the compound(s) is under investigation. Antimitotic effect of mycelium was not associated with any increase of catalase activity.

Table 1

Mitotic index (per cent) of dividing HeLa cells in the cultures growing in the presence of preparations from Inonotus obliquus

For details see Methods

Fungi preparation	Time of growth (h)		
	24	48	72
Intact HeLa cells	34.9	49.1	46.3
Mycelium	10.5	10.4	5.3
Medium	12.0	10.7	10.8
Sephadex G-25			
dialyzate	18.5	18.0	20.9
eluate			
ether extract	9.3	7.1	6.6
water phase	6.8	7.2	7.5

Table 2

Activity of catalase in the cells of two kinds of Nocardia after growth in the mineral medium with the addition of different sources of carbon and mycelium preparations

Numbers in the Table (in nkatals) are the difference between catalase activity in the cells growing with and without addition of the *Inonotus obliquus* mycelium preparations

Number of strain carbon source in the medium	Mycelium preparation		
	medium	dialyzate	mycelium
DSM 43100			
Glucose	21.45	50.0	55.0
Succinic acid	24.75	26.43	49.0
Vanillic acid	36.33	49.05	50.50
Ferulic acid	48.55	49.05	49.05
PCM 2186			
Glucose	40.25	49.56	49.05
Succinic acid	48.25	41.25	55.20
Vanillic acid	46.20	33.24	48.60
Ferulic acid	16.50	50.05	41.25

Preparations from *Inonotus obliquus* had no effect in *Nocardia* (Table 2) indicating specificity of the reaction in eukaryotic cells.

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