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The effect of low temperature on the enzyme activities and the level of SH groups in benign gastric ulcer and gastric carcinoma

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Abstract—Homogenates of human benign gastric ulcers and gastric carcinomas were stored at a low temperature (4°C). The activities of L-lactate dehydrogenase (LDH), malic dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PDH), glutathione reductase (GR) and the concentrations of SH-containing compounds showed changes. Increased activities of LDH, MDH, G6PDH and GR occurred in the gastric carcinomas stored at 4°C compared with controls at 37°C. In contrast, the activities of G6PDH and GR decreased in the tissues of benign gastric ulcers. On incubation at 4°C the SH-containing compounds incorporated into protein molecules, SH groups binding with protein and non-protein SH groups decreased in both the benign and malignant tissues.

Very little is known about the response of the gastric mucosa or the susceptibility of the stomach to carcinogenesis at low temperatures. Malignant tissues represent unrestricted growth not controlled by the growth regulation mechanisms in the organs.

This study examined the effect of prolonged hypothermia, persisting from 1 to 72 h, on the activities of several enzymes and on the SH groups, in benign gastric ulcer and gastric carcinoma. I attempted to discover whether low temperature could be used as a therapeutic agent to control tumour cell growth.

Materials and methods

Postoperation material (benign gastric ulcer and gastric carcinoma) was used. Tissues were obtained from patients who underwent surgery for ulcers and advanced gastric carcinoma at the Surgical Clinic in Lublin. Fragments of the tissues were incubated in air, in Eagle's minimum essential medium (MEM 1959) at 4°C for 1, 5, 10, 24, 48 and 72 h. Control tissues were incubated at 37°C. For determination of the enzyme activities and levels of SH groups, the weighed tissue was cut into small pieces and transferred to cold homogenizing vessels containing homogenization buffer. The homogenates were then centrifuged, and the supernatant was filtered through glass wool to remove surface lipids.

The content of the protein was estimated in homogenate samples according to Bradford's method (Bradford 1976). Samples were also taken for determination of activities of the following enzymes: L-lactate dehydrogenase (LDH), malic dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PDH) and glutathione reductase (GR) (Krawczyński 1972; Szczeklik 1974). The levels of SH groups incorporated into protein molecules (P-SH), SH binding with protein (PB-SH) and non-protein SH (NP-SH) were determined.

The enzyme activities were presented as nkat (units × 16·67) (mg protein)$^{-1}$ and the level of SH groups as nmol (mg protein)$^{-1}$ (Ellman 1959; Truscott & Augusteyn 1977).

All results were analysed using Student's *t*-test.

Results and discussion

Tissue sections of pathological gastric mucosa obtained from stomachs surgically resected, were incubated at 4 and 37°C. The sections incubated at 37°C served as controls.

On the basis of these experiments it was observed that the activities of LDH, MDH, G6PDH and GR in cancer tissues increased at 4°C, and those of MDH, G6PDH and GR in benign gastric ulcer tissues decreased in comparison with control cultures (Table 1).

The level of SH groups, whether incorporated into protein molecules, bound to protein or free, increased in the carcinoma cells (Table 2).

Two tissues were thought to be the sites of lactate metabolism. The possibility that high levels of L-lactate may slowly be reduced by tumour cells should not be ignored. The production and subsequent reoxidation of L-lactate is dependent not only upon the concentration of the substrate and product but also on the reaction properties of the enzyme. LDH is an indicator of the presence of metastatic cancer. It is important to note that a high activity of LDH occurs in a number of cancer types and it may be a helpful parameter in cancer without any other markers (Petrelli et al 1985). Malignant changes are associated in all organs with a considerable increase of the slowest migrating LDH isozymes (Manly et al 1987). The MDH activity was found to decrease in the ulcer cells in relation to carcinoma cells. A significantly higher level of G6PDH activity was found in carcinoma compared with ulcer cells. The results suggest that the determination of G6PDH activity could be a valuable method to distinguish ulcers from tumours. It is possible that intensification of G6PDH activity in cancer is a sign of the shift of the carbohydrate metabolism from the aerobic pathway, or that the activity of pentose is higher in tumour cells because of an increased need for nucleic acid precursors in tissues with faster growth rates. The results which showed a higher level of G6PDH activity in carcinoma than in ulcer cells are in accordance with the reports of other authors (Weber 1977; Bokun et al 1987). It is likely that glutathione reductase acts to protect critical sulph-

Table 1. The effect of low temperature (4°C) on enzyme activities.

Time of incubation (h)	Protein (mg mL^{-1})	LDH	MDH	G6PDH	GR
		(nkat (mg protein)$^{-1}$)			
Ulcer (n = 8)					
Control	0·26	14·40	9·82	1·79	0·21
1	0·25	19·08	4·70**	0·37**	0·20
5	0·26	25·12	3·23**	0·09***	0·17
10	0·26	27·63**	5·00*	0·03***	0·09*
15	0·28	26·80*	9·28	0·10***	0·20
24	0·27	27·05**	6·61	0·30**	0·20
48	0·27	27·55**	6·41*	0·48*	0·20
72	0·26	26·90*	4·57**	0·20**	0·11*
Tumour (n = 15)					
Control	0·26	15·05	4·82	3·40	1·89
1	0·25	16·84	7·15	5·89	5·14*
5	0·26	18·23	10·04*	9·15*	7·43***
10	0·27	18·25	7·66*	10·11*	5·40*
15	0·27	20·88	9·71*	8·91*	6·76**
24	0·26	23·36*	7·39*	11·56**	6·08**
48	0·26	22·59*	7·80*	10·50*	2·29
72	0·26	23·56*	5·47	10·00*	7·51***

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ compared with control values.

Table 2. The effect of low temperature (4°C) on the level of SH groups.

Time of incubation (h)	P-SH	PB-SH	NP-SH
	(nmol (mg protein)$^{-1}$)		
Ulcer (n = 8)			
Control	0·068	0·18	0·28
1	0·054*	0·13*	0·21
5	0·059*	0·18	0·18
10	0·057*	0·17	0·19
15	0·055*	0·20	0·29
24	0·056*	0·20	0·28
48	0·060	0·23	0·26
72	0·060	0·22	0·28
Carcinoma (n = 15)			
Control	0·054	0·14	0·13
1	0·054	0·13	0·11
5	0·050	0·13	0·13
10	0·045	0·12	0·12
15	0·055	0·17	0·13
24	0·060	0·15	0·13
48	0·073*	0·17	0·20*
72	0·075*	0·16	0·20*

*$P < 0·05$ compared with control.

hydryl groups from inactivation by low temperature. It has been suggested that the glutathione level in some tumour cells is near the minimum level required for survival, while normal cells have an excess of it. If this is the case, the depletion of glutathione reductase, glutathione and SH groups may sensitize tumour cells to the cytotoxic effect of low temperature without promoting toxicity in normal host cells (Romine & Kessel 1986). Hypothermia stimulates intestinal cell proliferation, but its cocarcinogenic effect is mild (Rainey et al 1987).

It is concluded that a low temperature may depress the synthesis of several enzymes in the tissue of gastric ulcer and induce the synthesis of the same enzymes in the tissue of gastric carcinoma.

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