ACUTE CELL DEATH INDUCED BY INHIBITION OF DNA SYNTHESIS IN VARIOUS PARTS OF THE GASTROINTESTINAL TRACT

O. S. Frankfurt

UDC 612.398.145.1.015.36.014.46:547.497.6]-08:612.32/.33.014.2

Injection of hydroxyurea into mice led after 4 h to degeneration of nearly all DNA-synthesizing cells in the crypts of the small intestine, but only about 10% of these cells in the epithelium of the glandular part of the stomach underwent necrosis under these conditions. Hydroxyurea inhibited DNA synthesis equally in both tissues. The resistance of the gastric cells, as shown by the criterion of acute death, reflected their ability to withstand inhibition of DNA synthesis without degeneration. This property is perhaps preserved in tumors of the stomach and may be one factor determining their resistance to chemotherapy.

KEY WORDS: *stomach; intestine; DNA synthesis; hydroxyurea; cell death.*

Inhibitors of DNA synthesis and certain alkylating compounds produce acute death by karyorrhexis of DNA-synthesizing cells in the epithelium of the crypts of the small intestine and also of certain other renewed tissues (epithelium of the tongue, lymphatic tissue). By contrast, cells synthesizing DNA in tissues with stimulated proliferation (liver, kidney, salivary gland) are virtually completely resistant to the action of alkylating compounds. The suggestion has been made that this points to fundamental differences in the organization of the cells of rapidly renewed tissues and of tissues with stimulated proliferation [3-5].

The object of this investigation was to study the sensitivity of cells in different parts of the gastrointestinal tract of mice to the necrotic action of hydroxyurea, a specific inhibitor of DNA synthesis. Investigations of this type are important because of their bearing on the problem of tissue-specific differences in the response of cells to antitumor preparations.

EXPERIMENTAL METHOD

To detect acute death of cells synthesizing DNA, thymidine-H^3 was injected intraperitoneally into noninbred mice in a dose of 2 μCi/g body weight, followed by hydroxyurea in various doses 30 min later. Various parts of the gastrointestinal tract were fixed 4 h after administration of the hydroxyurea. To determine the effect of hydroxyurea on DNA synthesis its injection into mice was followed after 1 h by injection of thymidine-H^3, and the tissues were fixed 30 min later. The method of preparing autoradiographs was described earlier [2].

Sector of Kinetics of Chemical and Biological Processes, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 8, pp. 33-35, August, 1975. Original article submitted December 16, 1974.

TABLE 1. Acute Cell Death in Epithelium of Mouse Gastrointestinal Tract following Inhibition of DNA Synthesis by Hydroxyurea

Hydroxyurea (in mg/kg)	Degeneration of labeled nuclei (in %)		
	small intestine	glandular part of stomach	large intestine
500	94	12	52,6
1500	98	7,3	—
5000	98	8	73
500—3h —500	100	14,2	—
1500—3h —1500	100	14,8	—

TABLE 2. Corrleation between Acute Cell Death and Degree of Inhibition of DNA Synthesis (in %)

Hydroxyurea (in mg/kg)	Small intestine		Glandular part of stomach	
	degen. of lab. nuclei	inhib. of DNA synth.	degen. of lab. nuclei	inhib. of DNA synth.
500	95	⩾90	10	⩾90
250	90	⩾90	8	⩾90
50	10	46	0	50
10	0	0	0	0

EXPERIMENTAL RESULTS

Nearly all cells synthesizing DNA in the crypts of the small intestine were necrotic 4 h after the injection of hydroxyurea (Table 1). If two injections of hydroxyurea were given at an interval of 3 h no intact labeled cells could be found in the crypts. The effect was virtually maximal after injection of 500 mg/kg of hydroxyurea.

A completely different picture was observed in the epithelium of the glandular part of the stomach. Following administration of hydroxyurea in all doses only about 10% of DNA-synthesizing cells in the epithelium of the glandular part of the stomach were necrotic. Repeated injection of hydroxyurea did not significantly increase the mortality of the cells. Consequently, the epithelium of the glandular part of the stomach was highly resistant to injection of the inhibitor of DNA synthesis as shown by the criterion of acute cell death. A tenfold increase in the dose of hydroxyurea or an increase in the duration of inhibition of DNA synthesis by repeated injection of the inhibitor did not lower the resistance of the cells. Death of labeled cells was not observed 24 h after injection of hydroxyurea.

The sharp difference in the intensity of acute cell death in the small intestine and stomach after injection of the inhibitor of DNA synthesis was unconnected with differences in the degree of inhibition of DNA synthesis. When thymidine-H^3 was injected 1 h after 500 mg/kg of hydroxyurea, no incorporation was found on autoradiographs of the small intestine or glandular part of the stomach. In the control series, the mean number of granules above the labeled cells was 20-30 in both tissues. Consequently, in this dose hydroxyurea inhibited DNA synthesis in both tissues by over 90%. With a decrease in the dose of hydroxyurea the degree of inhibition of DNA synthesis was lowered equally in the small intestine and glandular part of the stomach (Table 2). DNA synthesis was inhibited equally in the cells of both types, but under these circumstances the cells in the small intestine broke down rapidly whereas most cells of the stomach remained intact.

Cells synthesizing DNA in the crypts of the large intestine were sensitive to the necrotic action of hydroxyurea, but less so than the cells of the small intestine (Table 1).

By contrast with other rapidly renewed tissues, the cells of the glandular part of the stomach thus do not degenerate when DNA synthesis is inhibited. The nature of this resistance remains unexplained. All that is known is that this death is connected with protein synthesis, for its inhibition protects the cells against death when DNA synthesis is inhibited.

A definite parallel exists between the effectiveness of preparations against tumors of this type and their effect on the corresponding normal tissue, for primary tumors preserve to a considerable degree the morphological and biochemical specificity of the original tissue [1]. It can accordingly be postulated that the ability of preparations or their combinations to induce acute cell death in the gastric epithelium may reflect their potential value when tested against tumors of the stomach. However, it must be remembered that acute cell death is not the only or the most important type of necrosis resulting from the action of chemotherapeutic preparations. In most cases cells which have lost their reproductive activity remain viable for a long time. The possibility cannot therefore be ruled out that some cells of the glandular part of the stomach, although remaining intact for 24 h after the administration of hydroxyurea, die later. Nevertheless definite correlation exists between acute cell death and the sensitivity of a tissue to chemotherapeutic preparations. For instance, the epithelium of the small intestine, in the crypts of which certain preparations induce acute cell death, suffers severe damage during chemotherapy. On the other hand, the epithelium of the glandular part of the stomach is resistant, as manifested by the criterion of acute cell death, and tumors arising from this epithelium are relatively insensitive to the harmful action of antitumor preparations. The epithelium of the large intestine has intermediate sensitivity, as shown by the criterion of acute cell death, and tumors arising from this epithelium also exhibit intermediate sensitivity to antitumor preparations, compared with tumors of the small intestine and stomach.

The author is grateful to Academician N. M. Émanuél' for his interest in the work and for discussing the results.

LITERATURE CITED

1. L. F. Larionov, The Chemotheraphy of Malignant Tumors [in Russian], Meditsina, Moscow (1962).
2. O. F. Frankfurt, "Proliferation and differentiation of cells in the stratified squamous epithelium of the mouse forestomach (autoradiographic investigation)," Tsitologiya, No. 2, 175 (1967).
3. E. Farber and R. Baserga, "Differential effects of hydroxyurea on survival of proliferating cells in vivo," Cancer Res., 29, 136 (1969).
4. L. Lenaz, S. S. Sternberg, and F. S. Philips, "Cytotoxic effect of 1-βD-arabinofuranosyl-5-fluorocytosine and 1-β-D-arabinofuranosylcytosine in proliferating tissues in mice," Cancer Res., 29, 1790 (1969).
5. M. W. Lieberman, R. S. Verbin, M. Landay, et al., "A probable role for protein synthesis in intestinal epithelial cell damage induced in vivo by cytosine arabinoside, nitrogen mustard, or X-irradiation," Cancer Res., 30, 942 (1970).