ACTION OF VINBLASTINE ON EXPERIMENTAL GASTRIC CARCINOGENESIS

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Neurotropic drugs (atropine, reserpine, caffeine, nicotine, clofelin, adrenoblockers, etc.) have been shown to modify the effect of certain carcinogenic nitrosamines and aromatic amines, causing malignant tumors of the gastrointestinal tract in animals [8-10, 15]. Synergism or antagonism in the action of neurotropic drugs and carcinogens indicate that neurochemical reactions lying at the basis of neuronal and receptor function can be regarded as targets for the primary action of resorptive chemical carcinogens [10]. We know that axoplasmic transport of neurotransmitters plays an important role in the realization of neurotrophic control in innervated structures [6]. It has been shown recently that the well-known *Vinca rosea* alkaloids, vinblastine and vincristine, widely used in the combination treatment of certain forms of cancer, block axoplasmic transport of catecholamines and protein [7, 12]. The final effects of this blockade is inhibition of adrenergic processes, manifested in vivo as acute and delayed complications. To develop measures and methods of correction of activation of the adrenergic component of the autonomic nervous system during the action of carcinogens, there is good reason to study the effect of inhibitors of axoplasmic transport on chemical carcinogenesis.

In the investigation described below the effect of vinblastine on carcinogenesis was studied on a model of malignant tumors of the rat stomach arising after chronic administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

METHODS

Experiments were carried out on 100 noninbred male albino rats. In experiments on 30 rats the maximal tolerated dose (MTD) of vinblastine (Gedeon Richter, Hungary) was determined for chronic administration. This was 0.25 mg/kg body weight once a week by subcutaneous injection. Chronic experiments were carried out on 70 rats, divided into four groups: the animals of group 1 received 0.5 ml of physiological saline subcutaneously one a week, the animals of group 2 received vinblastine subcutaneously in the MTD, and the animals of groups 3 and 4 received MNNG continuously with the drinking water in a concentration of 85 mg/liter for 8 months. Besides the carcinogen, throughout the experiment, which lasted 10 months, the animals of group 3 received physiological saline, whereas those of group 4 received vinblastine in MTD. To assess the time course of changes in the gastric mucosa (GM) the animals in the groups were killed at different times: 1, 5-6, and 10-12 months after the beginning of the experiments. Visually distinguishable regions of pathological changes in GM were mapped on models. The stomachs, cut along the greater curvature, were spread out flat, with the external surface onfirm cardboard, and fixed in 10% formalin. Later, on the basis of these models histological sections were cut from the altered regions of GM and neighboring regions, and stained with hematoxylin and eosin.

RESULTS

GM of the rats of group 1, which forms the age control and were killed at various times, showed no abnormality (Table 1). In GM of the animals of group 2, killed 1 and 5-6 months after the beginning of vinblastine administration, signs of karyoclastic changes, dystrophy, and destruction of the basal portions of the glands followed by encapsulation of their remains by connective tissue, were observed over the whole extent of the antral and pyloric portions of the stomach. Characteristic changes described previously [3, 4, 8, 9, 13] were

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TABLE 1. Results of Investigation of Gastric Mucosa of Rats at Different Stages of the Experiment

Group of rats	Experimental conditions	Total number of animals	Time after beginning of experiment, months					
			1	5–6	10–12			
					total number of animals	number with histological diagnosis of		
						no change	intestinization	focal dysplasia (precancer)	adenocarcinoma
1	Intact control	18	6	6	6	6	—	—	—
2	Vinblastine, per se	12	8	2	2	2	—	—	—
3	MNNG and physiological saline	17	5	2	10	—	—	3	7
4	MNNG and vinblastine	23	4	6	13	—	10	1	2*

Legend. Asterisk indicates difference in frequency of adenocarcinomas from group 3 is significant at the $P < 0.05$ level.

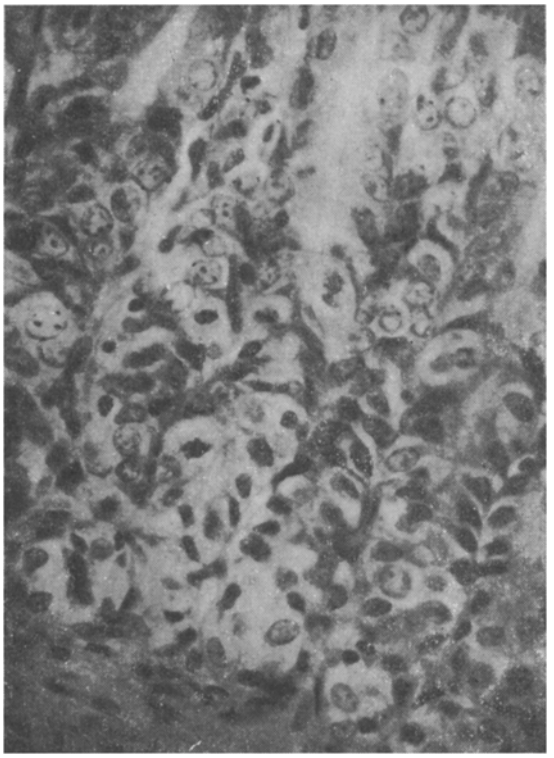

Fig. 1. Dystrophic changes in epithelium of basal portions of crypt-like glandular structures in pyloric part of rat stomach 6 months after beginning of combined administration of MNNG and vinblastine. Hematoxylin and eosin, 520×.

observed in GM of animals receiving MNNG. In the animals of group 3, killed after 5-6 months, manifestations of "intestinization" of the glandular structures were observed. In the animals of group 4 the morphological changes were characterized by a unique combination of "intestinization" and shortening of the crypt-like remnants of the glands, with signs of aplasia of their cambial stem cells and also by the development of strong bands of connective tissue in the basal zones of GM (Fig. 1). In the rats of group 2, killed 1 month after administration of vinblastine ceased (11 months), residual manifestations of the changes observed previously were noted, and normal mitotic activity of the epithelium was restored. In the animals of group 3, marked evidence of dysplasia of proliferating elements of the epithelium of the "transitional" zone of the glands, and also of the small epithelial outgrowths arising from this zone (precancer), predominated in GM. Besides dysplasia, signs of malignant

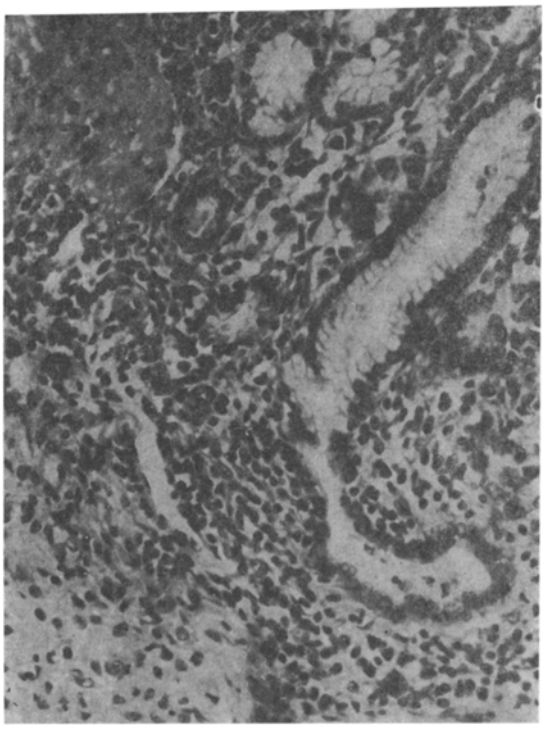

Fig. 2. One part of the wall of the pyloric portion of a rat stomach 1 year after beginning of systematic injections of MNNG.

transformation of the epithelium also were found (Fig. 2). Carcinomas were found in the GM of seven (70%) rats of this group, including submucosal adenocarcinoma in two rats, intramucosal adenocarcinoma in one rat, and both forms of tumors simultaneously in four animals. In the animals of group 4 during this period the signs of karyoclastic effects discovered previously were no longer present. Meanwhile shortening of the crypt-like structures and a distinctive kind of simplification of the connective-tissue basis still remained, surrounding their terminal portions and separating them from the muscular basis of the membrane (Fig. 3). In this group adenocarcinomas were discovered in only two rats (15%). The results of morphologic investigations of GM of the animals of group 3 thus demonstrate that adenocarcinomas were the main type of pathological changes in this case. By contrast, in animals receiving vinblastine, the development of the pathological changes had reached the depth virtually of only the "intestinization" stage. These data, in our view, justify the conclusion that administration of vinblastine inhibits carcinogenesis and reduces the frequency of malignant tumors of the stomach.

The antitumor effect of *Vinca rosea* alkaloids is considered to be based on their ability to "freeze" mitoses of tumor cells at the metaphase stage [12]. However, the chemotherapeutic effect of the alkaloids on tumors of the gastrointestinal tract, when used alone, is extremely weak [11]. It has been shown that vincristine, another *Vinca* alkaloid, induces destructive changes in the epithelium of rat intestinal crypts in the early period of its action and attacks glial cells and neurons of intramural nerve plexuses later [1]. It can be postulated on the basis of these data that the direct action of vinblastine, administered parenterally, on mitotic activity of the gastric epithelium is alternative in the effect of inhibition of tumor growth which we have discovered.

Adrenergic granular synaptic vesicles have been shown to be formed in neuron bodies and are transported along axons to the periphery, where further synthesis of catecholamines and their packing into granules take place. The action of vinblastine, as an inhibitor of axoplasmic transport, is based on blocking of movement of these granules, containing catecholamines and enzyme proteins, along the microtubular structures of the axon [2, 5-7, 14]. In the course of administration of *Vinca* alkaloids, investigators have observed definite changes in the behavior of the experimental animals, which exhibited hypodynamia, a state resembling sleep, and unsociability [1]. These changes, and also the clinical picture of the toxic

Fig. 3. Crypt-like glandular structures with signs of mild dystrophic changes of terminal portions in mucosa of pyloric part of rat stomach 1 year after beginning of combined administration of MNNG and vinblastine. Signs of development of connective tissue are visible in basal portions of mucosa.

action of vinblastine (a reduction in body weight, hypothermia, a tendency toward development of infectious diseases, diarrhea, etc.), are evidence of reduced activity of central adrenergic processes under the influence of these alkaloids. The present writers and others showed previously that any pharmacologic agency, aimed at reducing activity of adrenergic processes, is a factor in anticarcinogenesis [10, 15]. The results of the present investigation, together with data in the literature on morphological changes in the wall of the gastrointestinal tract under the influence of *Vinca* alkaloids [1], and the clinical picture of disturbances of the emotional sphere and autonomic responses in animals against this background, suggest that the ability of vinblastine to block axoplasmic transport of catecholamines is an alternative to its direct action on the epithelium of GM in the mechanism of its inhibitory effect on carcinogenesis. The importance of the second mechanism is increased in the case of chronic administration of *Vinca* alkaloids. The basis of adrenergic influences on innervated tissues and organs in the intact organism is evidently formed by the components of homeostasis that are associated with the regulation of proliferation and are carried by axoplasmic transport.

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CYTOSTATIC ACTION OF CELLS OF THE IMMUNE SYSTEM ON TUMOR CELLS

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Several types of cells of the immune system capable of producing lysis of tumor target cells exist. They include activated cells, such as macrophages, T killer cell, normal killer cells (NKC), and polynuclear lymphocytes [2]. However, the cytotoxicity of effector cells is unable to explain certain experimental data obtained during the study of interaction of tumor cells with cells of the immune system. Cheever et al. [5], for instance, raised the question of the existence of "nonclassical" NKC, on the basis of the absence of their cytotoxic action in the test with ^{51}Cr *in vitro*, despite the presence of cytotherapeutic activity *in vivo*. Other workers [3] also have reported the possibility of immunotherapy with cells noncytotoxic for leukemia EL-4 cells.

It has been shown that tumor cells can remain for a long time in the body in a dormant state [8]. These facts are evidence of the possible existence not only to cytotoxic, but also of cytostatic mechanisms of immunologic surveillance of tumor growth.

The aim of the present investigation was to study the ability of cells of the immune system to exert a cytostatic action on tumor cells.

METHODS

To test the cytostatic action of effector cells we used a modified method [9] based on recording inhibition of RNA or DNA synthesis in target cells. As effector cells we used thymus, bone marrow, and spleen cells from C57BL/6 mice, and also splenocytes of DBA/2, CBA, AKR, and BALB/c mice as well as (CBA × C57BL/6)F_1 hybrids. As target cells we used mastocytoma P-815 (H-2^d), leukemia EL-4 (H-2^b), lymphoma YAC (H-2^a), and sarcoma MCh-11 (H-2^b) cells, maintained in the ascites form in mice of the corresponding strains. All the target cells used were insensitive to the cytotoxic action of the effector cells in the test with ^{51}Cr. To prevent incorporation of ^3H-uridine by the effector cells they were treated beforehand with actinomycin D in a concentration of 1 μg/ml per 10^7 cells in 1 ml at 37°C for 1 h. As was shown previously, this kind of treatment does not affect activity of killer cells [4]. Macrophages were removed by adsorption on plastic Petri dishes.

Nonadherent fractions of spleen, bone marrow, and thymus cells were used in the experiments. To obtain peritoneal macrophages, C57BL/6 mice each received an injection of 1.5 ml of 10% peptone, and on the 3rd day after the injection the peritoneal cavity of the mice was flushed out with Eagle's medium with 10 U of heparin. The cells thus obtained were adsorbed on plastic Petri dishes. The adherent cell fraction was harvested with the aid of a siliconized tube. Effector cells and target cells were incubated for 4 h in 96-well plates in medium RPMI-1640, containing 10% fetal serum and 100 U each of penicillin and streptomycin in

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