The electron-histochemical investigation of ATPase of cancer cells with this type of ultrastructure showed that the reaction product was connected with the membranes of the villi of the intracellular tubules, the basal plasmalemma, and the nucleoli (Fig. 1e, f). Some cancer cells with different enzyme activity on the membranes of the microvilli were found. For instance, some cells had relatively high enzyme activity (Fig. 1e), whereas others had low activity, reflected in some cases in the almost complete disappearance of reaction product (Fig. 1f).

This comparative study of the distribution of ATPase activity in normal parietal cells and cancer cells with a similar ultrastructure thus shows that in cells of the latter type the part of the mechanism of hydrochloric acid secretion which is connected with H^+ and Cl^- transport remains intact. The observed decrease in hydrochloric acid production by the gastric mucosa in the presence of cancer and by the cancer itself is evidently attributable to other mechanisms.

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EFFECT OF CHLORAMPHENICOL ON THE CARCINOGENICITY OF N-NITROSOMETHYLUREA

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Chloramphenicol was shown to reduce the carcinogenic action of N-nitrosomethylurea on CBA mice. The mean latent period of onset of the tumors was increased but the number of tumors was reduced both at the site of injection of the carcinogen and in remote organs during observation for 53-66 weeks.

KEY WORDS: chloramphenicol, nitrosomethylurea, carcinogenesis.

The possible role of nitroso compounds in the etiology of human malignant neoplasms is being widely discussed at the present time. These substances have also become widely used for chemotherapy in clinical oncology. Attempts to discover substances which can reduce the toxic and carcinogenic action of these compounds are therefore natural and essential.

In this investigation the possibility of reducing the carcinogenicity of N-nitrosomethylurea (NMU), which has carcinogenic properties, by means of the antibiotic chloramphenicol was studied; chloramphenicol has a

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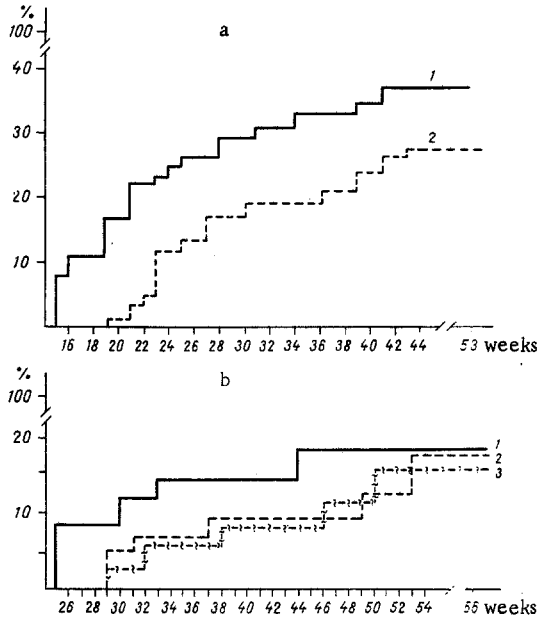

Fig. 1. Effect of chloramphenicol on tumor development at site of injection of NMU. a) Dose of NMU 0.07 mg/g, animals killed 53 weeks later; b) dose of NMU 0.025 mg/g, animals killed 66 weeks later. 1) Single injection of NMU; 2) chloramphenicol daily for 5 days, single dose of NMU 2 h after last dose of chloramphenicol; 3) the same as before plus a single dose of chloramphenicol 24 h after NMU. Abscissa, time after beginning of experiment, in weeks; ordinate, number of tumors developing, in per cent.

polyvalent anticarcinogenic action and is known to reduce the toxicity and carcinogenicity of several chemical carcinogens [2, 3, 5-8].

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice: males aged 2 months and females aged 4.5 months, weighing 20-25 g.

Analytically pure NMU (yellowish-silver crystals, mp 124°C, from methanol; synthesized in the Oncologic Scientific Center, Academy of Medical Sciences of the USSR, by Candidate of Chemical Sciences O. A. Pan'shin) was dissolved in physiological saline immediately before subcutaneous injection in doses of 0.025 and 0.07 mg/g body weight.

Chloramphenicol (the pharmacopoeial product, in tablet form) was administered via gastric tube in a dose of 1 mg/g. The chloramphenicol suspension was made up as described in [4].

In the course of 5 days the mice received chloramphenicol, and 2 h after the last dose they were given an injection of NMU. Animals receiving NMU in a dose of 0.025 mg/g received a further dose of chloramphenicol, 24 h after the carcinogen. Intact mice (23) and mice receiving chloramphenicol alone for 5 days (25) served as the control. NMU was given to 73 animals in a dose of 0.07 mg/g, 58 mice received NMU in a dose of 0.07 mg/g plus chloramphenicol, 35 received NMU in a dose of 0.025 mg/g, 35 received NMU in a dose of 0.025 mg/g plus chloramphenicol, and 20 received NMU in a dose of 0.025 mg/g plus chloramphenicol plus extra chloramphenicol.

All the animals were palpated once a week to determine if tumors were present at the site of injection of the NMU.

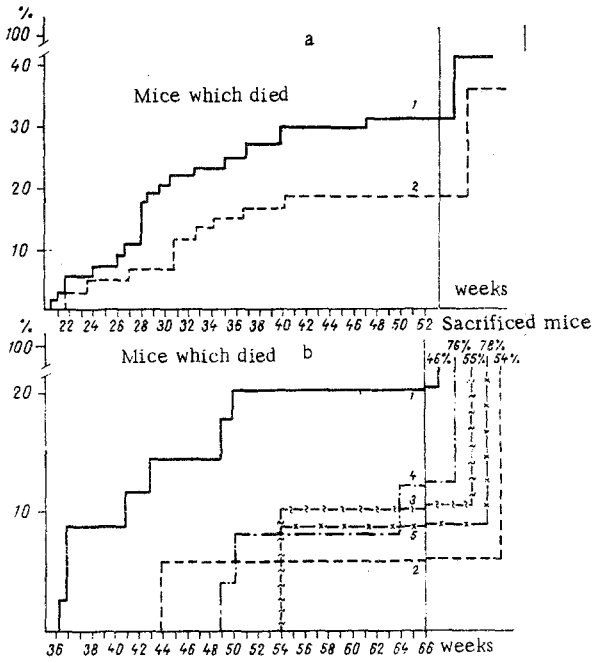

Fig. 2. Effect of chloramphenicol on development of remote tumors after induction by NMU. 4) Chloramphenicol for 5 days; 5) intact mice. Remainder of legend as in Fig. 1. For administration of NMU and different combinations of NMU plus chloramphenicol, integral displacement of curves of appearance of tumors relative to each other is statistically significant: $P < 0.01$.

A histological investigation of internal organs in which pathological changes suggesting the presence of neoplasms were discovered macroscopically was carried out on all the mice (those which died and those sacrificed after 53 and 66 weeks).

Statistical analysis of the results was carried out by means of Wilcoxon's two-sample criterion [1].

EXPERIMENTAL RESULTS

After injection of NMU in a dose of 0.07 mg/g (Fig. 1a) the main latent period of development of subcutaneous tumors at the site of injection of NMU was 158 days, whereas after administration of NMU plus chloramphenicol it was increased to 208 days. By the end of the experiment 36.5% and 27.5% of animals respectively had tumors. After NMU in a dose of 0.025 mg/g (Fig. 1b) the mean latent period (216 days) also was increased under the influence of chloramphenicol to 279 and 272 days, depending on the mode of administration. By the end of the experiments the number of animals with subcutaneous tumors was virtually the same in all groups: 16.7% after NMU, and 16.1 and 14.3% after NMU plus chloramphenicol. Incidentally, after administration of NMU plus chloramphenicol the first tumors were found later, and in fewer mice, than after administration of the carcinogen alone; moreover, when chloramphenicol was given, fewer tumors were observed in the course of observation for 48 h. By the end of the experiment the number of animals with tumors was somewhat smaller in the groups receiving 0.07 mg/g NMU plus chloramphenicol than in mice receiving the carcinogen alone, and approximately the same as in the group of mice receiving the smaller dose of NMU, with or without chloramphenicol.

After histological examination the subcutaneous tumors were classified as round-cell, spindle-cell, and polymorphocellular sarcomas. In some cases adenocarcinomas and a squamous-cell keratinizing carcinoma of the skin also were found. Chloramphenicol had no effect on the relative proportions of the different types of tumors. Among the control animals no subcutaneous or cutaneous tumors were found.

The effect of chloramphenicol on induction of remote tumors by NMU is illustrated in Fig. 2.

After NMU in a dose of 0.07 mg/g (Fig. 2a) chloramphenicol did not affect the minimal latent period, although the rate of appearance and the number of tumors at all times of observation until sacrifice were always less in animals receiving NMU plus chloramphenicol than in animals receiving the carcinogen alone. The number of tumors in the sacrificed animals (tumors appearing at later stages of observation) was somewhat greater in the group receiving NMU plus chloramphenicol than in animals receiving the carcinogen alone. The total number of neoplasms in the dying and sacrificed mice of both groups was thus roughly the same.

With the smaller dose of MNU (0.025 mg/g; Fig. 2b) the minimal latent period (35.8 weeks) was increased by administration of chloramphenicol to 44 and 54 weeks. In this dose, NMU induced tumors in only 20% of the animals in the course of 66 weeks. When chloramphenicol was given the percentage of animals with tumors fell to 5 and 10, i.e., practically to the control level (10 and 12%).

The total number of tumors in the dying and sacrificed animals was about equal after NMU alone and NMU plus chloramphenicol, but in the control groups it was 20-30% greater. The reason for this was evidently the fact that in all groups (including the control) hepatomas were found in about 80% of the sacrificed animals; the number of these tumors as a fraction of the total number of tumors in the control, moreover, was significantly greater, for at the time of sacrifice the number of animals surviving after NMU alone was 31%, after NMU plus chloramphenicol about 55%, and in the control groups, up to 80%.

Histological examination of mice receiving 0.07 mg/g NMU revealed lymphomas, leukemias, hepatomas, cystic adenomas of the kidneys, solid carcinomas and adenocarcinomas of the uterus, carcinoma of the ovaries, and a squamous cell keratinizing carcinoma of the forestomach. In animals of the control groups in these experiments there were no tumors. In mice receiving 0.025 mg/g NMU a solid carcinoma of the mammary gland and a squamous-cell keratinizing carcinoma of the forestomach were found. In all animals in this experiment, including the controls, hepatomas were found, and in some animals leukemias also were present, probably on account of the longer duration of the experiment. Tumors of the lungs also were found in the animals in both experiments receiving NMU, but these findings will be published separately.

These experiments thus showed that the antibiotic chloramphenicol reduces the local and remote tumorigenic effect of the carcinogenic nitroso compound NMU.

The protective effect of chloramphenicol was manifested as the later appearance of the tumors and their smaller number in animals dying in the course of long-term observation. However, the total number of tumors in the dying and sacrificed animals (i.e., the total number of tumors developing in the early and late periods of observation) in most cases was practically the same under these circumstances.

This result evidently supports the view that the anticarcinogenic effect of chloramphenicol is connected with an increase in the latent period of tumor development and not with a decrease in the total number of tumors which it is possible for NMU to induce. A similar mechanism of protection was observed by the writers in their study of the "antiurethane" effect of chloramphenicol [4].

The writers have previously discussed the possible mechanism of the protective action of chloramphenicol and have explained it by ability to compete with active metabolites of the carcinogens for binding with cellular macromolecules [3]. The possibility that chloramphenicol may act on the system of nonspecific microsomal oxidases, activating procarcinogenic compounds [3, 4], also was examined. NMU, however, is an agent which does not need metabolic activation and, consequently, which is independent of the action of chloramphenicol on the corresponding enzymes. The fact that chloramphenicol protects animals to some extent against this carcinogen also can only indicate that it acts on a certain terminal stage of interaction between carcinogen and cell that is common to agents of both direct and indirect action, or that the mechanism of the protective action of chloramphenicol against different carcinogens is not the same.

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