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CHLORPROMAZINE-INDUCED HYPOTHERMIA IN TUMOUR-BEARING MICE, ACUTE CYTOTOXIC DRUG LETHALITY AND LONG-TERM SURVIVAL

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Abstract

The acute lethality of various cytotoxic drugs (doxorubicin, vinblastine and nitrogen mustard) and long-term survival in a syngenic mouse-tumour system were studied at normal body temperature and at 28°C induced by chlorpromazine. Chlorpromazine-induced hypothermia itself neither caused acute toxicity nor influenced long-term survival. Doxorubicin (15 mg/kg) and nitrogen mustard (6 mg/kg) lethality was reduced at decreased temperature. The median survival time increased significantly from 35 days in normothermic to 52 days in hypothermic doxorubicin-treated mice. With nitrogen mustard, no increase in long-term survival was seen in the hypothermic group. The acute lethality of vinblastine was enhanced by hypothermia and the long-term tumour survival was unaffected. Hypothermia or possibly chlorpromazine considerably modulates drug toxicity and possibly anti-tumoural activity.

Key words: Chemotherapy, animal tumour, chlorpromazine, hypothermia, toxicity, doxorubicin, vinblastine, nitrogen mustard.

Treatment of malignant diseases with cytotoxic drugs is seriously limited by the narrow therapeutic index. This applies to most solid tumours, in advanced disease with a large tumour mass as well as in adjuvant treatment with a smaller tumour mass. Doses high enough to eradicate the tumour can often not be given owing to the risk of lethal complications, e.g. myelotoxicity. An important goal for the clinical oncologist is therefore to widen the therapeutic index.

Body temperature influences the sensitivity of tissues and organisms to ionising radiation (1–4) and presumably to chemotherapy as well (5–7). Hyperthermia is in clinical use (8) but for whole-body hyperthermia the relation between tolerability and temperature increase and duration is steep, limiting its usefulness in disseminated malignancy.

Enzymatic processes are temperature-dependent and quantitative differences in energy-producing metabolic pathways exist between normal and neoplastic tissues (cf. 9, 10). Few studies have been published on the relative cytotoxic effect of chemicals or irradiation on neoplastic and normal tissues at temperatures below that of the normal body (5, 11, 12). It was therefore decided to test various clinically used cytotoxic drugs at normal and subnormal temperatures, being feasible also for humans (cf. 13), on a syngenic mouse tumour system.

Material and Methods

MCG101-AA is an ascites-growing line of the methylcholanthrene-induced sarcoma MCG101-SS in C57Bl/63 mice (14, 15). Intraperitoneally, the ascites tumour grows as an almost monocellular suspension. It is transferred by i.p. injections of 0.1 ml ascites and was used in transfer generations 50–60.

In these experiments, the ascites was harvested from the abdomen of routinely transplanted mice and diluted 1 : 10 with Hanks' BSS. After centrifugation (10′, 100 g), blood was removed from the tumour cell suspension by repeated washings with hypotonic Hanks' (Hanks' BSS: sterile water 1 : 1) until the cells formed a white pellet after centrifugation (1′, 10 g). The cells were then suspended in Eagles' MEM. After hemacytometer counting, including a trypan blue vitality test, the suspension was diluted to 5×10^5 cells per ml.

On day 1 of the experiment, all the C57 mice, 10–13 weeks old, were given 0.1 ml of tumour cell suspension, i.e. 5×10^4 cells, into a lateral tail vein.

After an interval of three days to allow undisturbed

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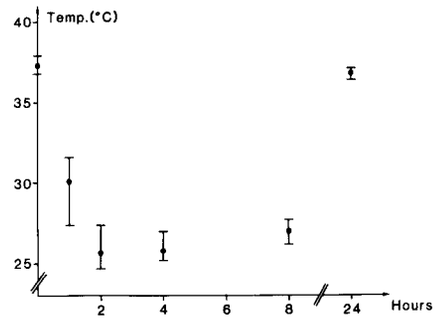

Fig. 1. Rectal temperatures in mice given chlorpromazine 15 mg/kg and kept at 26°C for 24 h after injection. Experiments involving drugs were performed at 28°C.

lodgement of the tumour cells, the mice were divided into four treatment groups: normothermic with saline (controls), hypothermic with saline, normothermic with cytotoxin and hypothermic with cytotoxin.

Hypothermia was achieved by i.p. administration of chlorpromazine. In separate experiments, a dose of 15 mg/kg (0.2 ml) was found to be non-lethal and lowered the temperature about 10°C to ambient temperature of 28°C, as measured by a rectal thermistor. The effect lasted for more than 8 h (Fig. 1). After this time the animals regained normal temperature. Mice given chlorpromazine were caged at 28°C for 24 h after the injection and subsequently at 22°C. The normothermic animals were given saline, 0.2 ml i.p. and kept at 22°C.

Three different cytotoxic drugs were used: doxorubicin, vinblastine and nitrogen mustard. For each drug, the LD_{50} for normothermic non-tumour-transplanted mice was roughly estimated by choosing three or four different dose levels with 6 mice in each group. From these tests, the following doses were chosen in the actual experiments: doxorubicin 15 mg/kg body weight i.p., vinblastine 12 mg/kg body weight, and 30 mg/kg body weight i.p. and i.v. respectively, and nitrogen mustard 6 mg/kg body weight i.v.

One hour after chlorpromazine/saline administration, the animals were given the cytotoxic drug i.p. or i.v. and controls saline by the same route and in the same volume (0.1 ml). One day later, the tails were amputated to avoid any tumour growth at the injection site. Survival was followed daily. Autopsy was performed to establish the cause of death. Macroscopically tumour-negative lungs and any doubtful metastates were examined by means of serially cut histological sections.

Statistics. Survival was classified as short-term, days 0–19, and long-term, days 20 and onwards. Log-rank tests according to Breslow-Gehan were used for analyses of difference between treatment groups.

Results

The effect of chlorpromazine-induced hypothermia on survival was compared between four paired groups of animals with a total of 112 treated and 111 control mice. No effect on tumour progression was found (Figs 2–5). The slight difference between the four control groups is probably due to differences in the tumour suspensions.

The acute early deaths from doxorubicin at normal temperature were reduced ($p < 0.02$) in the hypothermic group. The long-term survival was significantly increased ($p < 0.001$) in the hypothermic group compared to the normothermic animals (Fig. 2). An increase in long-term survival was seen for doxorubicin in normothermic mice as compared to untreated mice ($p < 0.05$). The median survival time in hypothermic doxorubicin-treated animals was increased by more than 50% as compared to the normothermic group.

The acute toxicity of nitrogen mustard at normal temperature (72% died within 2 weeks) was completely eliminated ($p < 0.001$) in the hypothermic mice (Fig. 3). The few animals surviving for long-term analysis in the

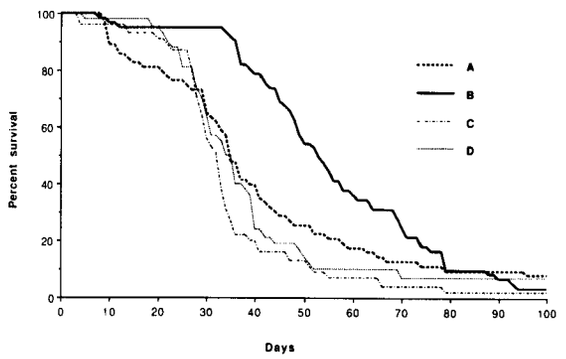

Fig. 2. Survival curves for the doxorubicin experiments. A = normothermic + doxorubicin (n = 63); B = hypothermic + doxorubicin (n = 61); C = hypothermic control (n = 45); D = normothermic control (n = 42).

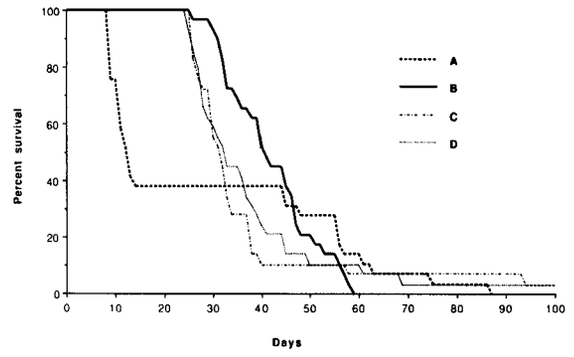

Fig. 3. Survival curves for the nitrogen mustard experiments. A = normothermic + nitrogen mustard (n = 29); B = hypothermic + nitrogen mustard (n = 29); C = hypothermic control (n = 22); D = normothermic control (n = 29).

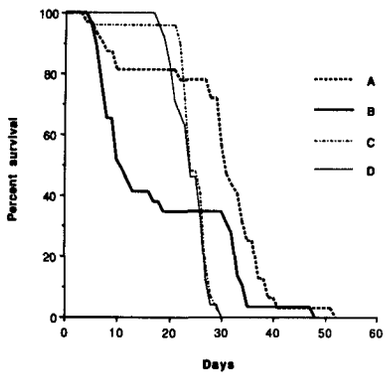

Fig. 4. Survival curves for the intravenous vinblastine experiments. A = normothermic + vinblastine i.v. (n = 32); B = hypothermic + vinblastine i.v. (n = 29); C = hypothermic control (n = 29); D = normothermic control (n = 24).

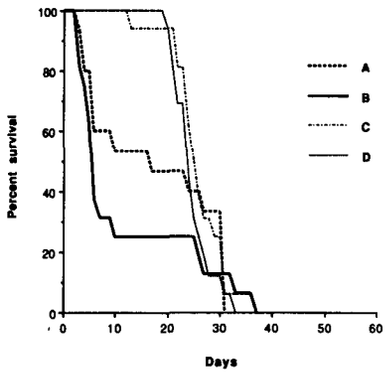

Fig. 5. Survival curves for the intraperitoneal vinblastine experiments. A = normothermic + vinblastine i.p. (n = 15); B = hypothermic + vinblastine i.p. (n = 16); C = hypothermic control (n = 16); D = normothermic control (n = 16).

normothermic group had a significantly better survival than those in the hypothermic group ($p < 0.001$). The long-term survival time for nitrogen mustard mice was significantly increased in normo- and hypothermic mice as compared to controls not treated by the cytotoxin ($p < 0.001$).

The acute toxicity of vinblastine was significantly increased ($p < 0.001$) in the hypothermic mice when the drug was given i.v. (Figs 4 and 5). No influence on long-term survival could be seen in intraperitoneally treated hypothermic mice, while this occurred in both normo- ($p < 0.001$) and hypothermic ($p < 0.001$) intravenously treated mice as compared to mice not treated by vinblastine (Figs 4 and 5).

At autopsy, microscopic pulmonary metastases could not be seen in mice dying within 2 weeks after cytotoxic therapy. Animals dying within two weeks of doxorubicin or nitrogen mustard administration exhibited widespread visceral haemorrhage, presumably a result of myelotoxicity, while those succumbing within 2 weeks of administration of vinblastine showed congested intestines as a major feature. Most animals dying two weeks or more after treatment showed macroscopic pulmonary metastases, some also cardiac and subcutaneous deposits.

Discussion

Mammalian cellular metabolism normally occurs at approximately 37°C, at which temperature most enzymes seem to have an optimal efficiency, although metabolism is speeded up at increased temperature. Metabolic activities are interrelated and intervention at a certain level may well produce a cascade of secondary effects at other levels. It is well-established that the energy producing metabolic pathways differ considerably between normal and neoplastic cells, the latter having a predominantly anaerobic and aerobic glycolysis. A large number of individual enzymes have been found to differ between normal and neoplastic tissue (cf. 16).

Each individual enzymatic reaction is temperature-dependent, the degree of dependence presumably varying between different enzymes. Since a considerable difference exists between the energy-producing systems of normal and neoplastic tissue, a net difference in temperature dependence is not unlikely to occur, which in turn may influence other metabolic activities, e.g. those involved in the processing of drugs and cellular proliferation. Against this background, it was considered of interest to test whether cellular damage caused by cytotoxic drugs could be changed to a different degree in normal and tumour tissue so as to alter the therapeutic index, hopefully broadening it.

The drugs to be tested were selected since: a) doxorubicin was previously studied, indicating temperature-dependent effects (7, 12) and the well-established alopecia-sparing effect of cold application (17), b) nitrogen mustard is an alkylating agent (18) like ionizing radiation, the latter with a complex temperature dependence (1–4), c) vinblastine interacts with microtubular proteins (19), being thermolabile (cf. 20).

A syngenic tumour system was chosen to avoid undue immunological reactions, possibly also prone to temperature dependence. No effect of decreased temperature and/or chlorpromazine per se was found in the system.

The amount of tumour cells injected, 5×10^4, was chosen to result in 100% tumour take in untreated mice, but to kill the mice at a time well-separated from the acute cytotoxicity period (i.e. days 0–19). This resulted in easy discrimination between acute drug toxicity and long-term survival, mainly depending on tumour progression, but possibly also on late drug toxicity.

The use of chlorpromazine to induce hypothermia introduces an uncertainty as to whether observed effects are to be ascribed to the decreased temperature per se or to chlorpromazine itself, or both. Chlorpromazine is a polycyclic

heterocyclic compound with similarities to anthracyclines and it has been shown to exert a number of potentially anti-proliferative actions in tumour cells (21). In our study, however, chlorpromazine given alone did not change tumour progression. Gailis et al. (22) investigated whether the myeloprotective effect of chlorpromazine-induced hypothermia was to be ascribed to the drug itself or to the decreased temperature and found the latter to be true.

The considerably increased long-term survival produced by doxorubicin during chlorpromazine-induced hypothermia merits further investigation, however, since these structurally related compounds may well interact. No such increase in anti-tumour activity was seen with nitrogen mustard and vinblastine, structurally unrelated to chlorpromazine.

Both doxorubicin and nitrogen mustard toxicity was abolished by hypothermia, while that of vinblastine was enhanced significantly when given intravenously. Vinblastine has a well-known neurotoxic action by damaging microtubular proteins, resulting, for example, in intestinal paralysis. Chlorpromazine is an anticholinergic drug, thereby probably potentiating the intestinal paralysis, which is further aggravated by the hypothermic state. This hypothesis is strengthened by the post-mortem findings of intestinal congestion exclusively in vinblastine-treated mice.

The use of hypothermia to widen the therapeutic index of chemotherapy and radiotherapy has attracted little interest so far (5, 11). Our results, indicating considerably reduced toxicity of certain drugs concomitant with unaffected or possibly enhanced anti-tumour efficacy, merit further investigation of the mechanisms involved.

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