Oral Administration of Anti-Doxorubicin Monoclonal Antibody Prevents Chemotherapy-induced Gastrointestinal Toxicity in Mice

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ABSTRACT

Gastrointestinal mucositis is a common and painful condition that affects a proportion of cancer patients receiving chemotherapeutic drugs including anthracyclines, and it has become the dose-limiting toxicity for a number of chemotherapeutic regimens. The murine monoclonal antibody MAD11 recognizes the anthracycline doxorubicin, and systemic administration of this antibody in mice treated with doxorubicin was found previously to prevent the toxic effects of the drug. The purpose of this study was to determine whether gastrointestinal toxicity associated with doxorubicin can be reduced by oral administration of anti-doxorubicin MAD11 in mice. Our experiments show that orally administered MAD11 antibodies: (a) are essentially not absorbed in the blood circulation since less than 0.5% of protein-associated radioactivity was recovered from blood samples; (b) reduce the extent of doxorubicin-induced apoptosis in murine intestinal crypts, as determined by labeling strand breaks with modified nucleotides in an enzymatic reaction; and (c) reduce the body weight loss in mice treated with 12 mg/kg body weight of doxorubicin and decrease the early mortality in mice treated with 16 mg/kg body weight. This type of treatment may be useful in preventing anthracycline-induced gastrointestinal mucositis in cancer patients.

INTRODUCTION

Although chemotherapy affects virtually every organ system in the body, the cell populations that typically exhibit rapid cell turnover, such as those of the bone marrow and gastrointestinal mucosa, are the most sensitive. Gastrointestinal disturbances are common side effects of antineoplastic therapy and drugs such as doxorubicin, methotrexate, 5-fluorouracil, bleomycin, cytarabine, and actinomycin D routinely produce mucositis (1, 2). The incidence and severity of mucositis are closely related to the drug dose, but even conventional doses induce mucositis, which results in severe local discomfort and pain, poor nutrition, dehydration, delays in subsequent chemotherapy, and possible dose reduction in the patient (3). The reduction of standard doses of effective agents results in the lowering of survival or complete remission rates (4-6). Additionally, several recent clinical trials in cancer patients have indicated the benefit of increased doses or dose intensities of chemotherapeutic agents (7). The pancytopenia associated with intensive dose protocols has been ameliorated by the use of bone marrow transplantation or autologous infusion of circulating hematopoietic progenitors and hematopoietic growth factors (8). However, for mucositis, no method has been developed to date to diminish this serious and potentially life-threatening complication.

Doxorubicin, one of the most valuable anticancer drugs for the treatment of solid tumors, such as carcinomas of the breast, lung, thyroid, and ovary, and soft tissue sarcomas causes bone marrow suppression and mucositis, which are dose limiting (9). We have derived a murine IgG2a MAb named MAD11 against doxorubicin, which also reacts with other anthracycline derivatives. The systemic administration of this antibody in mice injected with a high dose of doxorubicin antagonizes the toxic effect of the drug (10). Moreover, topical application of this MAb to the skin of rats prevents doxorubicin-induced alopecia (11).

Immunoglobulins of isotype G are usually used for systemic administration; however, several clinical trials in patients have suggested that passive immunity against diarrheal infectious agents can be achieved by oral administration of specific IgG antibodies (12). Those studies have also shown that a portion of the IgG resist degradation during passage through the gastrointestinal tract, and that even partially digested IgG retains its opsonic and antitoxic properties and its ability to bind to potentially pathogenic agents (13-16).

In this study, we investigated whether the oral administration of anti-doxorubicin MAD11 MAb in mice treated with high doses of doxorubicin antagonizes the toxic effect of the drug as concerns gastrointestinal mucositis prevention.

MATERIALS AND METHODS

Animals. Six- to 8-week-old female BALB/c mice were obtained from Charles River (Calco, Italy). All mice were treated in accordance with institutional guidelines. For oral treatments, a catheter was inserted into the stomach. Before sacrifice, animals were anesthetized with 0.2 ml/20 g body weight of 10 mg/ml Ketalar (Parke-Davis) and 0.05% Rompun (Bayer).

Reagents. Doxorubicin hydrochloride (Adriamycin) was supplied by Farmitalia Carlo Erba (Milan, Italy). A fresh solution of doxorubicin was prepared just before use. The anti-doxorubicin MAD11 MAb (IgG2a) selectively recognizes epitopes located at or near the aromatic D ring of the anthracycline molecule. An unrelated isotype-matched murine MAb was used as control in the experiments on doxorubicin-induced body weight loss. Ascitic fluids contained about 1 mg/ml of specific antibody. Human immunoglobulins (Sandoglobulina; Sandoz, Milan, Italy) were used in immunohistochemistry experiments to avoid the background reactivity on murine tissue, due to the use of biotinylated anti-murine IgG antibodies.

Immunohistochemistry. BALB/c mice were treated orally with 50 mg/kg of human immunoglobulin and sacrificed 2, 6, or 24 h later. Samples from the middle third of the small intestine were removed, carefully washed to flush out the luminal contents, frozen in liquid nitrogen, separated into 7-μm transverse sections with a freeze microtome at −30°C, and transferred to microscope slides. The immunoperoxidase technique was performed with an avidin-biotin-peroxidase complex (ABC) kit (Vector, Burlingham, CA). Sections were rehydrated with 1% BSA in PBS for 15 min at room temperature before a 30-min incubation with 300 μl/slide of antiserum. After rinsing in PBS, sections were blocked for endogenous peroxidase activity with 0.3% hydrogen peroxide-PBS solution for 40 min, incubated with avidin-biotin complex for 30 min, and stained with diaminobenzidine (Sigma Chemical Co., St. Louis, MO) for 5 min. Slides were counterstained with hematoxylin.

Quantitation of MAD11 Recovery in the Blood. MAD11 was purified by affinity chromatography on a protein-A Sepharose CL4B column and radio-labeled by lactoperoxidase-catalyzed iodination to a specific activity of 10.4 μCi/μg. MAD11 was mixed with unlabeled MAb to obtain 37 μCi/μg and then was orally administered to nine BALB/c mice. Animals were aneste-
To verify that IgG antibodies can penetrate the intestinal mucosa, human IgG antibodies were administered orally to mice and, after 2, 6 and 24 h, the small intestine was removed, sectioned, and examined immunohistochemically. The antibodies were clearly detected in the epithelium and muscularis mucosa of the small intestine at 2 h after the treatment (Fig. 1b). The presence of the antibodies was also detected in sections of gut obtained at 6 and 24 h after oral MAb administration (data not shown). No human antibodies were detected in lung, kidney, liver, heart, or spleen.

To determine whether intact IgG molecules were able to cross the intestinal epithelial cell surface into the serum, mice were treated orally with 125I-labeled MAD 11. At 2 h after treatment, 2.2 ± 0.36% of the total administered radioactivity was detected in the blood, less than 15% of which was bound to trichloroacetic acid-precipitable proteins, and only 3.1 ± 0.3% was in a molecular weight form >14,000. After 6 and 24 h, blood radioactivity recovery was less than 0.3%. Doxorubicin has been described (17, 18) to induce cytotoxic effects on mouse small intestine cells characterized by nuclear and cytoplasmic condensation and preservation of the organelles in the early stages. The peak incidence of these changes, typical of apoptosis, was observed at 4–6 h after doxorubicin treatment, and cell death occurred predominantly, although not exclusively, in the base of the crypt. A remarkable inhibition of apoptotic changes, compared to animals treated with doxorubicin only, was observed in mice treated orally with MAD 11 2 h before doxorubicin treatment (Fig. 2).

To test whether oral treatment with MAD 11 might partially reverse the marked decrease in body weight consistently associated with i.p. administration of sublethal doses of doxorubicin, 30 BALB/c mice were randomly divided in three groups, and 2 h before injection of 12 mg/kg of the anthracycline, mice were treated orally with 1 ml of MAD 11, with unrelated ascitic fluid, or with saline. Animals in the MAD 11-treated group demonstrated markedly less weight loss than did animals in the control groups (Fig. 3). Repetition of this study in two other separate experiments confirmed this observation. The percentage of weight loss in MAD 11 treatment groups (Fig. 3) was statistically different from control groups (P < 0.05 on day 4, P < 0.03 on day 7, P < 0.01 on day 10, and P < 0.02 day 12 by t test).

The effect of oral treatment with MAD 11 MAb on mortality was evaluated in mice injected with a lethal dose of doxorubicin. Forty-eight BALB/c mice were randomly divided in two groups and treated orally or not with 1 ml of MAD 11 ascitic fluid 2 h before administration of doxorubicin (16 mg/kg body weight). As shown in Fig. 4, partial protection of early mortality was observed in MAD 11-treated mice at around day 7 ($\chi^2$ test, P’ = 0.03), whereas a similar slope in the curves was observed thereafter.
FIG. 2. Photomicrograph of transverse sections through murine small intestines, illustrating protection from doxorubicin-induced apoptosis. Mice were orally treated with saline (a), doxorubicin alone (b), or doxorubicin plus MAD11 (c). Apoptotic nuclei in the crypts were visualized by labeling the fragmented DNA with biotinylated dUTP via the TdT reaction, followed by the addition of avidin-FITC. The red staining observable at the level of muscularis mucosa is due to doxorubicin autofluorescence.

DISCUSSION

The present study demonstrates that orally administered anti-doxorubicin MAbs prevent the intestinal toxicity of the drug. Like many other cytotoxic agents, doxorubicin targets cells that are intimately involved in cell proliferation, and previous studies have shown that the cell death induced by doxorubicin in the murine small intestinal tract has the morphological characteristics associated with apoptosis (17, 18). Our immunohistochemical data show that orally administered immunoglobulins can penetrate and remain for several hours in the small intestine mucosa. The administration of the anti-doxorubicin MAb 2 h before the chemotherapeutic treatment was effective in preventing apoptosis induced by doxorubicin.

Pharmacokinetic analyses of orally administered radiolabeled MAD11 revealed less than 0.5% of protein-associated radioactivity in the blood, almost all in a molecular weight form <14,000; this corresponds to a maximum of 5 μg of fragmented MAb, which is at least 100 times less than the minimal dose required to obtain an antidotal activity by systemic administration (10, 19). Our pharmacokinetic data are in agreement with findings in a previous study in which 2–4% of the radioactivity associated with orally administered immunoglobulins in infants was recovered in the blood but was not associated with high molecular weight molecules (15).

Since the presence of gastrointestinal mucositis adversely affects the uptake of nutrient substances, changes in body weight can provide an objective measurement of intestinal damage. In three separate experi-
doxorubicin. The treatment schedule was as described in "Materials and Methods."

ments, a significant reduction of body weight loss was observed at 7 days in animals orally pretreated with the anti-doxorubicin MAb. The similar
donor diffusion into the intercellular spaces. A direct effect of MAD11 against doxorubicin-induced body weight loss was observed when
MAD11 was administered only 30 min before doxorubicin treatment (data not shown), suggesting that the mere presence of the antibody in the
intestinal lumen is not sufficient to obtain protection; instead, the antibody must diffuse into the intercellular spaces.

Oral administration of the antibody also decreased mortality when lethal doses of doxorubicin were used. As expected, not all of the animals were protected, since bone marrow toxicity was not prevented. Indeed the dose-limiting toxicities for most chemotherapeutic drugs are bone marrow suppression and mucositis (3, 9). The discovery and development of colony-stimulating factors have reduced the severity and duration of hematopoietic toxicity (20, 21). Mucositis still represents an obstacle of paramount importance; moderate or severe (grade 3 or 4) mucositis that has not fully resolved at the time of retreatment usually suggests that not only should treatment be withheld until the mucosa has healed but also that the drug dose must be decreased, with consequent reduced therapeutic results.

Approaches to prevent and treat mucositis, which is induced by a wide range of therapeutic regimens, are under study (22). No protection of
cyclophosphamide-induced gastrointestinal toxicity was observed in mice pre-
treated with glutathione (22). Partial protection, confined to oral mucosa,
against 5-fluorouracil-induced stomatitis was obtained by cryotherapy
(23) and by topical administration of transforming growth factor 3, a
potent inhibitor of epithelial as well as hematopoietic stem cell growth (24).

Several observations on oral administration of immunoglobulins (12) suggest that oral MAD11 treatment of patients scheduled for
treatment with doxorubicin will not have negative side effects. Moreover,
we found no evidence of significant levels of intact IgG in the serum; therefore, oral MAD11 should not compromise the therapeutic activity
of drugs used to treat tumors that do not metastasize to the gastrointestinal tract. Finally, even in the absence of a convenient animal model, our results suggest that the topical application of MAD11 on oral mucosa would also prevent stomatitis.

In conclusion, since the antibody dosage used in mice is likely to be transferable to a clinical setting, our data suggest that the oral administration of MAD11 in patients undergoing doxorubicin chemotherapy should allow dose or schedule intensification of drug therapy by preventing
the gastrointestinal mucositis associated with this therapy. MABs specific for different chemotherapeutic agents might be considered in conjunction with other chemotherapeutic drugs that induce mucositis.

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