Macrophage Inflammatory Protein 1α Attenuates the Toxic Effects of Temozolomide in Human Bone Marrow Granulocyte-Macrophage Colony-forming Cells

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ABSTRACT

Macrophage inflammatory protein 1α (MIP-1α) is a chemokine that may act principally by preventing hemopoietic cells from entering G1, thereby attenuating the cytotoxic effects of cell cycle-specific chemotherapeutic agents. Here we examine the effect of MIP-1α on the sensitivity of human granulocyte-macrophage hemopoietic progenitor cells (granulocyte-macrophage colony-forming cells; GM-CFCs) with the cytotoxic effects of antitumor agents that act mainly via alkylation at the O6 position of guanine in DNA. Mono-nuclear cell preparations from human bone marrow were used in an in vitro GM-CFC colony-forming assay. The GM-CFC survival from individual patients displayed a range of sensitivities to the methylation agent temozolomide ([Tz] 20–55% survival at 10 μg/ml Tz). However, in all 16 cases, MIP-1α (50 ng/ml) protected against GM-CFC killing: survival in the presence of MIP-1α ranged from 65–97% at 10 μg/ml Tz, with GM-CFCs being 1.5–4.5-fold more resistant than control cells from the same patient. The highest levels of protection were seen in the GM-CFCs with the highest sensitivity in the absence of MIP-1α. Similar degrees of protection were seen for the methylation agent streptozotocin, but no protection was detected for the chloroethyliating agents carmustine or mitozolomide in the samples for which there was protection against the toxic effects of Tz. Whereas the mechanism of this effect remains to be established, the results may have potential immediate clinical application in the attenuation of hematological toxicity after administration of methylating antitumor agents.

INTRODUCTION

Hemopoietic toxicity is the major dose-limiting factor for a number of commonly used chemotherapeutic agents. Circumvention of such effects would allow dose intensification and possibly improved tumor response and long-term survival in a range of tumor types. Hemopoietic growth factors such as granulocyte colony-stimulating factor have allowed some dose intensification, but the practical gains have been disappointing (1).

An alternative approach to growth stimulation is to maintain stem cells in a quiescent state during periods of chemotherapy treatment so that the cells have an increased period during which the repair of potentially lethal damage may proceed prior to replication. This strategy has been shown to attenuate the cytotoxic effects on hemopoiesis (2–5) and epithelium (6), with the former being reflected in accelerated bone marrow and peripheral blood cell recoveries. One agent that has been demonstrated to be a specific hemopoietic stem cell proliferation inhibitor is MIP-1α (3, 7, 8). Although MIP-1α is well tolerated (9), thus far, only modest (10) or no (11) myeloprotective effects have been demonstrated clinically.

Alkylating agents that are methylation (e.g., dacarbazine, procarbazine, or Tz) or chloroethylating (e.g., BCNU or lomustine) are referred to as O6-alkylating agents because they exert their cytotoxic effects principally via the O6 position of guanine in DNA. We have shown previously that Tz is toxic to human GM-CFCs and that this effect is exacerbated by inactivation of the DNA repair protein, ATase (12), which normally attenuates the toxic effects of such agents.

To establish whether MIP-1α might protect hemopoietic cells against the toxic effects of O6-alkylating agents, we investigated the sensitivity of primary human bone marrow GM-CFCs to the cytotoxic effects of these agents in the presence and absence of MIP-1α using an in vitro colony-forming assay. Tz was used most extensively because it is currently undergoing clinical development (13).

MATERIALS AND METHODS

Alkylating Agents and MIP-1α. Tz was provided by the Cancer Research Campaign formulation unit. Steptozotocin...
and BCNU were obtained from the Christie Hospital Pharmacy, and mitozolomide was obtained from May and Baker. Stock solutions were made in DMSO and stored at −20°C. MIP-1α was supplied by British Biotechnology Limited (Oxford, United Kingdom) as a nonaggregating, genetically engineered variant of human MIP-1α (LD78) known as BB-10010. BB-10010 was dissolved in PBS at 50 mg/ml and stored at −20°C.

Bone Marrow Samples. Ethics approval for the study was obtained from the South Manchester Ethics Committee. Human bone marrow samples were obtained by aspiration from the right posterior iliac crest after informed consent was obtained from patients undergoing staging investigations for previously untreated lung or breast cancer. Up to 2 ml of marrow were aspirated, mixed immediately with transport medium [10 ml of Iscove’s modified Dulbecco’s medium (Life Technologies, Inc.) containing 40 units of preservative-free heparin and 2% FCS], and stored on ice. Each sample was assessed microscopically (by J. C.) for the presence of cancer cells, and if cancer cells were present, the sample was not utilized further. Erythrocytes were removed by density centrifugation (14). Samples were diluted 1:1 with PBS layered over an equal volume of J-Prep (1.077 mg/ml; TechGen International) and centrifuged at 400 × g for 30 min at room temperature. The mononuclear cells at the interface were collected, washed twice with PBS, and used in colony-forming assays.

GM-CFC Assay. The isolated bone marrow cells were diluted to 1–2 × 10^5 cells/ml in medium [300 mosmol Iscove’s modified Dulbecco’s medium containing 20% FCS and 10% 5637 conditioned medium as a source of growth factors (15)]. MIP-1α (final concentration, 50 ng/ml) was added to the cells, and 5–10 min later, agar noble (Life Technologies, Inc.; final concentration, 0.3%) was added, and the cells were plated into 35-mm Petri dishes containing the appropriate concentrations of each chemotherapeutic agent (diluted in medium). Control plates contained an equivalent volume of DMSO. After incubating for 9 days at 37°C in an atmosphere of 5% CO₂ and 95% air, colonies that arose from the GM-CFC lineage and contained more than 50 cells were counted. In the absence of any drug, these conditions gave ~50 colonies/plate. A protection factor was calculated by dividing the percentage survival with MIP-1α by the percentage survival without MIP-1α, which was then plotted against the control survival with Tz (10 μg/ml) alone.

RESULTS

Effect of Alkylating Agents and MIP-1α on GM-CFC Survival. The survival of GM-CFCs after exposure to increasing doses of Tz in 6 of the 16 bone marrow mononuclear cell preparations examined is shown in Fig. 1. GM-CFCs displayed a wide range of sensitivities, but in all cases, pretreatment with MIP-1α decreased sensitivity to Tz. Fig. 2 shows that for all samples studied, survival at 10 μg/ml Tz ranged from 20–55% (mean, 43%) and 65–97% (mean, 88%) in the absence and presence of MIP-1α, respectively. The mean protection ratio factor was 2.2. However, there was no direct correlation between survival in the absence of MIP-1α and the protection afforded by MIP-1α treatment (i.e., cells that were inherently more sensitive to Tz were afforded the greatest extent of protection by MIP-1α; Fig. 3).

In the Tz-untreated control samples, there were generally fewer colonies in the presence of MIP-1α than in the absence of MIP-1α. This was only statistically significant in 6 of the 16 samples, for which the protection factors ranged from 1.5–3.9; the range for those samples for which there was no statistically
significant reduction in colony-forming ability was 1.5–4.5. Therefore, there was no indication of any relationship between MIP-1α-induced reduced colony-forming ability and increased resistance to Tz.

The effect of MIP-1α pretreatment on GM-CFC sensitivity to other chemotherapeutic agents was examined for a smaller number of samples. The results show that MIP-1α was able to protect GM-CFCs against the toxic effects of the methylating agent streptozotocin but not against the effects of the chloroethylating agents mitozolomide and BCNU (Fig. 4).

DISCUSSION

Hemopoietic toxicity is frequently the major dose-limiting factor for a number of commonly used chemotherapeutic agents. Circumvention of such effects would allow dose intensification and possibly improve tumor response and patient survival in a range of tumor types. Hemopoietic growth factors have allowed some dose intensification, but the practical gains have been limited (1). An alternative approach to growth stimulation is to maintain stem cells in a quiescent state during periods of chemotherapy treatment so that the cells have an increased period during which the repair of potentially lethal damage may proceed before replication.

MIP-1α is a member of a large family of small, inducible, and secreted cytokines (7, 16) that, in vivo, rapidly reduce the cycling and numbers of progenitor cells in bone marrow and spleen (17). These inhibitory effects appear to be specific for multipotential hemopoietic precursor cells that are intermediate to late in the stem cell hierarchy (18–20). Murine models using MIP-1α in combination with cell cycle-specific (2, 3) and non-specific chemotherapeutic agents as well as repeated exposure to sublethal doses of ionizing radiation (21, 22) have confirmed that MIP-1α-treated mice show attenuated levels of hemopoietic damage, increased recovery of leukocyte numbers, and improved progenitor cell mobilization. Recent studies suggest that these chemoprotective effects of MIP-1α are mediated through inhibition of primitive progenitors from entering S phase (23).

To examine the extent to which MIP-1α might protect hemopoietic cells against the toxic effects of methylating and chloroethylating agents, primary human bone marrow mononuclear cell preparations were exposed to these agents in the presence and absence of MIP-1α. As reported previously (12), GM-CFCs showed variable sensitivity to the methylating agent Tz over the concentration range used, the highest dose of which equates to the plasma levels achieved clinically (24). However, in all samples, MIP-1α exposure resulted in the protection of the GM-CFCs against toxicity. The extent of this protection was variable: those samples that were intrinsically more sensitive to Tz were afforded the greatest degree of protection. In the untreated controls, MIP-1α caused a statistically significant reduction in the number of granulocyte macrophage colonies in <40% of the samples, and there was no correlation between this and protection against Tz toxicity. Therefore, unless protection was mediated by a transient growth suppression that had no effect on the number of colonies seen, cell cycle delay does not appear to explain our observations.
We have reported previously that inactivation of the DNA repair protein ATase, the principal mechanism of resistance to methylating agents, resulted in a considerably increased sensitivity of human GM-CFCs to the toxic effects of Tz (12). Hence, another possible explanation for the protection against Tz toxicity observed is that MIP-1α might up-regulate the expression of ATase. If so, protection against the toxic effects of other methylating agents might be expected, and, indeed, this was seen with streptozotocin.

The toxic effects of the chloroethyliating agents are also mediated by alkylation at the O6 position of guanine, in this case via lethal DNA interstrand cross-link formation (25, 26). Toxicity can be increased by inactivation of ATase (27, 28). Therefore, if MIP-1α increased ATase levels, it would be expected to attenuate resistance to this class of agents. However, protection against neither mitozolomide nor BCNU was observed in samples that were shown simultaneously to display increased MIP-1α-mediated resistance to Tz.

It is possible that MIP-1α is acting not via DNA repair mechanisms but via effects on cell cycle regulation, because delaying DNA replication would be expected to allow more time for ATase recovery by de novo synthesis, and this would then allow further repair of the potentially cytotoxic lesions and hence attenuate the toxicity of Tz. This would be expected to have much less impact on the toxicity of the chloroethyliating agents that kill cells via the relatively rapid formation of DNA interstrand cross-links because only the precursors of these cross-links are substrates for ATase action. In this case, slow recovery of ATase activity would not be expected to have any effect on survival. Unfortunately, the small number of bone marrow cells available from each individual sample precluded simultaneous assessment in the same sample of ATase expression levels and survival after treatment with Tz or a chloroethyliating agent. It would be clinically useful if it could be established that, as with human tumor xenographs (29), ATase levels can predict response to these agents.

An attractive strategy for protecting bone marrow cells against the toxic effects of O6-alkylating agents is to introduce the ATase cDNA into human hemopoietic cells ex vivo via retroviral transduction, followed by reintroduction into the host before chemotherapy (30–33). The present report indicates that MIP-1α may be a viable alternative to such gene therapy approaches. Irrespective of the mechanism of protection of GM-CFCs by MIP-1α, the possible clinical exploitation of the observation should be considered, not only with Tz, but also for other methylating agents such as dacarbazine, for which dose escalation is clinically useful. In addition to its potential role in dose intensification, MIP-1α has the advantage of maintaining stem cell numbers and viability, which should result in reduced risks of both short- and long-term bone marrow toxicity.

REFERENCES


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