Studies on the antitumor activities of pyrimidinone-interferon inducers. Part 2 Potentiation of antitumor resistance mechanisms

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In continuation of studies on antitumor activities of pyrimidinone interferon inducers [11], we report here that 2-amino-5-bromo-6-mF-phenyl-4(3H)-pyrimidinone (ABmFPP) is similarly effective to 2-amino-5-bromo-6-phenyl-4-pyrimidinone (ABPP) in its ability to reduce the number of metastatic nodules of a spontaneous fibrosarcoma (NFSa) and a spontaneous mammary carcinoma (MCa-K) in the lungs of C3Hf/Kam mice. Both compounds were more effective when given to mice prior to, rather than after, intravenous transplantation of tumor cells. In studies on the mechanism of the antitumor activity of pyrimidinones, 2-amino-5-iodo-6-phenyl-4-pyrimidinone (AIPP) was used in addition to ABPP and ABmFPP. These agents were capable of activating peritoneal macrophages that thus became capable of lysing in vitro 3T12 transformed cells but not syngeneic BALB/c embryo fibroblasts. Also, these agents were capable of augmenting significantly the natural killer (NK) cell activity in the spleen of C3Hf/Kam mice. Spleen cells from treated mice admixed to NFSa cells inhibited in vivo tumor take of these cells when the admixture was injected subcutaneously. Pyrimidinones were also effective against the development of NFSa nodules in the lungs of T-cell deficient mice implying that the presence of T-cells is not a prerequisite for the induction of antitumor activity by these agents. A further observation was that pyrimidinone compounds reduced the metastasis formation enhancing effect of cyclophosphamide. Therefore, pyrimidinone interferon inducers exhibit an appreciable antimetastatic activity mediated through antitumor resistance mechanisms involving activation of macrophages and stimulation of NK-cells.

Introduction
Recently, several pyrimidinone compounds have been identified as inducers of interferon production in several animal species and in cultured human tissues [20,21]. These include: 2-amino-5-bromo-6-phenyl-4-pyrimidinone (ABPP), 2-amino-5-iodo-6-phenyl-4-pyrimidinone (AIPP), 2-amino-5-bromo-6-methyl-4-pyrimidinone (ABMP), and 2-amino-5-bromo-6-mF-phenyl-4(3H)-pyrimidinone (ABmFPP). These compounds vary quite widely in their ability to induce interferon production. For example, while AIPP is a poor interferon inducer, ABPP is a strong

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interferon inducer [11, 20]. In general, however, these compounds exert strong antivirus activities and modulate different immunologic reactions [8, 9, 21].

We recently reported that ABPP and AIPP induced an appreciable antimetastatic activity against three different murine tumors [11]. The effect did not correlate either with tumor immunogenicity or the potency of the agent to induce interferon production. Neither induction of interferon production nor the antitumor activity was observed in mice exposed to whole body irradiation (WBI), suggesting that these agents acted against metastases by augmenting antitumor resistance responses. Here we report that these agents are capable of augmenting natural killer (NK) cell activity and of activating macrophages, which may be important factors in the antitumor effect of these pyrimidinones. In addition, we report antimetastatic activity of ABmFPP.

Materials and methods

Mice

Inbred C₃Hf/Kam mice were used in most experiments. They were bred and maintained in the specific pathogen-free mouse colony of the Department of Experimental Radiotherapy, The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Texas. Conventional BALB/c mice were used in one experiment as a source of activated peritoneal macrophages. Mice were 11–14 weeks old at the beginning of the experiments, with the exception of thymectomized mice; thymectomy was performed when C₃Hf/Kam mice were 5 weeks old. Within each experiment, the mice were of the same sex and were housed four to seven per cage.

Pyrimidinone compounds

Three pyrimidinone compounds were used: 2-amino-5-bromo-6-phenyl-4-pyrimidinone (ABPP), 2-amino-5-iodo-6-phenyl-4-pyrimidinone (AIPP), and 2-amino-5-bromo-6-mF-phenyl-4(3H)-pyrimidinone (ABmFPP), synthesized by W. Wierenga and H. I. Skulnick (The Upjohn Company, Kalamazoo, MI). ABPP and ABmFPP are potent interferon inducers, and AIPP is a weak interferon inducer [11, 21]. The compounds in the dose of 250 mg/kg per mouse were administered intraperitoneally in 0.2–0.3 ml of 1 per cent carboxymethylcellulose (vehicle). Because of poor solubility of these compounds, they were ground together with the vehicle in a tissue grinder to achieve a uniform suspension.

Tumors

Experiments on the antitumor effect of pyrimidinone compounds were performed using a spontaneous fibrosarcoma (NFSa) and a spontaneous mammary carcinoma (MCa-K) syngeneic to C₃Hf/Kam mice. NFSa is a poorly immunogenic tumor as determined by ability of specifically sensitized mice to influence the take of NFSa cells injected either intravenously or subcutaneously [1]. NFSa was in its 12th isotransplant generation when used in these experiments. The MCa-K tumor is moderately immunogenic as determined by the lung colony assay [12]. In these experiments we used 7th generation MCa-K isotransplants.

Single-cell suspensions from NFSa were prepared by trypsin digestion of nonnecrotic tumor tissue [13]. Viability of the cells was more than 95 per cent as assessed by phase-contrast microscopy. Single-cell suspensions of cells from MCa-K were prepared by a mechanical method [12]. The viability of cells was approximately 60 per cent.