Short Communication

Site-specific antitumour effects of 2 pyrimidinone compounds in rats

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Recently a series of 5-halo-6-phenyl pyrimidinones has been found to induce interferon production in several animal species (Stringfellow et al., 1980) and in man (Earhart et al., 1985). These compounds modulate a variety of immune responses. Most prominently they enhance the activity of natural killer (NK) cells (Taggert et al., 1980) and macrophages (Li et al., 1985). In the experiments reported here we evaluated the effects of two pyrimidinones that differ greatly in their ability to induce interferon (IFN) production. ABPP (2-amino-5-bromo-6-phenyl-4-pyrimidinone) induces high serum levels of IFN whereas AIPP (2-amino-5-ido-6-phenyl-4-pyrimidinone) does not. Yet, both agents are equally active in enhancing NK cell activity (Lotzova et al., 1983). In a previous communication (Marquet et al., 1983) we have shown that subcutaneous growth of liposarcoma LS175 in BN rats is inhibited by IFN. This nonimmunogenic liposarcoma is of spontaneous origin and has been found to be NK resistant. In order to investigate whether the induction of IFN plays a part in the antitumour effects of ABPP and AIPP we compared the two agents in the subcutaneous and in the artificial lung metastasis model, using tumour LS175. Ten to 12-week old male BN rats were used in all experiments. The NK cell activity in peripheral blood lymphocytes (PBL) after the intraperitoneal administration of 250 mg kg⁻¹ of ABPP or AIPP was assessed by a standard 3h²⁵¹Cr release assay as described by Ortaldo et al. (1977). Control animals received an equal volume of PBS i.p. The results are shown in Figure 1. A rapidly established and longlasting +4-fold increase in NK cell cytotoxicity is seen after the administration of either agent.

The influence on macrophage activity was assessed by measuring the ingestion of latex particles by peritoneal exudate (PE) cells after a single injection of 250 mg kg⁻¹ i.p. of either pyrimidinone. Phagocytosis was rapidly 3–4 fold enhanced (within 4 h) and remained elevated for 4 days (data not shown). In the s.c. tumour model 2 mm cubes of tumour LS175 were implanted s.c. in the left flank of BN rats. Tumour growth was assessed on days 4, 7 and 12 by measuring the two largest perpendicular diameters of the tumour with calipers. The average diameter was taken as the measure of tumour size. The inoculation of the tumour was preceded by i.p. injections of AIPP or ABPP at a dose of 250 mg kg⁻¹ on days −3, −2 and −1. The effect of this pretreatment on tumour growth is illustrated in Figure 2. A significant inhibition of tumour growth was observed in the ABPP-treated group (P<0.003) whereas some, but

Figure 1 Enhancement of NK cell activity in peripheral blood lymphocytes (PBL) 1, 3 and 9 days after a single i.p. injection of 250 mg kg⁻¹ of AIPP or ABPP. Control animals received PBS i.p. The percentage of specific lysis is shown as determined in a 3h²⁵¹Cr release assay at an effector-to-target (Yac-1) ratio of 40:1.

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Figure 2 Effect of pretreatment with AIPP or ABPP on s.c. growth of tumour LS175. AIPP or ABPP were administered at a dose of 250 mg kg⁻¹ i.p. on days −3, −2 and −1. The difference between the mean tumour diameter in the ABPP treated group (●) and the control group (○) was statistically significant (P < 0.003). No significant inhibition of tumour growth was observed in the AIPP treated group (the AIPP treated group (△)). Each group consisted of 5 animals.

insignificant inhibition was seen after pretreatment with AIPP.

The same pretreatment schedule was used in the lung metastasis model. Here a single cell suspension of 10⁵ tumour cells was injected in the tail vein on day 0. The rats were sacrificed on day 14 and the number of lung colonies was counted with the naked eye after fixation of the lungs in Bouin's solution. As shown in Table I, a significantly (P < 0.02) lower number of lung colonies was observed in the rats pretreated with ABPP or AIPP than in the control group. No difference existed between the two treated groups.

This inhibitory effect on the development of lung metastases is in agreement with the results reported by Milas et al. (1982) concerning immunogenetic as well as non-immunogenetic murine tumours.

The activity of both NK cells and macrophages at the time of i.v. tumour inoculation was strongly enhanced as a result of the pretreatment with either ABPP or AIPP. The activation of these two cell populations may well explain the results in the lung metastasis model.

Hanna et al. (1980) have shown that tumour cells are most sensitive to NK cell cytotoxicity when they are in a bloodborne or early lodging phase. The fact that tumour LS175 is NK resistant does not rule out an important role for activated NK cells to account for the observed lower number of lung metastases in the pyrimidone treated groups.

Table I Effect of pretreatment with AIPP or ABPP on the development of lung metastases after i.v. injection of 10⁵ LS175 cells

| Treatment | Mean number of lung metastases ± s.d. | Range |
|-----------|--------------------------------------|-------|
| PBS       | 39 ± 12                              | 24–56 |
| AIPP      | 18 ± 7                               | 10–23 |
| ABPP      | 13 ± 3                               | 10–16 |

(i) In vitro NK resistant tumour cells may be less resistant in vivo to highly activated NK cells (Talmadge et al., 1984). (ii) Very high NK cell activity can be induced in the lungs of rats by pyrimidinones (Lotzova et al., 1984). (iii) Activated NK cells have been shown to produce a variety of lymphokines like IFN-gamma and IL-2 (Kasahara et al., 1983).

IFN-gamma especially and a putative more rapidly working macrophage activating factor (MAF) secreted by NK cells in the rat and in man (Gomez et al., 1985) are very potent activators of alveolar macrophages.

The tumour site specific setting of a high concentration of (pyrimidinone-activated) macrophages further activated by NK-cell-secreted lymphokines could well explain the observed inhibition of the development of lung metastases. The difference in tumour reponse to ABPP and AIPP in the s.c. tumour model may be related to the difference in IFN serum levels after the administration of the two agents involved. When we observe the growth curve of the tumour in the ABPP pretreated group it is noted that the tumour seems to disappear over the first few days.

This is the only antitumour effect observed since tumour growth follows a parallel course to the other experimental groups when the tumour becomes measurable. Apparently tumour suppression is only short lived and no prolonged inhibitory effect can be observed.

This could be explained by the fact that high IFN levels are induced by ABPP for a period of only 24 h (Oku et al., 1984; Lotzova et al., 1984). Since LS175 has been shown to be IFN sensitive and IFN can have a direct inhibitory effect on tumour growth, a high IFN level at the time of s.c. tumour inoculation may have created the ±2 day
lag time in tumour growth that is observed in the animals pretreated with ABPP. Since multiple injections with ABPP induce a hyporeactive state and a fall in IFN levels (Oku et al., 1984), as is observed with many biological response modifiers (Talmadge et al., 1985), the antitumour effect in the s.c. model offers little prospect for an effective role for ABPP in this setting.

The anti-metastasis effect of these pyrimidinones is the more important observation, especially since the tumour used was NK resistant.

Interactions between activated NK cells and activated macrophages, both of which are present in high numbers in the lungs, may have overcome this resistance and may have brought about the effective lysis of the tumour cells by either cell population. Since the administration of pyrimidinones is virtually without toxicity or side effects (Earhart et al., 1985) these agents may hold some promise in boosting anti-metastasis protective mechanisms in a peri-operative or otherwise adjuvant setting.

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