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*Short communication*

# Histamine in immunotherapy of advanced melanoma: a pilot study

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**Abstract.** Sixteen patients with advanced metastatic malignant melanoma were treated with a high-dose infusion of interleukin-2 (IL-2; $18\times10^6$ IU/m$^{-2}$ day$^{-1}$) together with daily subcutaneous (s.c.) injections of interferon α (IFNα; $3\times10^6$ U/m$^{-2}$ day$^{-1}$) in 5-day cycles. Nine of these patients were given histamine (1 mg s.c.) twice daily during treatment with IL-2 and IFNα. In the seven patients who did not receive histamine, one partial response (that is a reduction of more than 50% in the total tumour burden) was observed in a patient with skin and lymph node melanoma. In the eight histamine-treated patients evaluable for response, four partial responses were observed. Two other patients showed regression at one site of metastasis but tumours remained unchanged at other sites. Two histamine-treated patients showed complete resolution of extensive liver metastasis. Sites of response in histamine-treated patients also included the subcutis, lymph nodes, skeleton, spleen and muscle. Lung melanoma did not respond to histamine/IL-2/IFNα. Three patients with lung tumours responded with significant (more than 50%) reduction of the volume of soft-tissue tumours, suggesting that the response to histamine may be organotropic. Survival was significantly prolonged in patients receiving histamine. Our data suggest that treatment with histamine may improve the antitumour efficacy of immunotherapy in metastatic melanoma.

**Key words:** Histamine – NK cells – Melanoma – Interleukin-2 – Interferon α – Immunotherapy

## Introduction

Interleukin-2 (IL-2) and interferon α (IFNα) are cytokines that effectively enhance the antitumour activity of natural killer (NK) cells in vitro [16] and exert significant antimetastatic effects in experimental animals in vivo by NK-cell-dependent mechanisms [3]. However, treatment of patients with solid cancers with IL-2 or IFNα has not been successful. Only approximately 15% of patients with metastatic malignant melanoma respond with significant reduction of tumour burden to IL-2 or IFNα used as single agents [13]. Combination therapy with IL-2 and IFNα has not significantly improved the response rate [11, 16].

One reason why potent immunostimulants such as IL-2 and IFNα are insufficiently effective in human cancer could be that suppressive signals counteract the activation of antitumour effector mechanisms. Recently it was shown that phagocytes, such as monocytes and granulocytes, inhibit functions of human NK cells including the killing of tumour cells, proliferation and cytokine production in vitro by a cell-contact-dependent mechanism. The biogenic diamine histamine, specifically acting via $H_2$-type histamine receptors ($H_2R$), maintains NK cell function in the presence of suppressive phagocytes by inhibiting the phagocyte-derived, suppressive signal [4, 5, 7]. By this mechanism, histamine and IL-2 [6] or histamine and IFNα [9] synergistically enhance the killing activity of NK cells against cultured and freshly recovered human tumour cells.

Similar mechanisms have been described in tumour-bearing experimental animals in vivo. Thus, treatment of mice with histamine, or $H_2R$ agonists, reduces the number of metastatic foci produced by B16/F1 melanoma cells by a mechanism that requires intact NK cells. Endogenous histamine seems to be critical for the antitumour activity of NK cells in vivo. Further, treatment with histamine and IL-2 completely eliminates mouse B16/F1 melanoma metastasis [8].

With this background, we chose to treat nine patients with advanced metastatic melanoma with histamine, at doses sufficient to activate $H_2R$, in addition to immunotherapy with IL-2 and IFNα. The results were compared with those obtained in seven melanoma patients treated with the same regimen of IL-2 and IFNα but without histamine.

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## Materials and methods

All patients were treated at the Department of Surgery, Sahlgren's Hospital, Göteborg, Sweden. The study was approved by the Ethical Committee at Sahlgren's Hospital, Göteborg. Informed consent was obtained from each patient. To be eligible for treatment, the patients had to have a histologically confirmed diagnosis of melanoma with one or more sites of distant metastatic involvement. Exclusion criteria were a history of myocardial infarction, congestive heart failure and significant cardiac arrythmias. All patients were subjected to brain computed tomography (CT) or magnetic resonance imaging (MRI) prior to onset of treatment; patients with cerebral melanoma were excluded. None of the patients had received chemotherapy before the onset of immunotherapy.

Patients 1–7 (see Table 1) were treated in 5-day cycles with continuous intravenous IL-2 (kindly provided by the Cetus Corporation) at $18\times10^6$ IU/m$^2$ for 24 h. IFNα (IFNα2b; Schering) was administered daily during the treatment cycles at $3\times10^6$ U/m$^2$ as a s.c. injection. Two such cycles were given with a 48-h interval. After a 4-week intermission, most patients received another two 5-day cycles of treatment. Patients with stable disease or a response after two cycles were continued on therapy consisting of single 5-day cycles, aimed at treatment every 4–6 weeks. Patients 1–7 entered the trial in March 1989.

Histamine dihydrochloride (1 mg) was given twice daily to patients 8–16 as a s.c. injection during treatment with IL-2/IFNα; otherwise, the treatment was identical to that given to patients 1–7. Patients 8–16 entered the trial in November 1990.

The response to therapy was evaluated after four completed cycles of treatment. Each site of tumour was evaluated as a complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) with reference to the tumour volume. CR was defined as complete resolution of all detectable tumour mass. PR was defined as more than 50% regression of tumour volume. PD was defined as an increase of the tumour volume by more than 25%. SD was defined as anything less than PR without PD. Table 1 shows the sites of tumour for each patient, the number of lesions at each site, the mean diameter of each lesion, and the local response to therapy. Table 1 also shows the overall objective responses recorded (PR) on the basis of more than 50% reduction of the entire tumour volume of each patient.

The side-effects induced by IL-2 and IFNα were similar to those described in detail by others [11, 13], i.e. fatigue, hypotension, fever, dermatitis, bacterial infections, lethargy, fluid retention, and nausea. Histamine produced a short-lasting flush in several patients. One patient (patient 12) experienced pulsating headache after each histamine injection, which required dose reduction; otherwise, histamine was well tolerated.

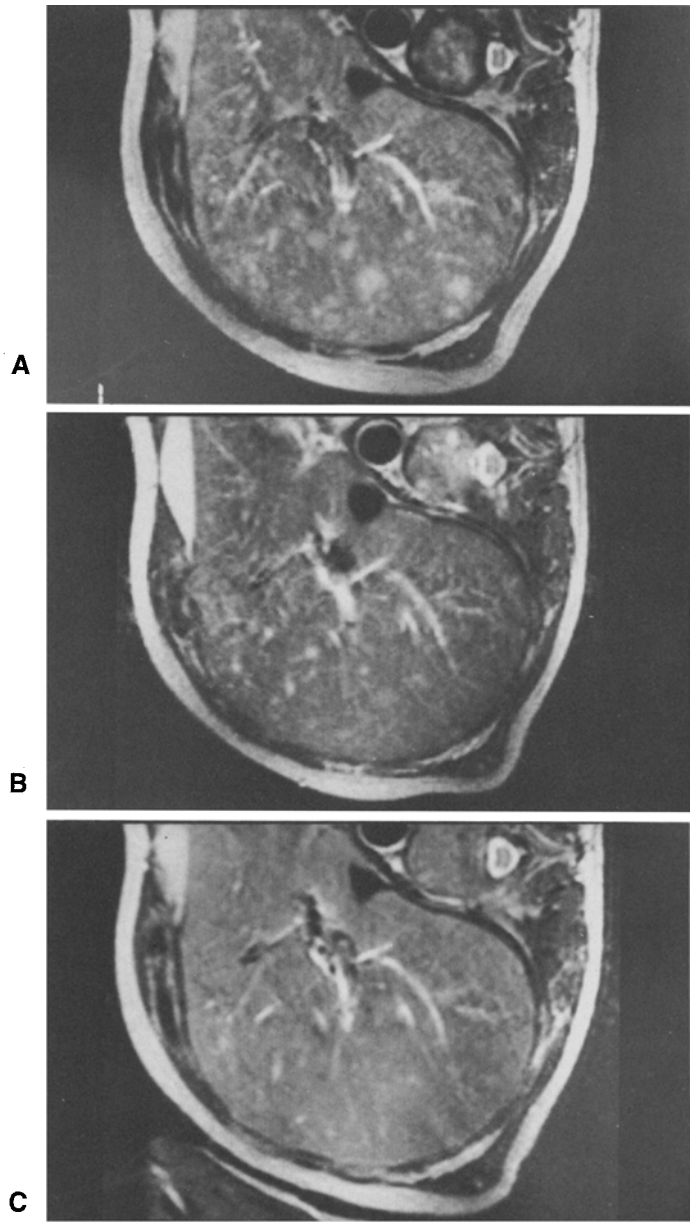


**Fig. 1A–C.** MRI scans of the upper abdomen in a patient (patient 11 in Table 1) treated with histamine in addition to immunotherapy with interleukin-2 and interferon α: before treatment (**A**), after four cycles (**B**), and after seven cycles of treatment (**C**)

## Results and discussion

In the seven patients who received IL-2 and IFNα without histamine, an objective overall response (CR or PR) occurred in one (14%; Table 1). In this patient (patient 3 in Table 1), the volume of subcutaneous and lymph node tumours was reduced by more than 75% (PR). Similar results were obtained in a study by Oldham and co-workers, who used an identical regimen of IL-2/IFNα. These authors reported regression of more than 50% of the total tumour burden in 7/66 (11%) melanoma patients treated with IFNα and high-dose infusion of IL-2 [11].

Objective responses in overall tumour volume were observed in four of the eight evaluable histamine-treated patients (50%; patients 8, 10, 11 and 14 in Table 1). In two patients (9 and 13) discordant responses with partial regression at one site without objective regression at other sites were observed. One patient (15) showed regression at one site but progressive growth of another lesion (Table 1). An additional patient (not included in the table; a 30-year-old man with abdominal melanoma) received four 5-day cycles of treatment including histamine, but no post-treatment evaluation of tumours was performed. The abdominal tumour showed significant regression 3 months later, but the patient had received chemotherapy prior to this evaluation.

None of the patients receiving IL-2 + IFNα without histamine is alive, and the mean survival time was 6.8 months (range 1–14.5 months) after the onset of treatment. Four patients in the group receiving histamine in addition to IL-2/IFNα are alive at 20, 18, 10 and 10 months; the survival time in the remaining five histamine-treated

**Table 1.** Immunotherapy of metastatic malignant melanoma with histamine and interleukin-2 (*IL-2*)/interferon α (*IFNα*) (*CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease)

| Patients | | | | | | |
|---|---|---|---|---|---|---|
| Sex | Age (years) | Treatment | Site of tumour (no.) | Diameter (cm) | Local response | Overall response |
| 1. F | 45 | IL-2/IFN | Lymph nodes (1)<br>Liver (2) | 5<br>2 | PD<br>PD | |
| 2. M | 59 | IL-2/IFN | Subcutis (2)<br>Liver (5) | 2<br>1–3 | PD<br>PD | |
| 3. F | 55 | IL-2/IFN | Subcutis (4)<br>Lymph nodes (1) | 3, 4, 5, 7<br>8 | **PR**<br>**CR** | **PR** |
| 4. M | 68 | IL-2/IFN | Subcutis (1)<br>Lymph nodes (1)<br>Liver (>10) | 1<br>4<br>1–3 | SD<br>SD<br>SD | |
| 5. M | 52 | IL-2/IFN | Subcutis (1)<br>Lungs (>10)<br>Liver (1) | 10<br>2<br>1–3 | SD<br>PD<br>SD | |
| 6. M | 53 | IL-2/IFN | Subcutis (1)<br>Lymph nodes (2) | 2<br>2, 3 | PD<br>PD | |
| 7. M | 62 | IL-2/IFN | Subcutis (2)<br>Lungs (4) | 2, 3<br>1–2 | PD<br>PD | |
| 8. M | 41 | IL-2/IFN<br>+ histamine | Lymph nodes (1)<br>Liver (5) | 2<br>1–5 | **PR**<br>**CR** | **PR** |
| 9. M | 54 | IL-2/IFN<br>+ histamine | Lymph nodes (1)<br>Lungs (>10) | 6<br>1–3 | **PR**<br>SD | |
| 10. F | 55 | IL-2/IFN<br>+ histamine | Subcutis (4) | 2–3 | **PR** | **PR** |
| 11. M | 49 | IL-2/IFN<br>+ histamine | Subcutis (5)<br>Liver (>10)<br>Skeleton (>10)<br>Spleen (4) | 2–3<br>1<br>1<br>1–2 | **CR**<br>**CR**<br>**CR**<br>**PR** | **PR** |
| 12. F | 33 | IL-2/IFN<br>+ histamine | Lungs (1) | 2 | SD | |
| 13. F | 57 | IL-2/IFN<br>+ histamine | Subcutis (5)<br>Lungs (1) | 1–2<br>2 | **PR**<br>SD | |
| 14. M | 38 | IL-2/IFN<br>+ histamine | Intramuscular (1) | 3 | **PR** | **PR** |
| 15. M | 41 | IL-2/IFN<br>+ histamine | Intramuscular (1)<br>Lungs (1) | 4<br>5 | **PR**<br>PD | |

patients was 24, 18, 9, 7 and 4 months. The mean survival time of the histamine-treated patients is in excess of 13.3 months. Survival time was significantly longer in patients receiving histamine in addition to IL-2 and IFNα ($2P < 0.05$, Mann-Whitney *U*-test).

Two histamine-treated patients with extensive liver metastasis showed complete resolution of all detectable liver melanoma. Patient 8 (Table 1) had 5 metastases (as verified by CT scan) with a diameter of 1–5 cm, all of which had completely disappeared after four cycles of treatment. Patient 11 had a miliary spread of liver melanoma with more than 50 small tumours (Fig. 1 A). After four cycles of treatment, approximately 10 of these tumours remained (Fig. 1 B). After seven cycles, liver tumours were not detectable (Fig. 1 C). The findings in these two patients are encourageing, since liver melanoma is considered relatively refractory to immunotherapy with IL-2 used as a single agent [1, 10, 12, 15] or combined with IFNα [2, 11, 16]. To our knowledge, complete regression of liver melanoma after treatment with IL-2 or IL-2/IFNα has not been reported before.

It is noteworthy that melanoma metastases regraded significantly in patients treated with histamine at all anatomical sites evaluated, with the exception of lungs. Thus, while 11/11 extrapulmonary lesions regressed by more than 50%, none of four patients with pulmonary melanoma showed regression of lung tumours. That the response to histamine may be organotropic was further suggested by the findings that three patients (9, 13 and 15) with non-responsive lung lesions showed more than 50% reduction of lymph node, subcutaneous or intramuscular lesions.

Although the admittance criteria as well as the immunotherapy with IL-2 and IFNα were identical in the two treatment groups, it should be emphasized that our study

was non-randomized. A controlled trial is required to establish whether the addition of histamine improves the antitumour response to immunotherapy in metastatic melanoma.

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