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Short communication

Local treatment of cutaneous and subcutaneous metastatic malignant melanoma with fotemustine

Karin U. Schallreuter, John M. Wood, Hartwig Mensing, and Eckhard W. Breitbart

Department of Dermatology, University of Hamburg, Federal Republic of Germany

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Summary. Fotemustine is a highly reactive chloroethylnitrosourea anti-tumor drug that is currently undergoing phase III clinical trials in stage IV metastatic malignant melanoma. The drug is a potent alkylating agent and rapidly chloroethylates the active sites of the important thioproteins thioredoxin reductase (TR), glutathione reductase (GR) and ribonucleotide reductase (RR). These enzymes control ribonucleotide reduction and, consequently, DNA synthesis in the S phase of the cell cycle. Side effects are minor due to the rapid metabolism of the drug. [^{14}C]-Fotemustine exhibited a half-life of 90 min in the vascular system after the administration of 100 mg/m^2. Fotemustine was shown to yield the volatile degradation product acetylene (a) in distilled water (4.1%/h), (b) in melanoma cell culture medium (MCDB) supplemented with 10% fetal calf serum (33%/h) and (c) in fotemustine-sensitive human melanoma cells in culture medium (70.5%/h). Due to its rapid metabolism and its low toxicity, high concentrations of fotemustine (55×10^{-3} M) were injected directly into cutaneous and subcutaneous melanoma metastases ($n = 36$) of seven patients, resulting in minor necrosis followed by total remission of the metastases. Untreated metastases adjacent to the treated tumors were not affected by fotemustine, confirming that rapid local metabolism of this drug occurs only in the vicinity of injected tumors without producing any systemic effects.

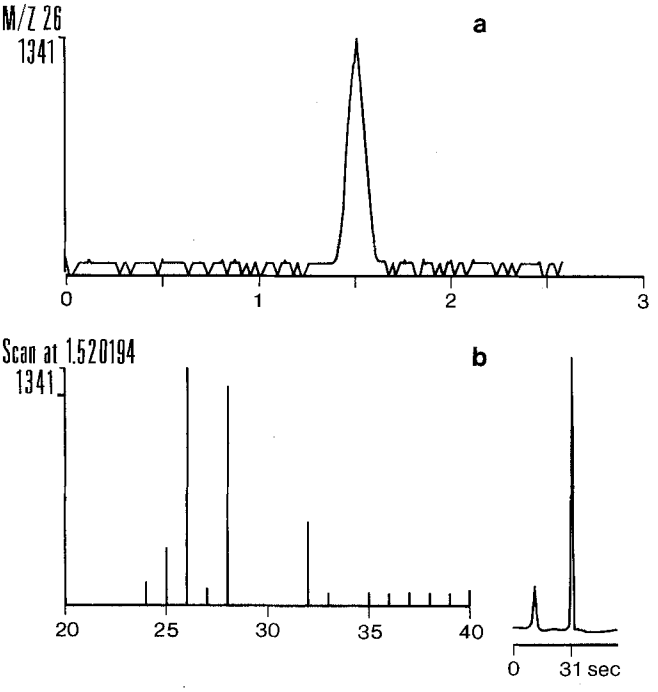

Fig. 1. a Mass spectrometry of the volatile reaction product of the degradation of fotemustine (0.58×10^{-3} M) in solution. The molecular ion has a mass of 26 [C_2H_2 (acetylene) = 26]. **b** Acetylene production as determined by quantitative gas chromatography based on a calibration of the peak height with C_2H_2 at a 31-s retention time

Introduction

Fotemustine is a novel chloroethylnitrosourea containing a diethylphosphonium group attached to an alanine residue as its non-polar constituent. The presence of the diethylphosphonium group enables rapid transport of the drug into all organs due to excellent penetration of the blood-brain barrier [1]. Recent preclinical studies using fotemustine have established that its principal mechanism of action involves alkylation of the thiolate-active sites of thioredoxin reductase (TR), ribonucleotide reductase (RR) and glutathione reductase (GR) to form chloroethylthioether-enzyme inhibitor complexes [6].

TR purified from human metastatic melanoma tissue has been shown to be 500 times more sensitive than GR to fotemustine [6]. Melanomas from patients responding to TR contained high amounts of TR and low levels of GR, whereas resistant tumors were found to contain high amounts of GR and low levels of TR [5]. Since TR and GR

Offprint requests to: Dr. K. U. Schallreuter, Department of Dermatology, University of Hamburg, Martinistr. 52, W-2000 Hamburg 20, FRG

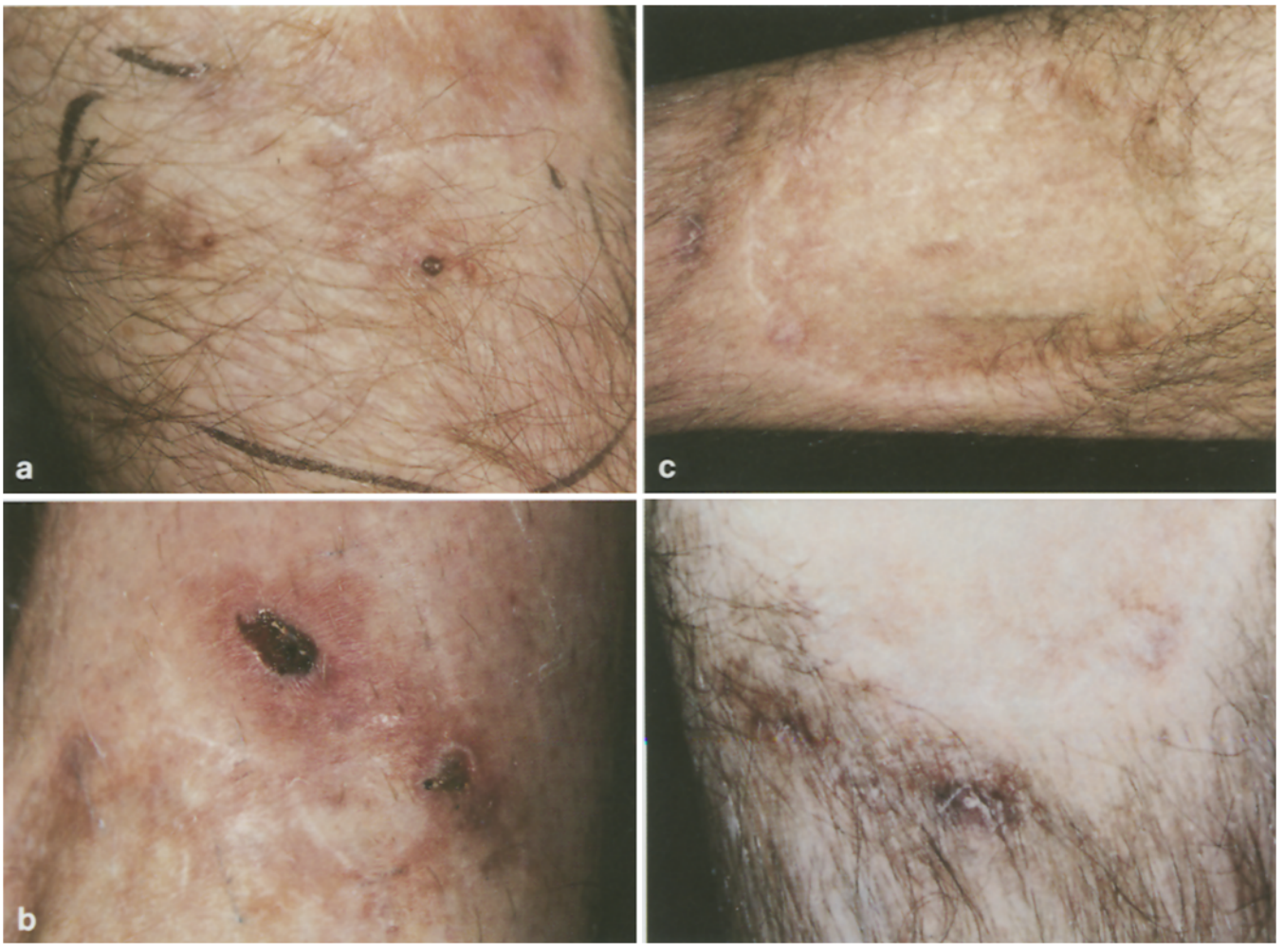

Fig. 2 a–c. This 22-year-old man presented with an ulcerated nodular malignant melanoma (Clark V, Breslow 6.3 mm) on the left lower leg and was admitted in October 1988 for surgical excision (diameter, 5 cm). The first intransit metastasis appeared in July 1989. The patient refused hyperthermic perfusion. In May 1990, he developed two *in loco* metastases in the vicinity of the old scars, and these were treated with fotemustine. **a** Two epicutaneous metastases on the old scars. **b** Metastases at days after the injection of fotemustine (55×10^{-3} M). **c** The result of local fotemustine therapy in November 1990 (lower port: close-up). The patient did not develop any new metastases for 10 months

Table 1. Stability of fotemustine in solution[a]

Conditions	% Acetylene derived from fotemustine/h
Distilled water	4.1
Serum-free MCDB culture medium	6.3
MCDB culture medium with 10% fetal calf serum	33
Human keratinocytes (10^6 cells) in serum-free MCDB	12
Human melanocytes (10^6 cells) in 0.5% fetal calf serum	58
Fotemustine-resistant melanoma cells (10^6 cells, Cal 7) in 10% fetal calf serum	31.3
Fotemustine-sensitive melanoma cells (10^6 cells, Cal 1) in 10% fetal calf serum	70.5

[a] Acetylene production was measured as described in Patients and methods

represent alternate electron donors for ribonucleotide reduction via RR in the S phase of the cell cycle, it appears that this metabolic difference may well determine the success or failure of fotemustine monotherapy [3, 5]. A clinical trial carried out in 19 patients presenting with metastatic melanoma (clinical stage IV according to the 1987 UICC classification system) yielded a 47% response rate using a modification of the EORTC protocol (confidence limit, 95%; range, 24–71%). Modifications involved a rapid infusion using *freshly* dissolved fotemustine and a reduction in the rest period from 5 to 3 weeks [4, 7].

Since TR, GR and RR can be inhibited by different concentrations of the drug, the rationale for the present study was to follow in detail the degradation of fotemustine under a variety of conditions with a view toward proving the efficacy of direct injections of high concentrations of the drug into cutaneous and subcutaneous metastases.

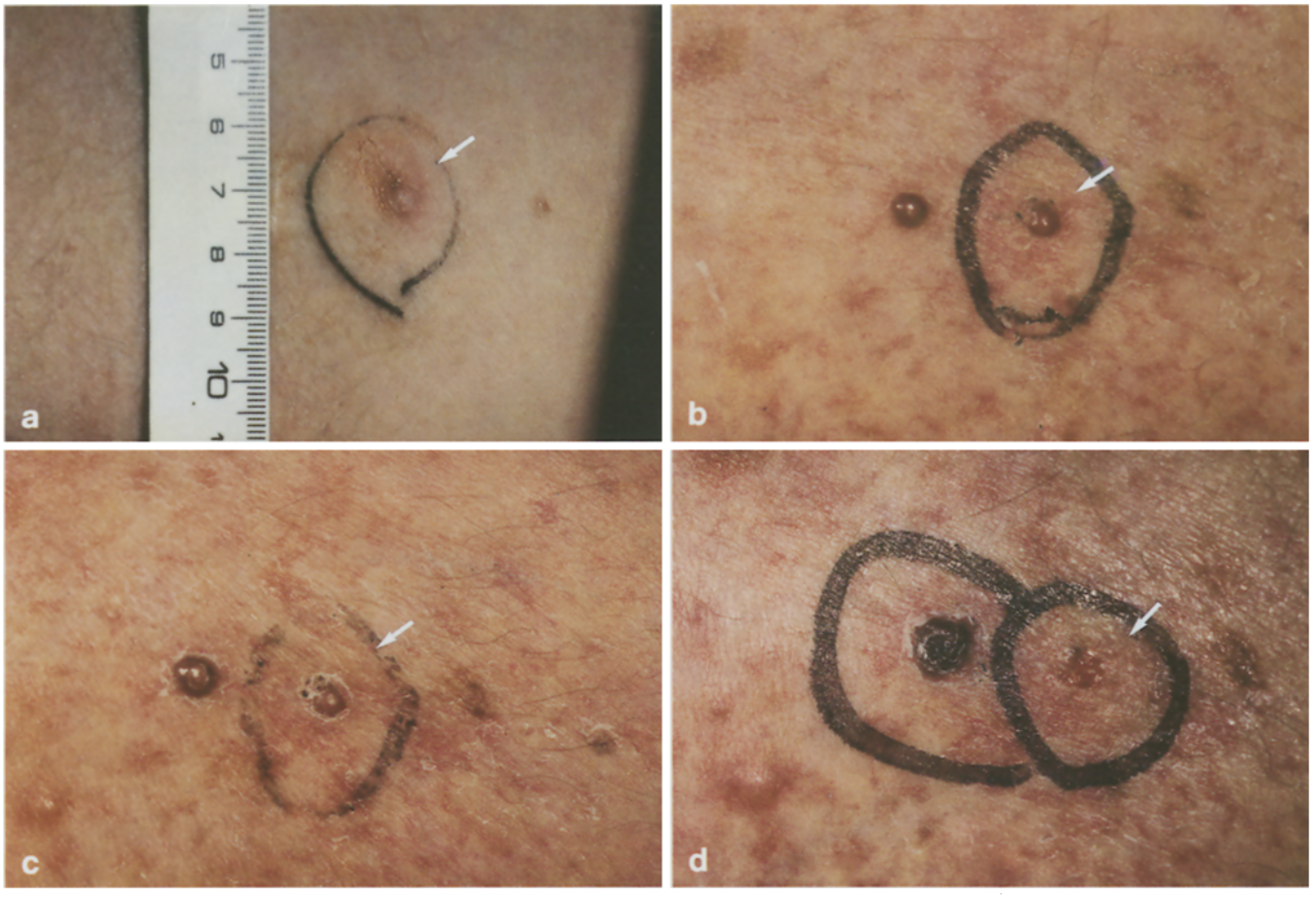

Fig. 3 a–d. This 54-year-old woman presented with nodular malignant melanoma (high risk) associated with lung metastases and multiple cutaneous metastases on the left lower extremity. **a** A metastasis treated with fotemustine (*arrow*), and an adjacent untreated tumor. **b** Response to fotemustine after 2 days. **c** Response after 9 days. **d** Response after 14 days. The untreated tumor increased in size; whereas complete remission of the treated metastasis was achieved

Patients and methods

Fotemustine was obtained from Servier Company (Paris, France) [2]. All other reagents were supplied by Sigma Chemical Company (St. Louis, Mo., USA).

Gas chromatography. Experiments to determine the stability of fotemustine were conducted in 3 ml sealed serum vials. Reactions were conducted in 1 ml solutions containing 0.58×10^{-3} M fotemustine. The gas volume in the sealed serum vials was 2 ml. In all, 40 µl, gas was injected into a Hach-Carle AGC-100 gas chromatograph (150° C isothermal) using N_2 as the gas carrier at a flow rate of 30 ml/min for flame ionization detection (FID). The major volatile product was acetylene (retention time, 31 s). The structure of the gaseous product was determined by mass spectrometry (Fig. 1).

Patients and treatment protocol. Seven patients (four men and three women: mean age, 52.7 years; range, 22–67 years) presenting with metastasized melanoma of clinical stage II–IV (according to the 1987 UICC classification system) were selected for the protocol. All patients exhibited cutaneous or subcutaneous metastases and received no other treatment during the observation period. Individual tumors were injected every 30 min for 2–3 consecutive h with 55×10^{-3} M fotemustine. The drug was freshly dissolved in 0.1 ml ethanol (95%) and then diluted with physiological saline to a volume of 2 ml. Aliquots were injected directly into and below the tumors. The follow-up included a weekly control using photographs. In three cases, biopsies were taken to assess the vitality of the tumor.

Histology. Biopsies of the injected tumor were taken at 21 days after medical treatment following local anesthesia with xylocaine. Both H&E and S100 staining were used.

Results

The stability of fotemustine in solution could be assessed by measuring the volatilization of acetylene from the drug using quantitative gas chromatography. The degradation rates of fotemustine/h were determined for a 0.58×10^{-3} M solution (a) in distilled water, (b) in MCDB cell culture medium in the presence and absence of 10% fetal calf serum, (c) in serum-free cultures of human keratinocytes, (d) in cultures of human melanocytes supplemented with 0.5% fetal calf serum and (e) in fotemustine-sensitive (Cal 1) and -resistant (Cal 7) human melanoma cell lines in MCDB supplemented with 10% fetal calf serum. Previous investigations had established that the resistance of Cal 7 cells to fotemustine was based on low rates obtained for the transport of and reactivity with ^{14}C-labelled drug as compared with those found for fotemustine-sensitive (Cal 1) cells [5]. The results of this study on fotemustine's stability are presented in Table 1. Based on our knowledge of the instability of fotemustine, it was anticipated that successful

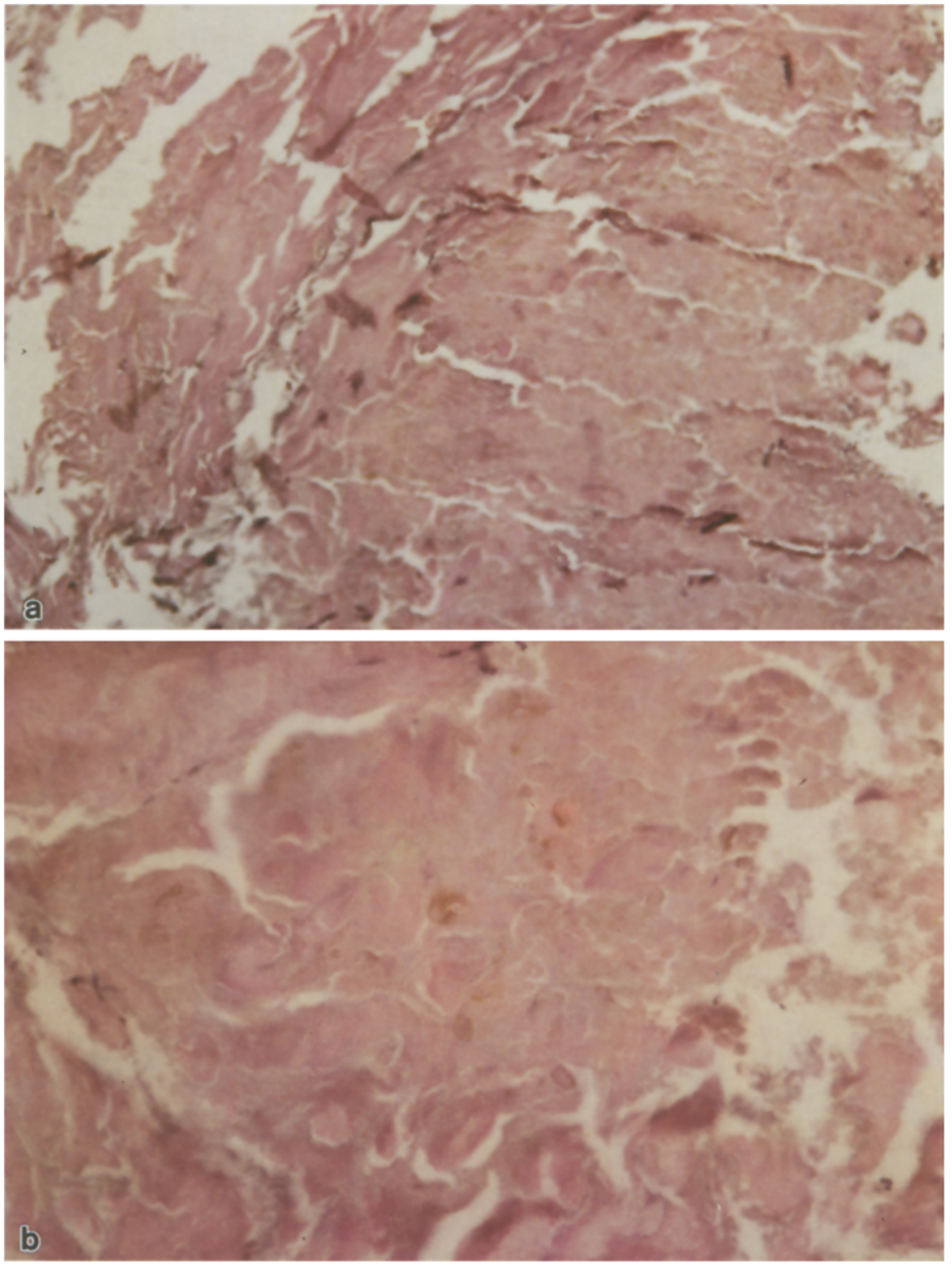

Fig. 4 a, b. Histologic examination of punch biopsies taken at **a** 4 weeks and **b** 6 weeks after treatment with fotemustine. **a** Total necrosis with a homogeneous eosinophilic mass, whereby the cells can be recognized only as shadows. **b** Detailed view, whereby some pigment from the tumor cells remains recognizable but cell structures are not visible

chemotherapy with this drug required its fresh preparation followed by its rapid infusion into patients so as to deliver an effective dose to the melanoma metastases. In addition, the biochemical mechanism of action of fotemustine indicated that the direct delivery of high concentrations of the drug to the tumor would have to be 100% effective.

Based on this rationale, 7 patients exhibiting 36 cutaneous or subcutaneous metastases were selected for local injections of fotemustine (55×10^{-3} M) directly into and below the tumors. Figure 2 shows the results obtained in one of these patients, who experienced some necrosis followed by complete remission of the metastases as shown by subsequent histologic examination (Fig. 3). However, the reactivity and degradation of fotemustine was limited to the zone of injection in each metastasis. Figure 4 shows two adjacent metastases; one was injected with fotemustine and the other was left untreated over a period of 2 weeks. Total remission of the injected tumor left little scarring, whereas the untreated metastasis continued to increase in size.

Discussion

The results of the present study demonstrate that the administration of fotemustine directly into cutaneous and subcutaneous melanoma metastases provides effective palliative treatment and good cosmetic results. The technique

is simple and was well tolerated by the patients without affecting their quality of life. All metastases (diameter, 0.5–1 cm) injected with fotemustine showed 100% remission as determined by standard histologic procedures. No systemic side effects were caused by cutaneous or subcutaneous injection of the drug. This local treatment could be rationalized only on the basis of the preclinical studies of fotemustine's instability, of its lack of toxicity, and of a detailed understanding of the mechanism of action of the drug [3, 5, 6]. The method described provides a new mode of palliative treatment of special cases of melanoma exhibiting recurrent cutaneous metastases. Our results also indicated that this treatment should be limited to metastases that measure $\leqslant 1$ cm in diameter so as to avoid major necrosis and complications during the healing process. In our experience, patients have favored this procedure over surgical and cryosurgical intervention due to the minor discomfort involved and the cosmetically satisfactory results.

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