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Azelaic acid decreases the fibrinolytic potential of cultured human melanoma cells in vitro

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Abstract

Azelaic acid (AZA) has been used successfully in the treatment of lentigo maligna and malignant melanoma. Since it is generally accepted that the fibrinolytic potential of tumour cells is related to their malignant phenotype, it was the aim of this study to investigate the effect of AZA on the fibrinolytic potential of three different human melanoma cell lines (Bowes, GUBSB and MJZJ). Melanoma cells were incubated with AZA in doses ranging from 10^{-2} M to 4×10^{-2} M for 5, 8 and 24 h. The expression of tissue-type plasminogen activator (t-PA), urokinase-type PA (u-PA) and PA inhibitor-1 (PAI-1) in such treated cells was investigated by specific ELISAs on the protein level and by Northern blotting on the mRNA level. AZA caused a time and dose dependent decrease in the fibrinolytic potential of all three cell lines investigated by decreasing t-PA antigen in Bowes, by decreasing u-PA antigen in GUBSB and by increasing PAI-1 antigen in MJZJ cells, respectively. There was no significant difference between the viability of cells in control cultures and those treated with AZA. The effect of AZA on specific mRNA for t-PA in Bowes cells, u-PA in GUBSB and PAI-1 in MJZJ was consistent with its effect on the secretion of these fibrinolytic proteins by the respective cells. The results show that AZA decreases the fibrinolytic potential of the three human melanoma cell lines in vitro. This decrease may be operative in the mechanism by which AZA has been shown to affect malignant melanoma in vivo.

Keywords: Azelaic acid; Melanoma cells; Fibrinolytic potential

1. Introduction

In 1978 Nazzaro-Porro and Passi [9] reported that certain lipid fractions, mainly C9–C11 dicarboxylic acids, are capable of inhibiting tyrosinase activity in

vitro. They suggested that such dicarboxylic acids administered locally, might therefore be used in the treatment of hyperpigmentary disorders. In fact, azelaic acid (AZA), a C9 dicarboxylic acid, has been used successfully in the treatment of lentigo maligna and malignant melanoma by them [10,12] and others [7] (for a review of the therapeutic efficacy of AZA, see also Ref. [3]).

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In vitro studies with AZA have suggested several mechanisms of action to explain the beneficial effects of AZA seen in vivo. These include inhibition of tyrosinase activity [11], inhibition of mitochondrial enzymes [13,14] and inhibition of RNA and DNA synthesis [4,7]. In recent publications Grammatico et al. reported on the effects of AZA on the viability, ultrastructure and karyotype of melanoma cells in long-term culture [5,6].

It is generally agreed that the fibrinolytic potential of tumour cells is related to their malignant phenotype [1]. We have reported that AZA decreases the plasminogen activator activity (PAA) in conditioned media of the human melanoma cell line CRL 1424 [15], and Mensing et al. showed a correlation between the effect of AZA on PAA and the migratory behaviour of melanoma cells [8]. In these studies only overall PAA had been determined and no attempt was made to differentiate between the two known types of PAs, urokinase (u-PA) and tissue-type plasminogen activator (t-PA) or to determine plasminogen activator inhibitors (PAI). We have described previously several human melanoma cell lines exhibiting different fibrinolytic patterns with preferential secretion of either t-PA, u-PA or PAI-1, respectively [16] and it was therefore the aim of this study to investigate the effect of AZA on the fibrinolytic system in three different melanoma cell lines secreting either of the fibrinolytic components preferentially in culture.

2. Materials and methods

Bowes, GUBSB and MJZJ melanoma cells [16] were obtained as indicated and cultured to confluence in 24-well plates (Costar, MA) in RPMI 1640 (Flow Laboratories, UK) containing 10% supplemented calf serum (SCS) (Hyclone, UT). Twenty-four hours prior to the experiments, the cells were washed with Hank's balanced salt solution (HBSS) and incubated in RPMI containing 1 mg/ml bovine serum albumin (BSA). Thereafter confluent melanoma cells were incubated with increasing doses ranging from 10^{-2} M to 4×10^{-2} M of AZA (Serva, Germany) for 5, 8 and 24 h.

t-PA, u-PA and PAI-1 antigen in conditioned media harvested from such cells was measured by commercially available enzyme linked immuno-sorbent

assays (ELISAs; Technoclone, Austria). AZA had no direct effect on the respective ELISAs when added to the samples to be tested.

Total RNA was recovered from cell cultures incubated under experimental and control conditions by acid guanidine thiocyanate–phenol–chloroform extraction as described [2]. Random primed [32 P]dCTP-labelled probes for human u-PA (ATCC, ML), human PAI-1 (PCR-amplified coding sequence from MJZJ cDNA), human t-PA (Dr. Pannekoek, Amsterdam) or rat GAPDH (Dr. Busslinger, Vienna) were used to probe for specific mRNAs.

The viability of the melanoma cells under experimental and control conditions was assessed by trypan blue exclusion. There was no significant difference in viability of cells as determined by trypan blue exclusion in experimental or control conditions.

All values shown in the respective figures represent means \pm SD of three experiments each performed independently in triplicate if not stated otherwise. Data presented in Fig. 2A–C were analysed statistically by performing one-way analysis of variance (Instat-Graphpads, CA).

3. Results

Under control conditions Bowes cells expressed only t-PA, MJZJ cells expressed only PAI-1 and GUBSB cells expressed predominantly u-PA, but also PAI-1 as well as minimal amounts of t-PA (Fig. 1).



Fig. 1. Basal production rate of t-PA (clear bars), u-PA (dotted bars) and PAI-1 (hatched bars) by 3 melanoma cell lines in ng/10⁵ cells determined in media conditioned for 24 h.

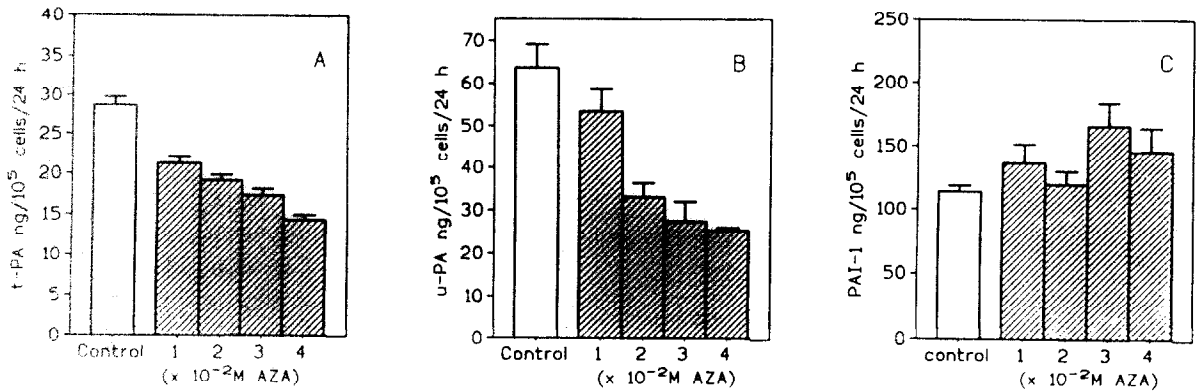


Fig. 2. t-PA antigen production by Bowes cells treated with increasing doses of AZA (A). u-PA antigen production by GUBSB cells treated with increasing doses of AZA (B). PAI-1 antigen production by MJZJ cells treated with increasing doses of AZA (C). The respective melanoma cells were incubated for 24 h with the indicated doses of the dicarboxylic acid or with serum free media only.

Increasing concentrations of AZA caused a significant dose dependent decrease in t-PA production by Bowes cells ($P < 0.0001$) (Fig. 2A) and a significant dose dependent decrease in u-PA production by GUBSB cells ($P < 0.0001$) (Fig. 2B). AZA also increased t-PA production and decreased PAI-1 production by GUBSB cells (data not shown). Increasing concentrations of AZA caused a slight but also significant dose dependent increase in PAI-1 production by MJZJ cells ($P < 0.003$) (Fig. 2C).

Fig. 3 shows the time course of the predominantly secreted fibrinolytic component in the conditioned media of the respective cell lines under control conditions and in the presence of 4×10^{-2} M AZA. No effect on expression of t-PA by Bowes cells can be seen until 8 h, and only after 24 h is t-PA in the media reduced (Fig. 3A). In GUBSB cells similarly a significant reduction in u-PA is seen after 24 h in conditioned media from AZA treated cells as compared to control cells (Fig. 3B). In the case of MJZJ the PAI-1 content in the conditioned media reaches a plateau after 8 h, being slightly higher in the presence of AZA (Fig. 3C).

When mRNA prepared from cells exposed to AZA for the time periods indicated in the figure legend were analysed by Northern blotting with mRNA probes for the respective predominantly found fibrinolytic component, the results shown in Fig. 4A were obtained. AZA caused a 30% reduction in t-PA mRNA in Bowes cells, a 35% reduction in u-PA specific mRNA in GUBSB cells and a 32% increase

in PAI-1 specific mRNA in MJZJ, respectively, as compared to the respective controls (Fig. 4B).

4. Discussion

We have shown that addition of AZA reduced the fibrinolytic capacity of three melanoma cell lines. t-PA in Bowes cell line was reduced by approximately 50%, u-PA in GUBSB was reduced by approximately 55% and PAI-1 in MJZJ was increased by up to 40%. In GUBSB, however, the reduction in u-PA was somewhat counterbalanced by a simultaneous increase in t-PA and a decrease in PAI-1 production caused by AZA. When calculated on a molar basis of the involved profibrinolytic (t-PA and u-PA) and antifibrinolytic (PAI-1) molecules, the fibrinolytic potential in these cells after treatment with AZA was only about 60% as compared to the untreated control cells.

Since the production of the respective fibrinolytic components in all cell lines was normalised for cell numbers the observed changes are not likely to be caused by antiproliferative effects of AZA. It has to be pointed out, however, that the actual fibrinolytic activity is also influenced by factors not determined in this study like u-PA receptors, PAI-2 and α_2 -antiplasmin.

The effects of AZA on the expression of the respective antigens were also reflected at the level of specific mRNA expression as demonstrated by Northern blotting. However, the magnitude of

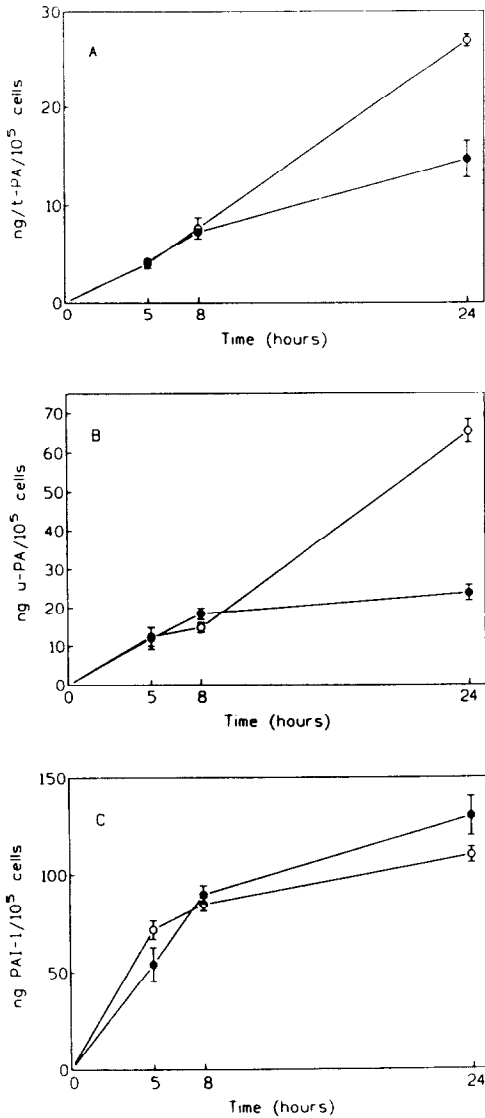


Fig. 3. Time course of the predominantly secreted fibrinolytic component in the conditioned media of Bowes cells (A), GUBSB cells (B) and MJZJ cells (C) under control and experimental conditions. The respective melanoma cells were treated with 4×10^{-2} M AZA (●) or left untreated (O).

changes seen in specific mRNAs was such that other mechanisms, e.g. on the level of translation or mRNA stability, cannot be ruled out.

In this study the expression of the fibrinolytic pa-

rameters by melanoma cells was affected by AZA used at concentrations between 1×10^{-2} and 4×10^{-2} M. In other studies AZA has been shown to inhibit tyrosinase activity, DNA synthesis and mitochondrial enzymes and result in karyotype modifications when used at similar concentrations ranging from 0.1×10^{-2} to 8×10^{-2} M [4,6,7,11,14]. In vivo AZA has been used topically at concentrations of 10-15 g/day [12]. Therefore it seems likely that therapeutic doses reached locally are comparable with doses of AZA which had been shown to be effective in vitro as described above.

It is generally accepted that the fibrinolytic potential of tumour cells correlates with their respective malignancy and might play an important role in tumour invasion and metastasis [1]. Our results show that AZA decreases the fibrinolytic potential of all three human melanoma cell lines investigated. This decrease might contribute to the mechanisms by

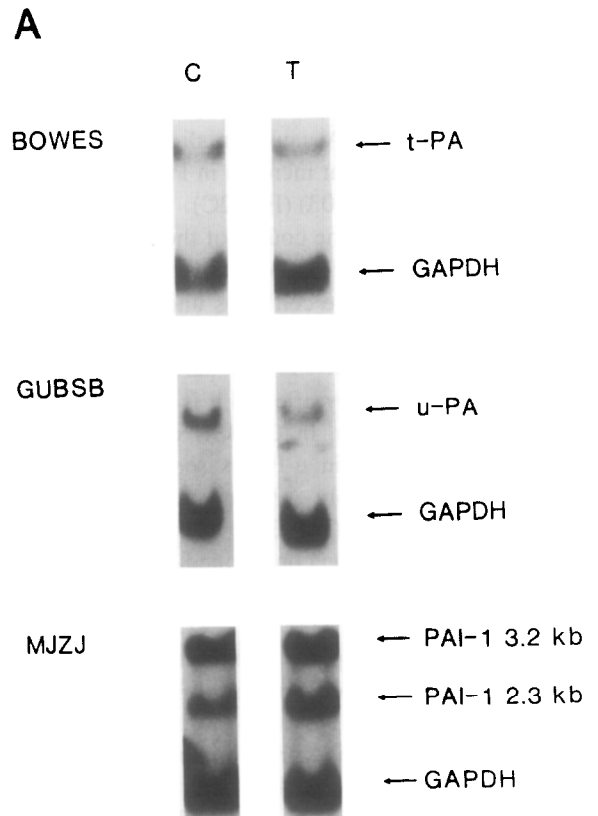


Fig. 4A.

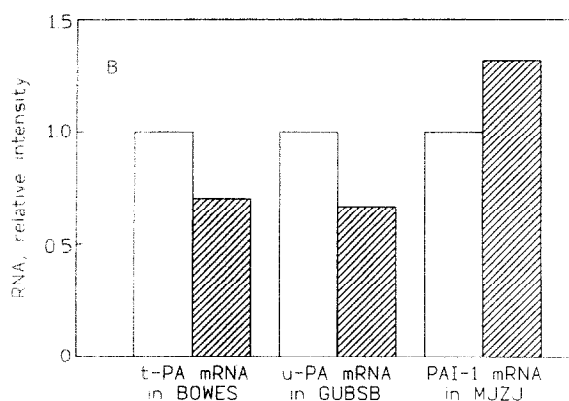


Fig. 4. (A) Northern analysis of t-PA specific mRNA in Bowes cells, u-PA specific mRNA in GUBSB cells and PAI-1 specific mRNA in MJZJ cells. GAPDH specific mRNA was used as a control in all cell lines tested. The respective melanoma cells were incubated in the absence (C) or presence of 4×10^{-2} M AZA (T) for 7 h (Bowes), 3 h (GUBSB) or 1 h (MJZJ), respectively. (B) Quantitation of the mRNA levels for t-PA, u-PA and PAI-1. Northern blots shown in (A) were scanned densitometrically and the absorbance values for t-PA, u-PA and PAI-1 were divided by the respective absorbance value for GAPDH to account for uneven loading. The score of 1 was arbitrarily assigned to the respective untreated controls. Open bars represent values from untreated controls. hatched bars give values for the respective melanoma cell line incubated with AZA.

which AZA has been observed to affect the behaviour of transformed melanocytes such as those seen in malignant melanoma, and might be one of the explanations for the beneficial effects seen upon local treatment of lentigo maligna or malignant melanoma with AZA.

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