INTRODUCTION

It is now seventeen years since a preliminary report was published describing a beneficial regressive effect of topical and oral azelaic acid on lesions of primary cutaneous malignant melanoma (1). Clinical regression of lesions was accompanied by destruction of malignant melanocytes and return to normal organization of epidermis and dermis, as seen by light and electron microscopy. At the time, no explanation could be given for these effects, beyond the observation that, in vitro, azelaic acid is a competitive inhibitor of tyrosinase, the key enzyme for melanogenesis.

Since then, chemical, biochemical, pharmacological, metabolic, toxicological, cytological, genetic, cell-cultural, radioautographic xenotransplantation and other studies have been carried out in the original investigator's laboratories and elsewhere, which have fully established the biological properties and activities of azelaic acid (see below and References), and which provide explanation and rationale for its clinical effects. These lie mainly in the areas of anti-tumoural, anti-bacterial, and anti-inflammatory activities. The latter two have already been successfully applied to the treatment of acne (2,3), and rosacea (4,5) and its anti-pigmentary activity to the treatment of melasma (6,7). Here, the anti-tumoural effects of azelaic acid come under examination from the point of view of its potential as a general antitumoural agent. It can be administered topically, focally, orally, intravenously, intra-arterially, and intralymphatically, all without local or general ill-effects, and is metabolized without harmful side-products. Simultaneous administration by different routes can ensure delivery of high concentrations at lesional sites and for sustained periods. Courses can be repeated. In addition to melanoma, cutaneous and bronchial squamous cell carcinoma, bladder and breast cancers, and leukaemia would seem to be ideal candidates for further clinical investigation and trial of the anti-cancer potential of azelaic acid, as prime, adjuvant, and palliative therapy, and for disseminated disease.

BIOCHEMISTRY AND PHARMACOLOGY OF AZELAIC ACID

Biochemistry

Azelaic acid (HOOC-(CH2)7-COOH) is a naturally occurring straight-chained, 9-carbon atom saturated dicarbo-
xylic acid obtained by oxidation of oleic acid by nitric acid and by chemical, physical, or biological oxidation of free and esterified fatty acids with the first double bond in position 9–10. It occurs in small amounts in the urine of normal individuals and in excess in the urine of patients with ketosis and in those with a congenital or acquired inability to β-oxidize monocarboxylic acids – dicarboxylic aciduria (8). It is probably generated in vivo by liperoxidation of free and esterified cis-poly-unsaturated fatty acids such as those normally present in cell membrane phospholipids and may act as a natural antioxidant in vivo (6). In vitro, azelaic acid is a competitive inhibitor of a number of oxidoreductive enzymes including tyrosinase (9), enzymes involved in the synthesis of DNA, such as thioredoxin reductase (10) and DNA polymerase (11), and of mitochondrial oxidoreductases of the respiratory chain (12). It is a potent inhibitor of microsomal 5-alpha-reductase (13), and also inhibits anaerobic glycolysis (14). In vitro, it is a scavenger of toxic oxygen species, particularly the toxic hydroxyl free radical, and inhibits oxynradical activity in cell cultures (15,16). It also inhibits generation of reactive oxygen species by neutrophils (17). Some of these activities are involved in its anti-tumoural properties.

Pharmacology
Azelaic acid lacks acute or chronic toxicity and is non-teratogenic and non-mutagenic (18,19). It can be administered to humans topically, orally, and in the form of the disodium salt by intra-tissue injection or infusion, intravenously, intra-arterially, and intralymphatically, all without local or general ill effects (20). Topically applied, up to 8.1% or 31% of the dose of azelaic acid is absorbed from gel, and viscosized/water micro-emulsion formulations (21). Orally, up to 20 g per day in capsule for six months may be given, with no side-effects apart from transient mild gastric irritation (20). After oral administration, the serum concentration peaks after 2–3 hours (75 mg/L after a dose of 5 g), and is negligible after 8 h. Intravenous administration of 10 g over 1–1.5 h achieves a serum level of 589 to 900 mg/L peaking at 2 h, dropping to negligible levels after 4 h due to rapid renal excretion (20). Twenty grams given over 4 h reaches a serum peak of 1450 mg/L after 4 h, dropping to 650 mg/L at 6 h, and 20 mg/L after 8 h. Prolonging the infusion with succes-sive doses of similar concentration produces sustained higher serum concentrations over a period. It has been estimated that 90% of maximal uptake should be reached in the plateau phase of constant infusion of 2.2 g per h, with a maximum cellular (normal) uptake of 0.657 g per h (22,23). The serum levels achieved are equivalent to 5 ×10^{-3} mol/L and above, which is the level at which azelaic acid has an anti-proliferative and cytotoxic effect on tumoural cells in culture.

However administered, 60% of azelaic acid is eliminated unchanged in the urine within 12 h, and part is metabolized by β-oxidation in the mitochondria via pimelic and glutaric acids to acetyl coenzyme A, which enters the Krebs cycle and gives rise to CO₂, and to malonyl coenzyme A, which may be involved in fatty acid biosynthesis (24). No toxic metabolites are generated. Azelaic acid crosses the blood–brain barrier (20).

ANTITUMOURAL ACTIVITIES IN VITRO
Cell culture
In cell culture azelaic acid has been shown to have a time- and dose-dependent reversible antiproliferative and cytotoxic action on the following tumoural cell lines: human cutaneous malignant melanoma (25–27), human choroidal melanoma (28), Harding–Passey and Cloudman murine melanoma (29,30), human squamous cell carcinoma (31), Raji, lymphoma, and leukaemia-derived cell lines (32), and fibroblastic lines (33). It has been shown to penetrate selectively tumoural cells as compared with normal cells (32), on which it has no effect at similar concentrations (34). The antitumoural effect is associated with inhibition of DNA synthesis (11), and damage to mitochondria (29,30,28). Azelaic acid also affects the karyotype of melanoma cells exposed to subtoxic doses in long-term culture, selectively affecting undifferentiated cells with a high growth rate and chromosomal abnormalities (35). It has an effect on plasminogen activator activity (36), and decreases the fibrinolytic activity of cultured melanoma cells in vitro (37). The fibrinolytic potential of tumour cells correlates with their respective malignancy and may play an important role in tumour invasion, progression, and metastasis (38). An anti-viral effect has been reported (39). Azelaic acid has an antibacterial activity against a wide range of organisms (40) and, in so far as secondary infection can be a complication in some tumoural situations, this property could prove useful.

ANTITUMOURAL ACTIVITIES IN VIVO
Xenotransplantation
Human melanoma cells implanted (tumours grown) on the peritoneum of mice who had been fed azelaic acid, regressed significantly as compared with controls (9).

Human melanoma cells xenotransplanted onto athymic nude mice were significantly affected by topical, perifocal, and intravenous administration of azelaic acid (41). There was a clear reduction in the mitotic index and in the autoradiographic [³H]-thymidine labelling index, the latter
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in vitro studies, workers used different concentrations of azelaic acid in the medium varying from $1 \times 10^{-5}$ to $5 \times 10^{-2}$ M. These seem to be relatively high concentrations, and led to the criticism that the effect might be due to perturbations of pH, or osmolarity. However, in our cultures (29), we always had a control with the C$_3$ dicarboxylic acid, adipic acid, and showed that even at much higher concentrations than that of the azelaic acid to which cells were exposed and inhibited or killed, there was no effect with the adipic acid. Leibl et al (41) have confirmed this. We also showed that when the disodium salt was used, the sodium content of the medium was not a factor in the effect on the cells.

Clearly, it is desirable that further xenotransplantation experiments involving other human tumoural cell lines, such as those referred to below, should be performed to establish which might also be sensitive. There is a rumour that experiments were conducted at the National Cancer Institute of the USA in the early 1980s but, as far as can be determined, the results were never published.

**CLINICAL**

**Melanoma in situ and malignant melanoma**

The only tumoural situations in which azelaic acid has been used and shown to be effective clinically are melanoma in situ, uncomplicated (42–44) and progressed to invasive melanoma (45), primary cutaneous malignant melanoma (1,46) and Grade IIIA melanoma with satellitosis (47). Our results with melanoma in situ (have been confirmed by others (21,41,48) but, to date, no other workers have published case reports of its realistic application to malignant melanoma. There are reports of a beneficial effect on penile lentiginosis (49) and reticulate acropigmentation of Kitamura (50).

Of all tumours, melanoma must be the most difficult to bring to double-blind, randomized, controlled clinical trials. What is needed further to evaluate the potential of azelaic acid in the treatment of melanoma is first of all to encourage, or persuade physicians to try it on suitable individual cases for whom no other current treatment is contemplated, on a named-patient basis. This is a prime purpose of this review. If shown to produce an effect, as evidence to date suggests, the results should be published as case reports. The cumulative effect of individual confirmed successes can persuade others to try a new therapy. With sufficient of these case reports published, Stage 1 trials might be organized. Fitzpatrick (51), doyen of pigment cell biologists and expert on melanoma, who was originally very sceptical of the effects of azelaic acid, later referred to it as ‘a major break-through in the quest for a rational chemotherapy of malignant melanoma’, and went on to advocate the institution of clinical trials of its adjuvant effect on surgery for primary melanoma. One might also suggest its trial by oral and systemic routes on cases of disseminated melanoma for which no further conventional treatment is available or contemplated. A palliative effect might well be expected contributing to remaining quality of life (45).

There is sufficient evidence with backing rationale already available to justify organization of such trials.

**SOME OTHER POSSIBLE APPLICATIONS**

The significant features of the anti-tumoural activities of azelaic acid are:

1. It acts selectively against abnormally hyperactive and tumoural cells, as compared with normal cells.
2. It acts against the synthetic and energy-producing mechanisms of the cell as a competitive inhibitor of the appropriate enzymes. The mitochondrion is a main target.
3. It can affect the karyotype and metastatic potential of tumoural cells.
4. Its antitumoural activity is not limited to melanogenic cells containing tyrosinase as originally thought, but is active against other tumoural cell lines.

Apart from melanoma, one could consider it applicable to different types of cancer in a variety of situations, as in the following examples:

**Cutaneous squamous cell carcinoma**

Azelaic acid is effective against a carcinoma line in vitro (41), and in vivo in the author's unpublished experience, against Bowen's disease (carcinoma in situ) and solar keratosis. Topical and oral admistration would be the lines of treatment here.

**Bronchial carcinoma**

Since Azelaic acid acts against cutaneous squamous cell carcinoma in vitro, (41) it might well act against the same type of bronchial carcinoma. This should be tested in culture and on xenotransplants. Whatever the result, treatment in vivo should also be tried in selected cases. Oral and systemic administration would be the routes.

**Bladder cancer**

Bladder cancer may be due to squamous cell carcinoma, transitional cell carcinoma, or adenocarcinoma. Cutaneous squamous cell carcinoma is susceptible to Azelaic acid in cell culture (41). Superficial lesions confined to the mucosa or submucosa could be attacked *a fronte* and
a tergo at first diagnosis by a combination of continuous oral administration for periods up to six months, and coincident intermittent trans-urethral deliveries of the disodium salt by catheter. As 60% of orally administered azelaic acid is excreted unchanged in the urine over 12 hours, this regimen could ensure continuous bathing of the bladder mucosa over periods. Intermittent intravenous infusions of concentrations indicated above (or higher, as might emerge) could also be given as augmenting systemic dosage. One could envisage this scheme as primary therapy with regular post-treatment surveillance for possible recurrence, and/or, especially with more aggressive superficial tumours, as adjuvant therapy before and after trans-urethral resection.

Administration by the same three routes could also be applied as adjuvant therapy before total bladder resection for invasive tumours in the expectation of reducing metastases, with oral and intermittent intravenous treatment continuing post-operatively for whatever length of time. Patients unfit for radical cystectomy and with generalized metastases might benefit in terms of prolongation, or improved quality of life by an appropriate schedule of a combination of oral and intravenous administration. Finally, lymph-node metastases could be attacked by injection of the sodium salt into appropriate peripheral lymphatic vessels. Azelaic acid might be of value as combination chemotherapy in reducing the dosage of other highly toxic agents currently in occasional use, such as methotrexate and vinblastine.

Similar courses of treatment might be applicable to benign enlargement and cancer of the prostate. Azelaic acid is a potent inhibitor of 5-alpha reductase (13).

In that urinary infection may complicate some of the above conditions, the wide anti-bacterial properties of azelaic acid (40) could be of associated value.

Breast cancer
Cancer in this situation seems ideally suited for applying the anti-tumoural potential of azelaic acid therapy, in that all methods of administration of the drug, topical, oral, intramammary infusion, intravenous, intralymphatic, even intra-arterial, can be applied in various combinations either as primary or adjuvant therapy depending upon stage and extent of disease from initial localized lesions to generalized metastases. Appropriate examples and situations will readily come to mind. Topical treatment is thought of in those, now thankfully few, cases of inoperable surface ulceration with infection, where quality of remaining life is the major consideration.

Leukemia
U-Taniguchi et al (52) suggest that TRX (thioredoxin) reductase inhibitors such as azelaic acid may have a potential therapeutic utility for treatment of HTLV-1(+)-T-cell leukemia. In fact, it has been shown that azelaic acid has a marked effect on the proliferative rate and survival of lymphoma- and leukemia-derived cell lines in culture (32). For leukemia, oral and systemic administration (intravenously or intra-arterially), combined or in succession, with intervals or sustained, depending on effect, would be indicated. Precise protocol and dosage, with possible variations for individual cases, could be based on the figures for serum levels attainable cited above. For local lymphadenopathy, it may conveniently be administered via the peripheral lymphatics, or even by direct injection.

CONCLUSION
Azelaic acid has been shown in cell culture and in vivo to be effective against malignant melanomal and, from the above review of its properties, it may fairly be concluded that it has potential as a general antitumoural agent, and rationale for its use in this area is provided by its now well-established biological activities. It has many of the properties of an ideal chemotherapeutic agent. It is non-toxic, non-teratogenic and non-mutagenic, and has no significant short- or long-term undesirable side-effects. At effective dosage, it acts specifically against tumoural cells, normal cells being unaffected. It can be applied as prime, adjuvant, and palliative therapy. It can be administered by a variety of routes which in appropriate combinations can be utilized to achieve a high and sustainable concentration of effective agent at lesional sites, both directly accessible and inaccessible, with minimal invasive procedures. Treatment need not be limited to one course, and repeated courses could be given at regular intervals. Is there an agent with similar potential and advantages in any pipeline? Suggestions are made here of cancer situations other than melanoma in which its likely efficacy might be investigated in vitro and in vivo.

REFERENCES
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