Eur J Cancer, Vol. 29A, No. 7, pp. 1045–1047, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00
Pergamon Press Ltd

From Triazines and Triazenes to Temozolomide

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NEW DRUGS don't just 'happen' by a process of spontaneous generation: rather, their emergence into the clinical spotlight is acheived in a tentative series of evolutionary nudges, with the 'fitness' of the candidate molecule moulded by the selection forces imposed by laboratory and clinical environments.

As in most human endeavours inquisitiveness and awareness are the catalysts to innovation, but luck plays a major role, especially when contrasting but complementary research strands converge at the right time in the right place. So it was with the discovery of temozolomide which is a story of a symbiotic relationship between two cultures; on the one hand the laboratory sciences of chemistry and pharmacology and on the other the clinical sciences which impose the ultimate test of efficacy — does the drug actually work in patients, or not.

The ancestor molecules of the bicyclic nucleus of temozolomide were originally synthesised in the early 1960s at Nottingham University and their structural similarity to temozolomide itself will be obvious (Fig. 1). The pyrazolo-1,2,4-triazines proved to have modest inhibitory properties against the mouse sarcoma 180 and a methylcholanthrene-induced rat sarcoma [1], but at the time there was no mechanism for evaluating the clinical potential of these leads. A series of imidazo-1,2,4-triazinones and 1,2,3-benzotriazinones prepared at Aston University in the 1970s were completely lacking in antitumour activity against the available tumour models of the day, the L1210 and P388 leukaemias of the NCI. Despite these disappointments, the experience gained in exploring the chemical properties of these reactive close relatives of temozolomide was the essential foundation stone in the whole programme [2].

In 1978 the pharmaceutical company May & Baker agreed to fund a programme of inquisitive chemistry extending the foregoing studies at Aston and a pharmacy research student Robert Stone was recruited and given the simple instruction by his academic and industrial supervisors: "Make some *interesting* molecules". In 1979, Ege published a paper describing a new synthetic route to bicyclic azolo-1,2,3,5-tetrazines [3]. By adapting Ege's route the transformation of useless imidazo-1,2,4-triazinones into *interesting* imidazo-1,2,3,5-tetrazinones was accomplished in 1980 with just the one extra nitrogen atom making all the difference [4].

The chemistry pathway was only one component in the discovery of temozolomide: the pharmacological experience could also be traced back over four decades. The antitumour properties of 1-aryl-3,3-dimethyltriazenes were first reported in 1955 [5]. These compounds are prodrugs and Tom Connors and his colleagues discovered that, irrespective of the nature of the substituent on the phenyl group, only those compounds which could undergo metabolic dealkylation to a monomethyltriazene displayed activity against the TLX5 lymphoma in mice [6]. The anti-melanoma drug dacarbazine (DTIC) which was launched into clinical practice in the 1970s was originally conceived as a prodrug of the DNA antimetabolic 5-diazoimidazole-5-carboxamide: further study showed that DTIC also required metabolic activation — to 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide MTIC [7].

Contemporaneous with these developments John Hickman and Andy Gescher were building a research programme at Aston University on the pharmacology of antitumour *N*-methyl compounds. In 1979 the Cancer Research Campaign (CRC) of the U.K. agreed to establish the CRC Experimental Chemotherapy Group and the new enterprise, reinforced by the appointment of Mike Tisdale in 1981, functioned as a comprehensive drug discovery and development team with synthesis, screening and toxicological expertise tightly embedded within the Pharmaceutical Sciences Institute. Crucially, John Slack one of the co-directors and an experienced pharmaceutical scientist created a formulation laboratory which was to develop a pivotal role in integrating the pharmaceutical expertise of the CRC,

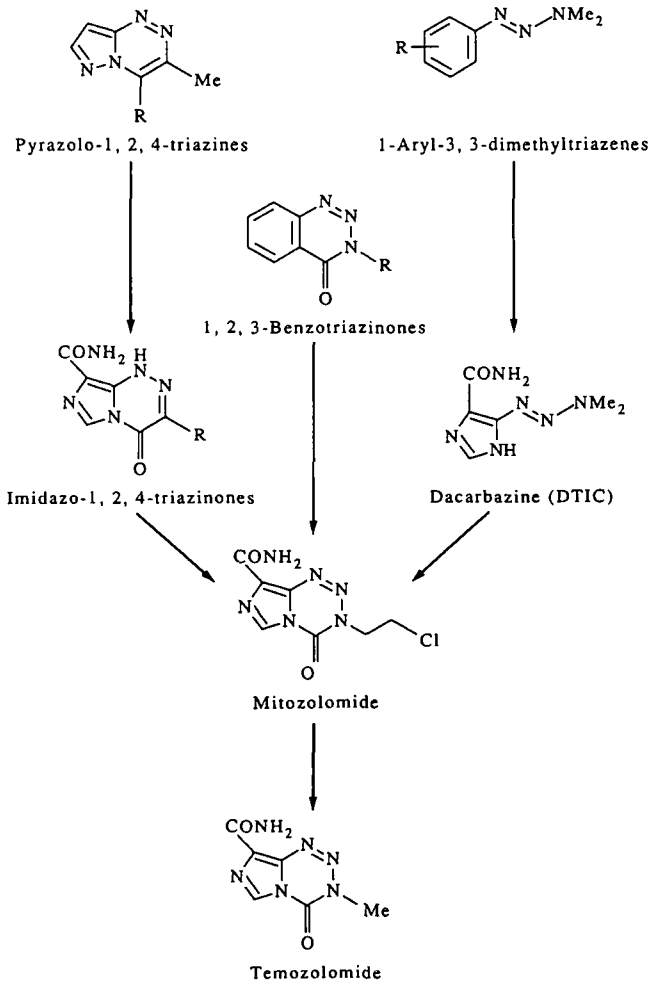

Fig. 1. Molecular milestones en route to temozolomide

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Received 3 Dec. 1992; accepted 10 Dec. 1992.

EORTC and NCI: this facility gave the group the resources to progress novel molecules from bench to clinic.

It was in this fertile environment that the newly invented imidazotetrazinones were screened against the mouse TLX5 lymphoma by another pharmacy research student, Neil Gibson, in 1981. As the antitumour results on the lead compound azolastone (CCRG 81010; mitozolomide) unfolded [8] the more excitable members of the Group really believed that the elusive 'magic bullet' lay within their grasp. New analogues from Aston, or from Eddy Lunt and his colleagues at May & Baker, which were synthesised one week, were on test the next. This excitement was sustained throughout the preclinical development of mitozolomide but evaporated rapidly when the drug entered clinical trial in 1983. In the phase I study the dose-limiting toxicity was identified as thrombocytopenia [9] and in phase II trials the recommended dose of 115 mg/m^2 proved to be too toxic; even with dose reductions to 90 and 70 mg/m^2 a delayed and profound thrombocytopenia, similar that elicited by chloroethylnitrosoureas, was encountered. Although limited clinical activity was seen in small cell carcinoma of the lung and malignant melanoma, the unpredictable thrombocytopenia spelt the end of mitozolomide and the abandonment of the project by May & Baker. The fall of mitozolomide also dealt a final blow to the mouse tumour models then used for selecting clinical candidates and their replacement by panels of human tumour cell lines.

So, the second chapter of the antitumour imidazotetrazinone story began with funding exclusively from the CRC.

Mice have a more robust bone marrow than humans and the myelosupressive property of mitozolomide in humans is probably related to the DNA cross-linking action of the monochloroethyltriazene decomposition product formed from mitozolomide *in vivo*, and species-dependent repair of the DNA lesions by proteins such as O^6-alkylguanine-DNA alkyltransferase [10]. Thus when considering the selection of a second-generation imidazotetrazinone, all analogues bearing a chloroethyl substituent were rejected despite the fact that some were more potent than mitozolomide itself [11]. Instead, the 3-methyl congener, temozolomide (CCRG 81045; formerly methazolastone), was chosen because it had a different profile of antitumour activity against mouse tumours and was less toxic (but less potent) than mitozolomide, presumably because it could not cross-link DNA [12]. Also, in mice, temozolomide displayed good tissue distribution, including into intact mouse brain [13].

In 1980, the CRC phase I/II clinical trials Committee was established in the U.K. by Connors and Fox with the mission to facilitate the safe introduction of new drugs into the clinic. This organisation has had a major impact on new anticancer drug development within Europe and also influenced the practices of the NCI in the U.S.A. In essence, four questions are asked of any potential new drug: does the drug probe a biological concept which hasn't been previously tested in the clinic? Can the drug be synthesised on a sufficient scale? Can the drug be formulated for human administration? Are experienced clinicians interested in conducting clinical trials? If the answer to all four questions is yes, then the drug is submitted to an abbreviated toxicological examination with the main aim of establishing a safe starting dose for a phase I trial [14].

But what was the rationale for proceding with temozolomide? Temozolomide, like mitozolomide, is a prodrug and undergoes ring-opening under physiological conditions to the monomethyltriazene MTIC [12]. This DNA methylating agent is the same reactive species that is formed by metabolic demethylation of DTIC. However, whereas mice metabolise DTIC most efficiently (and DTIC is an effective agent against mouse tumours), apparently humans only demethylate DTIC to a limited extent [15]. The conversion of temozolomide to the putative antitumour species MTIC is under chemical control and not subject to the vagaries of metabolism. Thus a clinical trial of temozolomide would test the concept that DTIC might have been an effective antitumour agent if only humans were more like mice in their ability to effect metabolic *N*-demethylation. In truth, it is a rather fragile rationale.

The phase I trial of temozolomide started at the Charing Cross Hospital, London, and the Queen Elizabeth Hospital, Birmingham, in 1987. Initially, temozolomide was given intravenously at doses between 50 and 200/mg/m^2 on a single dose schedule. At 200 mg/m^2 the good bioavailability of oral temozolomide was confirmed and all subsequent doses have been given with the oral formulation in gelatin capsules [16]. In the single dose part of the study, doses were escalated up to 1.2 g/m^2 and myelosupression became dose-limiting at around 1 g/m^2. The pharmacokinetics of temozolomide were studied concurrently and the area under the curve was linear with dose. A total of 51 patients were entered in the single dose schedule and no major clinical activity was seen apart from two clinical improvements in patients with recurrent high grade gliomas: in neither of these cases was there a reduction in computed tomography (CT) scan mass of tumour.

This was a disappointing outcome and the development of temozolomide might well have foundered at this stage. However, preclinical antitumour studies conducted by Simon Langdon at Aston had shown that, unlike mitozolomide, the activity of temozolomide was very schedule-dependent [12]. Therefore, without too much expectation of success, temozolomide was administered to patients on a 5 day schedule, with the dose split equally per day and the schedule repeated every 4 weeks. In contrast to mitozolomide, the repeat dosing was no more myelosuppressive than the single dose schedule with myelosupression becoming dose-limiting at 1 g/m^2. The current recommendation for future trials is for the initial course to be given at 750 mg/m^2 and, if no myelosuppression is seen on day 22, to escalate the dose to 1g/m^2 split over 5 days. Using this schedule, in an analysis to May 1992 at 750mg/m^2 no WHO toxicity in leukopenia or thrombocytopenia greater that grade 2 has been seen in 108 courses. However, as phase II trials have started in different patient populations, treatment with extensive field radiotherapy or prior treatment with nitrosoureas may be risk factors for severe myelosuppression requiring cautious dosing of temozolomide, perhaps dropping as low as 500 mg/m^2 split over 5 days for the initial course. But, in general, temozolomide is a well tolerated drug. Nausea and vomiting occur with higher doses, but this can be controlled with modern antimetics. Clinically detectable alopecia is uncommon and so far no other major organ toxicity has been identified.

On the repeat dose schedule clinical activity has been observed in patients with malignant melanoma, astrocytomas recurrent after surgery and radiotherapy, and also preliminary data in mycosis fungoides. As reported in the phase I paper [16] four responses [2 complete responses (CR), 2 partial responses (PR); 17%] were seen in 23 patients with metastatic melanoma. Given its ease of administration and better toxicity profile than DTIC, temozolomide warrants a confirmatory phase II study in melanoma; in the future, a randomised phase II trial against DTIC needs to be performed.

The most exciting clinical activity that has been identified so far is in astrocytomas this notwithstanding the fact that temozolomide shows no noteworthy activity in the NCI neural cell tumour panel. In the extended phase I trials using temozolomide on the 5 day schedule, repeated every 4 weeks, a major improvement was seen in the CT scans in 5 out of the initial 10 patients with astrocytomas recurrent after radiotherapy with a major clinical improvement on CT scanning in 1 further patient. In addition, reductions in the size of CT scan lesions were also observed in 4 out of 7 patients with newly diagnosed high-grade astrocytomas given two to three courses of temozolomide prior to irradiation [17].

In conclusion, temozolomide is a small and neat molecule of molecular weight 194 D; it is pharmaceutically robust and survives the chemical stresses of oral administration and absorption. Unlike DTIC it achieves adequate penetration through the blood–brain barrier. Although the precise mode of action of temozolomide has yet to be defined it is probable that a methylation reaction at the O^6 position of guanine residues by the reactive species MTIC is the initial molecular event, possibly occurring in a sequence-selective manner [18]. Perhaps significantly, a recent analysis showed that 22% of primary brain tumours had no detectable levels of the repair methyltransferase protein [19]. However, malignant gliomas are also known to express high levels of epidermal growth factor (EGF) receptors [20] and conceivably the drug might be influencing expression of the EGF receptor gene. Proposed positron emission tomography scanning studies employing temozolomide labelled with an ^{11}C isotope at C(3) and C(4) and NMR studies on ^{19}F-labelled temozolomide will answer some of these mechanistic points. Also, given the ease of administration of the drug and the availability of larger supplies following the signing of a licensing deal between CRC Technology and a major pharmaceutical company, fine tuning of the current scheduling of temozolomide may point to an extended role for this new drug against other tumour types.

Finally, we cannot resist quoting a comment by Corwin Hansch in a paper which analysed the structure–activity relationships in antitumour dimethyltriazenes:

> "Unless one had new biochemical or molecular biological information suggesting that a new triazene might be more effective in some specific way, we would not recommend the synthesis and testing of new congeners"

This paper, published in 1978 [21] coincided precisely with start of the project which led to temozolomide.

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