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Perspectives and Commentaries

TCNU: a Ray of Hope for Designer Nitrosoureas?

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(A COMMENT ON: Smyth JF, Macpherson JS, Warrington PS *et al.* A phase I study of TCNU, a novel nitrosourea. *Eur J Cancer Clin Oncol* 1987 **23**, 1845–1849 and Vibe-Petersen J, Bork E, Moller H, Hansen HH. A phase I clinical evaluation of 1-(2-chloroethyl-3-[2-(dimethylaminosulphonyl)-1-ethyl]-1-nitrosourea (TCNU). *Eur J Cancer Clin Oncol* 1987, **23**, 1837–1843.)

It is over 20 years ago since the chloroethylnitrosourea BCNU first entered clinical trial [1], to be followed by the analogues CCNU, MeCCNU, chlorozotocin, PCNU and others [2–4]. The preclinical appeal of these drugs lay in their astonishing broad spectrum of activity against experimental tumours in rodents, their lack of cross resistance with classical alkylating agents and their ability to penetrate the blood–brain barrier and thereby inhibit the growth of intracerebral tumours [5, 6]. Initial clinical optimism remained high in view of the apparent absence of serious adverse toxicities other than vomiting and delayed myelosuppression. This enthusiasm was not maintained, however, in the light of relatively modest antitumour activity and problems of prolonged cumulative myelosuppression and lung and kidney damage [7]. In reviewing the performance of BCNU, CCNU and MeCCNU, Wasserman *et al.* [3] pointed out the absence of compelling data to support an advantage of these agents over the original lead chemicals, the methylating agents MNNG and MNU.

Nevertheless, the nitrosoureas have found a limited role in clinical oncology, particularly for tumours of the brain [7]. Because of this and no doubt because their preclinical activity continues to appeal, further analogue development has been perpetuated in the hope that dose-limiting toxicities may be reduced or improved antitumour activity realized.

The ingenuity of the medicinal chemists has produced an impressive battery of molecules with a staggering variety of physicochemical and biological properties [4, 8]. Of particular interest has been the development of agents with special carrier groups, including sugar, nucleoside and amino acid derivatives. These designer nitrosoureas might be expected to exhibit altered disposition characteristics, although the rationale for specificity against tumour versus normal tissue is not always clear. It is against this background that we should consider the two reports in this issue from Smyth *et al.* [9] in Edinburgh and Hansen *et al.* [10] in Copenhagen describing phase I evaluation of the novel chloroethylnitrosourea TCNU.

Despite its stronger polar character compared to CCNU and MeCCNU, the drug has limited water solubility and because of this and its excellent activity po in mice, the drug was given orally, every 4–6 weeks. Gastrointestinal toxicity and myelosuppression were similar to those of the familiar chloroethylnitrosoureas, and the authors recommend starting doses of 90–130 mg/m^2 every 5 weeks for phase II evaluation. Clinical responses were observed in melanoma, renal cell carcinoma, carcinoma of the stomach and lung cancer, including squamous cell, adenocarcinoma and large cell histologies as well as small carcinoma. Although it is stressed that the majority of responses seen with non-small cell lung cancer were in untreated patients of good performance status, it is also emphasized that therapeutic activity was observed at non-toxic dose levels. The results are clearly very promising for a phase I study and suggest the possibility of an unusual antineoplastic profile for a chloroethylnitrosourea.

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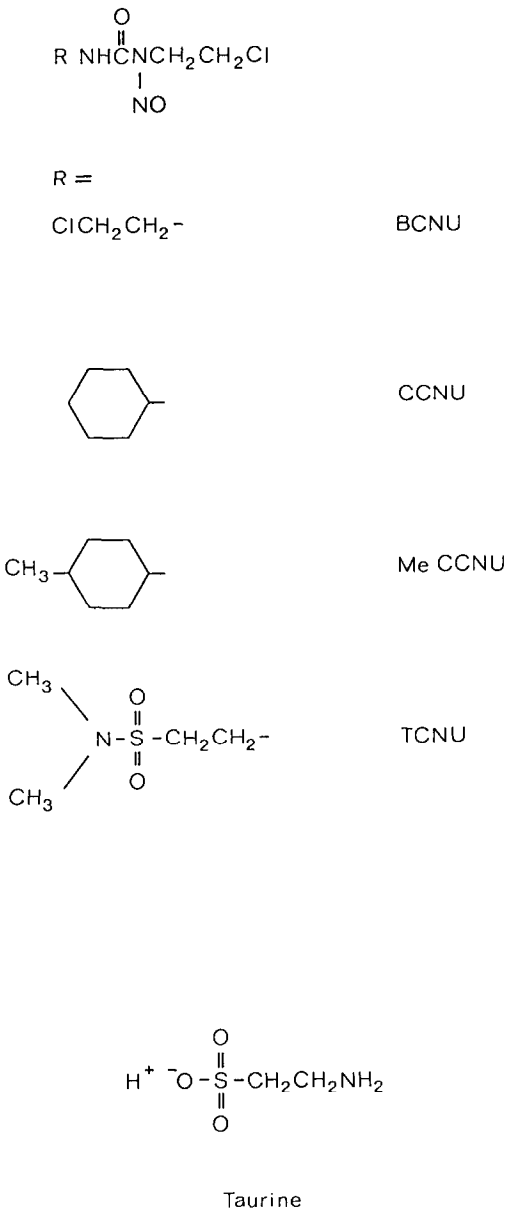

Fig. 1. Structures of the novel taurine-based chloroethylnitrosourea TCNU, together with those for the β-amino acid taurine itself, and BCNU, CCNU and MeCCNU for comparison.

TCNU is related to the classical nitrosoureas, but the structure is based on the naturally occurring β-amino acid taurine. The taurine is however extensively modified, with the amino group nitrogen forming one of the nitrogens of the nitrosourea, and a dimethylaminosulphonyl group replacing the sulphonic acid residue (see Fig. 1). Taurine occurs naturally in the body, and is especially abundant in excitable tissues and those generating oxidants and rich in membranes [11]. Its principal functions appear to involve membrane stabilization, detoxification of toxins and antioxidant activity [11]. However, both the amine and the sulphonic acid functions are thought to be required for activity and the substitution of these residues in TCNU may well eliminate the protective biological properties. It may nevertheless be possible that TCNU is targeted to particular biological structures, especially membranes.

It is important to ask why the chloroethylnitrosoureas have fared so poorly in the clinic compared to the laboratory and how TCNU is likely to differ. There appears to be no consistent difference in sensitivity at the cellular level between human and rodent tumour cell lines; survival curves are generally exponential with dose following a shoulder of variable size, and concentrations to give a 1 log cell kill are in the region of 3–30 μg/ml for nominal 1–4 h exposures [12]. Two reasons in particular have been advanced to account for the relatively modest clinical activity of these agents. The first is pharmacokinetic. For CCNU the chloroethylnitrosourea exposures which can be achieved with maximum tolerated doses in man are generally at the low end of those required for cytotoxicity, both for mammalian cells *in vitro* and for activity in murine tumours and human tumour xenografts in mice [12, 13]. Peak chloroethylnitrosourea concentrations of 6 μg/ml are readily achievable in mouse plasma, while those in humans are about 1–2 μg/ml. In addition, with oral administration of CCNU in man, first-pass metabolism prohibits the parent drug from reaching the systemic circulation where only the *cis*- and *trans*-4-hydroxy metabolites are seen; by contrast unchanged drug as well as five hydroxylated metabolites are identified in mouse plasma. Although some pharmacokinetic and metabolic characterization was carried out in the early development of the first chloroethylnitrosoureas [14], this was necessarily limited by the unavailability of sophisticated HPLC technology now in routine use for analysis of preclinical and clinical samples in expert centres. As a result, important differences in pharmacology, both quantitative and qualitative, were not fully appreciated at that time.

Bonus points are awarded to TCNU development for the characterization of its pharmacokinetic behaviour using a sensitive and specific HPLC assay, both in preclinical species and as part of the phase I clinical studies [9, 15]. In contrast to CCNU, unchanged TCNU is identified in plasma after oral administration with no evidence of metabolites to date. At doses of 70–150 mg/m^2 peak concentrations at 15 min to 2 h averaged about 1 μg/ml, which is similar to that for total chloroethylnitrosoureas 2–4 h after 130 mg/m^2 CCNU orally. On the other hand, the 60 min half-life for TCNU elimination is about half of that for total chloroethylnitrosoureas after CCNU and the area under the curve is correspondingly lower. Differences clearly do exist then in the plasma pharmacokinetics of oral CCNU and TCNU, but it is unclear whether these are of

sufficient magnitude to provide a major advantage in favour of TCNU. It should be borne in mind, however, that even greater differences may exist at the tissue, cellular and subcellular levels as a result of the taurine-based substituent.

The second important factor governing the cytotoxic effects of chloroethylnitrosoureas is the activity of a specific DNA repair protein. Chloroethylnitrosoureas decompose spontaneously under physiological conditions generating alkylating fragments (particularly chloroethyldiazonium hydroxide) which react with nucleophilic centres in DNA [16]. Alkylation at the O^6 position of guanine appears to be particularly critical, and removal of the monoadduct prevents cross-linking of DNA by the relatively slow reaction of the chloroethyl group attached to the guanine O^6 with the adjacent cytosine in the opposite strand [17, 18]. The removal of the initial chloroethyl monoadduct is carried out by the protein DNA O^6-alkylguanine alkyltransferase [19]. The transferase is analogous to the inducible product of the *ada* gene in *E. coli*; this has been cloned and sequenced, and its regulation worked out [20]. Removal of an alkyl or chloroethyl group is a stoichiometric suicide reaction, inactivating the protein which cannot be regenerated. Mammalian cells express this alkyltransferase in comparatively low activity and with variation between organs and individuals [19, 21]. The molecular genetics and regulation are less well understood than for the bacterial gene, and the mammalian gene is proving difficult to clone. However, very recent results show that tranfection of the bacterial gene into mammalian cells results in up to a 1000-fold increase in alkyltransferase activity and a concomitant resistance to methylating and chloroethylating agents [22, 23]. Similarly,human cell lines with low alkyltransferase (Mer^-) are more sensitive to chloroethylnitrosoureas than those with high activity (Mer^+) [24], and sensitivity can be increased by depletion of the transferase using DNA methylating agents or the alternative substrate O^6-methylguanine free base [25, 26].

Although studies with cell lines suggested that a high proportion of tumours might exhibit low alkyltransferase activity [24], evidence from human tissues shows that decreased activity in tumour compared to corresponding normal tissue is rather infrequent, and in most cases alkyltransferase levels are similar or higher in tumour [27]. Recent studies have also shown that the alkyltransferase is in fact particularly low in human bone marrow precursors [28].

It seems reasonable then to suggest that the limited clinical activity of chloroethylnitrosoureas in man may well be related to the unfavourable distribution of the alkyltransferase repair enzyme between tumour and normal cells, particularly in the bone marrow. Unless the normal–neoplastic differential is unequal between mice and humans, however, no immediate explanation is provided for the higher activity of these drugs in mice. This would require most preclinical model tumours to be repair deficient since mouse marrow appears to be low in activity compared to the human [28].

How does this information help us with the comparative activity of TCNU with respect to traditional chloroethylating agents? TCNU exhibited similar or improved activity compared to these agents in rodent screening models [29] and was active in small cell and non-small cell human lung tumour xenografts [30]. However, it shows minimal activity in a mouse sarcoma made resistant to CCNU *in vivo* and cross-resistant to other conventional chloroethylnitrosoureas, as well as the chloroethylating agent mitozolomide [31]. Similar resistance was seen to TCNU and mitozolomide in the above mentioned mammalian cell line transfected with the bacterial alkyltransferase gene [23]. So it seems unlikely that TCNU would exhibit clinical activity against human tumours with high levels of alkyltransferase which are normally resistant to conventional chloroethylnitrosoureas.

There are further additional features of the molecular pharmacology of chloroethylnitrosoureas that we also need to consider. Firstly, their decomposition gives rise not only to a common alkylating species, but also the corresponding organic isocyanate [16]. For example, BCNU, CCNU and MeCCNU produce chloroethyl, cyclohexyl and methylcyclohexyl isocyanates respectively while TCNU would be expected to yield the isocyanate of the substituted taurine. Isocyanates are highly reactive species which form adducts predominantly with protein by carbamoylation [5].

The role of carbamoylation in the antitumour activity and haemopoietic toxicity of chloroethylnitrosoureas has been controversial for many years. Results obtained with derivatives of differing alkylation and carbamoylation potential [32] show that the latter is not required for cytotoxicity, but does contribute to the overall effect, probably by inhibiting DNA repair [33]. Sugar derivatives with low carbamoylating activity, such as chlorozotocin, were considered to have reduced myelotoxicity while maintaining antineoplastic activity in mouse and man [34, 35].

The second additional issue concerns thiol chemistry and is also related to carbamoylation. BCNU is a substrate for glutathione-*S*-transferase [36] and conjugation with glutathione will function as a protective mechanism for the cell. However, carbamoylating nitrosoureas act as irreversible inhibitors of glutathione reductase [37] and both

reactions will serve to deplete cellular glutathione [38].

In view of these latter two considerations, it is important to note that TCNU is weakly carbamoylating compared to BCNU and CCNU in an assay based on inhibition of esterase activity and this was not due to inadequate cell permeability [39].

As for antitumour testing (see later) the predictive accuracy of preclinical toxicology screens is currently under debate [40]. Quantitatively, the delayed myelotoxicity of TCNU seen in man was correctly predicted in mouse, rat and dog, and the gastrointestinal and hepatotoxic effects were also anticipated from preclinical toxicology [29]. On the quantitative side, the human MTD for the oral route of 130–150 mg/m^2 was well predicted by the mouse i.p. LD_{10} of 127 mg/m^2, but the dog was oversensitive with an oral LD_{10} of 45 mg/m^2.

In many cases it has been possible to explain such differences on the basis of interspecies pharmacokinetic differences [41], but this is not the case for TCNU and the dog appears to be particularly susceptible to myelosuppression from this agent. Pharmacokinetically guided dose escalation is currently being recommended as a means of reducing the number of dose escalations in phase I trials [41, 42]. In the TCNU phase I studies the starting doses were 10 and 20 mg/m^2 (0.08 and 0.16 times mouse LD_{10}) and 9–11 dose escalation steps were carried out. In retrospective analyses for BCNU, CCNU and mitozolomide, pharmacokinetic information did not help the prediction of maximum tolerated doses from mouse to man [43]. Nevertheless it is likely that the number of dose escalations would have been reduced by the application of pharmacokinetically guided dose escalation for TCNU, as for other agents [41, 42].

The development and early clinical evaluation of TCNU has been professional. The responses seen are encouraging. There is a ray of hope for a substantial improvement over conventional analogues, though promising results in early trials are not always maintained in subsequent studies. TCNU does show some preclinical advantages, possibly related to its distinct pharmacokinetic properties, yet it is nevertheless a chloroethylating agent with a mechanism of action essentially identical to that of other chloroethylnitrosoureas and also such investigational agents as mitozolomide and clomesome [44].

The historical experience with the early nitrosoureas was repeated for mitozolomide, with the exciting broad spectrum clinical activity failing to be maintained in the clinic despite extensive thrombocytopaenia. We need to be particularly cautious in the further development of such compounds. It can be argued that they admirably illustrate the poor predictive capability of preclinical screens, though whether this is because of species-dependent pharmacokinetics, differences in alkyltransferase activity or both is not yet clear. Certainly, it is unlikely that such agents would be selected in the new NCI screen, which is designed to identify agents with high specificity for particular tumour types.

On the positive side, the various investigational chloroethylating agents do show differences between themselves in alkylation chemistry with respect to the DNA adduct formed (e.g. chloroethyl versus hydroxyethyl); the base sequence selectivity of guanine N^7 alkylation (the situation for guanine O^6 is unknown); the specificity for linker versus nucleosome DNA; and possibly the preferential reaction with guanosine–cytosine rich sequences in oncogenes [44, 45]. Nevertheless the activity of all these agents is predominantly governed by alkyltransferase activity, particularly those with low carbamoylating potential [33, 44].

A number of recommendations can be made to optimize the clinical evaluation of such agents. Only a small minority of patients may be likely to benefit. The ideal patient is clearly one expressing high alkyltransferase activity in the marrow and a low level in the tumour, and at the same time exhibiting a favourable pharmacokinetic profile, probably with maximum peak concentrations. The techniques are available to characterize both parameters. The capacity to develop resistance through up-regulation of alkyltransferase, as seen both *in vitro* and *in vivo* [46] (Workman P, Lee FYF, Margison GP, unpublished), can be determined. Success will encourage further development, particularly of the designer compounds with specific carrier substituents.

With TCNU in particular, we will want to know to what extent the taurine substituent is responsible for improved activity and whether delivery to specific sites are involved, for example in the membrane or the chromatin [45]. We will need to discover whether it crosses the blood–brain barrier and exhibits activity in brain tumours. We will have to find out more about its metabolism.

Success will also give added impetus to the use of these new drugs together with chemosensitizing agents such as nitroimidazoles [47]; thiol depleters such as buthionine sulphoximine and chemoprotectors like WR 2721 [48]; modifiers of chromatin structure and transcription such as steroids and sodium butyrate [45, 49]; and also hyperthermia [50].

What, on the other hand, if improved activity is not seen with the new chloroethylating agents like TCNU or clomesome, or with the novel investigational methylating agents such as temozolomide which react at the same locus in DNA? Resistance may be overcome by depletion of tumour alkyl-

transferase activity. Higher doses can be administered with bone marrow rescue, or potentially after transfection of marrow cells with the alkyltransferase gene. However, the benefits will be limited by depletion of the normal tissue enzyme in the first case and extramedullary toxicity in the second.

Let us hope that the promising activity of the new designer drugs is maintained in clinical trials. If not, we shall surely be forced to discard guanine O^6 as a target for chemotherapy and look elsewhere in the genome and the cell.

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