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Review paper

Anthrapyrazoles: true successors to the anthracyclines?

Ian R Judson
IR Judson is at The Institute of Cancer Research and Royal Marsden Hospital, Drug Development Section, Block E, 15 Cotswold Road, Belmont, Sutton, Surrey SM2 5NG, UK.

The anthrapyrazoles are a new class of intercalating agents which were synthesized in order to reduce the potential for free radical generation and subsequent cardiotoxicity. Selected compounds showed a reduction in superoxide formation compared with doxorubicin plus inhibition of lipid peroxidation. Broad spectrum activity was seen against experimental tumors comparable with doxorubicin, with incomplete cross-resistance. The anthrapyrazoles bind to DNA, intercalate, preferentially inhibit DNA compared with RNA synthesis and form DNA single and double strand breaks consistent with inhibition of topoisomerase II. Clinical studies have been performed with CI-937, CI-941 and CI-942. In each case the dose-limiting toxicity was leukopenia with other toxicities being minor. CI-941 has shown significant activity in patients with advanced breast cancer and these agents appear to have a bright future.

Key words: Anthrapyrazoles, breast cancer, cardiotoxicity, free radicals, intercalation, lipid peroxidation, superoxide, topoisomerase II.

Introduction

Intercalating agents which exert their effect through binding to DNA are extremely important in the treatment of human malignancy. In particular, the anthracycline antibiotics daunorubicin and doxorubicin have a well-established role and doxorubicin is probably the most widely used anticancer agent in current use.[1,2] Unfortunately, the anthracyclines cause both acute and delayed cardiotoxicity which in the latter case can lead to cardiomyopathy, congestive cardiac failure and death.[3] This form of cardiotoxicity is cumulative and hence the total dose of doxorubicin is recommended to be <500 mg/m^2, below which the incidence of congestive cardiac failure is very small.[4] There is a good deal of interpatient variation in the susceptibility to this toxicity but at higher doses the risk increases to 30–40%. This limits the use of these agents, particularly in recurrent disease.

Extensive analog development work has been devoted to the attempt to identify a derivative of doxorubicin with reduced cardiotoxic potential which retains broad-spectrum antitumor activity. A number of such agents have been evaluated, of which 4′-epidoxorubicin (epirubicin) is probably the most successful, being somewhat less potent in terms of myelosuppression but possessing similar activity and reduced cardiotoxicity.[5] The related anthracenediones, in which the amino sugar and tetracycline A-ring of the anthracyclines are replaced by aminoalkylamino side chains, were synthesized in the 1970s and found to be effective intercalating agents.[6] Mitoxantrone has demonstrated useful clinical activity with reduced cardiotoxicity but is clearly less potent than doxorubicin as a single agent and has a reduced spectrum of activity.[7,8] Its use is essentially limited to the treatment of breast cancer, acute myeloid leukemia and some lymphomas. Cardiomyopathy is still observed with large cumulative doses and care needs to be taken with patients who have already received doxorubicin.

It is thought that the cardiotoxicity of the anthracyclines and anthracenediones is at least partly due to the formation of free radical intermediates. Both classes of compound can undergo reduction to semiquinones with subsequent generation of reactive oxygen species.[9] These highly reactive radicals can cause membrane lipid peroxidation and other lesions.[10,11] The heart may be especially susceptible owing to a relative lack of the detoxifying enzymes superoxide dismutase, catalase and glutathione peroxidase in this organ.[12]

Rationale and synthesis of the anthrapyrazoles

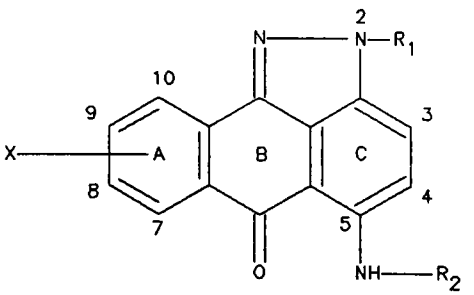
In order to make the quinone chromophore more resistant to enzymatic reduction, substitutions such as, for example, the replacement of a carbonyl group in the B-ring by an imine moiety giving 5-iminodaunorubicin and 5-iminodoxorubicin were shown to diminish the degree of redox cycling and free radical generation.[13] In experimental systems the cardiotoxic potential of these agents appeared to be reduced.[14] This observation led to the adoption of the anthra[1,9-*cd*]pyrazol-6(2H)-one, or anthrapyrazole, ring system by workers at Warner-Lambert.[15,16] This modification converts the central quinone into a quasi-iminoquinone and incorporates another ring into the chromophore. This is achieved while retaining the planar configuration and electronic requirements for DNA intercalation.[17]

The initial report by Showalter *et al.* described compounds with a single hydroxyl group in the A-ring and side chains identical to those of mitoxantrone and ametantrone.[16] A large number of analogs were synthesized to examine both the impact of varying the number and position of A-ring hydroxyl substitutions and variations in the side chains.[18,19] Cytotoxicity, DNA binding, inhibition of DNA synthesis, DNA damage and redox cycling were studied. The general structure of the anthrapyrazoles and details of the three compounds chosen for clinical development are shown in Figure 1.

Preclinical studies

Antitumor activity

The anthrapyrazoles showed a high level of activity against murine leukemias; in one study 67% of the compounds tested were curative in the NCI P388 pre-screen.[20] Similarly, these agents were highly active against several other tumors in the NCI panel, with curative activity shown by 82, 73, 45 and 80% of the compounds, respectively, vs the L1210 leukemia, B16 melanoma, M5076 sarcoma and the MX-1 mammary xenograft.[20,21] In order to rank the compounds, further testing was carried out against tumors which respond less favorably to doxorubicin, such as mammary adenocarcinoma 16C and colon 11a. In these tumor models relatively few anthrapyrazoles produced a net reduction in tumor burden, but those which did so showed activity similar to that of doxorubicin and superior to the anthracenediones mitoxantrone and ametantrone.[20] Against the Ridgeway osteogenic sarcoma, the anthrapyrazoles generally showed curative activity and were again superior to the anthracenediones. It was found that basic side chains at C-5 and N-2 were required and that in the series with identical upper and lower side chains, A-ring hydroxylation increased activity, the most active compounds having 7-(OH), 7,10-$(OH)_2$ and 7,8,10-$(OH)_3$ substitutions. On the basis of antitumor activity and biochemical assessments, three compounds were chosen for further development, CI-942, CI-937 and CI-941 (Figure 1).



	X	R_1	R_2
CI-937	7,10-$(OH)_2$	$(CH_2)_2NH(CH_2)_2OH$	$(CH_2)_2NHCH_3$
CI-941	7-OH	$(CH_2)_2NH(CH_2)_2OH$	$(CH_2)_2NH(CH_2)_2OH$
CI-942	7,10-$(OH)_2$	$(CH_2)_2NH(CH_2)_2OH$	$(CH_2)_3NH_2$

Figure 1. The general structure of the anthrapyrazoles and details of the three compounds chosen for clinical development.

DNA binding

A number of studies have been performed to investigate the mechanism of action of the anthrapyrazoles. Studies of ethidium bromide displacement[22] showed that the anthrapyrazoles are efficient DNA binding agents.[23] The efficiency of DNA binding is affected by the number and position of hydroxyl groups in the A-ring. For example, in a series with symmetrical side chains as in mitoxantrone, the C-7 monohydroxy compound was twice as efficient as the deshydroxy, whereas C-10 substitution diminished DNA binding.[18] The order of increasing binding affinity was 10-OH < deshydroxy < 7-OH < 7,9,10-$(OH)_3$ < 7,10-$(OH)_2$ < 7,8,10-$(OH)_3$.

Hartley *et al.* found that anthrapyrazole binding showed a preference for GC-rich regions of DNA, as reported for mitoxantrone.[24] Certain members of the series were found to be more efficient intercalators than mitoxantrone, as measured by a DNA unwinding assay, and intercalation was diminished by hydroxyl substitution. The trihydroxy compounds studied bound strongly to DNA but did not appear to intercalate. It had been noted previously that these compounds did not produce DNA strand breaks which had been attributed to instability or failure to enter cells.[18,25] It is possible that the explanation for this difference in behavior is their inability to intercalate.[24]

Inhibition of nucleic acid synthesis

The anthrapyrazoles are potent inhibitors of DNA synthesis as measured by tritiated thymidine incorporation in L1210 cells.[23] Once more, 7-OH and 7,10-$(OH)_2$ compounds were more active.[18] These agents share with amsacrine the ability to inhibit DNA synthesis more strongly than RNA synthesis, whereas doxorubicin and mitoxantrone inhibit both equally.[23] For example, the 50% inhibitory concentration (IC_{50}) for DNA synthesis was 0.33 and 0.57 μM for CI-937 and CI-942, respectively, compared with 2.0 and 11.3 μM for RNA synthesis. A similar effect on DNA and RNA polymerases was confirmed in permeabilized cells.[23] In this system the inhibition could be reversed by addition of exogenous DNA, tending to confirm that the DNA template was the site of action.

DNA damage

The anthrapyrazoles appear to be similar to other intercalators in causing single-strand, and to a lesser extent double-strand, protein-associated DNA breaks. These observations are consistent with inhibition of topoisomerase II. Differences were observed between compounds in the rate of strand breakage, which was more rapid with CI-937 than CI-942.[23] Following substantial strand breakage, repair was observed to take place slowly and further breaks were formed after drug removal, as also occurred with doxorubicin and mitoxantrone. This effect was particularly prominent with CI-937.[23] As discussed above, the trihydroxy-substituted compounds did not produce strand breaks, possibly because of a failure to intercalate.[24] The 7-OH and 7,10-$(OH)_2$ derivatives were most potent and a 10-OH compound studied had little strand-breaking ability.[18] In spite of the similarity with amsacrine regarding the preferential inhibition of DNA synthesis, strand breaks produced by amsacrine are rapidly repaired and continuing damage is not observed.

Free radical generation

As a result of the structural modification to the B-ring, the anthrapyrazoles are much more resistant to reductive metabolism than anthraquinones. This can be measured electrochemically and as with DNA interactions, hydroxyl substitution in the A-ring appears to be beneficial. The presence of a hydroxyl group adjacent to the carbonyl group on the B-ring renders the drug more difficult to reduce and further substitutions increase this effect.[18]

The formation of superoxide radical by CI-937 and CI-942 has been measured by oxygen consumption in the presence of NADPH and rat liver microsomes and compared with the effect of doxorubicin.[23] The two anthrapyrazoles studied caused 5- to 10-fold less oxygen consumption than doxorubicin. In all cases this was inhibited by superoxide dismutase indicating that oxygen consumption was related to superoxide formation. In a subsequent report of oxygen consumption in a series of anthrapyrazoles with symmetrical $(CH)_2NH(CH)_2OH$ side chains this was shown to be substantially greater for the 7,10-$(OH)_2$ derivative in comparison with either 7-H or 10-H.[18] Graham *et al.* studied free radical generation by CI-941 using electron spin resonance and NADPH utilization, also superoxide formation by measuring the reduction of acetylated cytochrome *c* or adrenaline to adrenochrome.[26] A drug free radical signal could not be detected by electron spin resonance either in the presence of NADPH-fortified rat liver microsomes or purified cyto-

chrome P-450 reductase. Unlike with doxorubicin, only minimal superoxide formation could be demonstrated in the presence of CI-941 with either system used.[27] Frank and Novak also reported reduced superoxide formation by a series of anthrapyrazoles compared with doxorubicin.[28]

Lipid peroxidation

One of the consequences of free radical formation is that of lipid peroxidation. Generally the anthrapyrazoles studied appear to be capable of inhibiting lipid peroxidation. This was demonstrated in the case of CI-941 which not only inhibited basal rate lipid peroxidation but also strongly inhibited doxorubicin-stimulated NADPH-dependent lipid peroxidation at concentrations as low as 5 μM.[26] A comparative study of anthrapyrazoles as inhibitors of doxorubicin and iron-stimulated lipid peroxidation suggested that CI-937 and CI-942 were much more potent than CI-941.[28] In these studies CI-941 was only 35% inhibitory at 100 μM, in contrast to 80% inhibition by CI-942 at 15 μM. This conflicts with the 52% inhibition of doxorubicin-stimulated lipid peroxidation by 5 μM CI-941 reported by Graham *et al.*[26] The reason for this discrepancy is unclear but is probably due to differences in methodology, including the use of rabbit rather than rat liver microsomes by Frank and Novak.[28]

Cardiotoxicity

The relationship between free radical formation and cardiotoxicity remains unclear. The information available does support the hypothesis that those intercalating agents which are more difficult to reduce and are less prone to produce drug-free radicals will be less cardiotoxic. Certainly, mitoxantrone has proved less cardiotoxic than doxorubicin, fulfilling its promise in that regard.[8]

A cultured mouse fetal heart model has been used to compare the potential cardiotoxicity of doxorubicin and the anthrapyrazoles.[29] Fagan *et al.* showed that the anthrapyrazoles produced less damage than doxorubicin or mitoxantrone, as measured by LDH enzyme loss and certain characteristic electron microscopic changes such as myofibrillar loss and mitochondrial swelling.[29] This work suggested that these agents would be significantly less cardiotoxic than doxorubicin but, nevertheless, a spectrum of cardiac cell damage was observed which varied according to the length and composition of the side chains.[29] A toxicity study in rats did report cardiotoxicity and nephrotoxicity with CI-937.[30] However, there is some doubt about the relevance of this model since there were no major toxicities in other animal species.[31,32] Furthermore, nephrotoxicity has not been reported in phase I clinical trials with CI-937, 941 or 942.[33-38]

An additional consideration is the role of iron chelation. Redox cycling involving drug–iron complexes has been implicated in cardiotoxicity. For example, ferric ion–doxorubicin complexes have been shown capable of inducing membrane damage by lipid peroxidation.[39] Additional evidence is provided by the clinical finding that the iron-chelating agent ICRF-187 can protect against the cardiotoxicity of doxorubicin.[40] Frank and Novak[28] showed that CI-937 did form a complex with ferric ion, and hence could become involved in redox cycling via this mechanism.

Resistance

Burchenal *et al.* reported activity with two anthrapyrazoles, CI-937 and CI-942, against a number of cell lines resistant to a wide variety of agents, although cross-resistance with doxorubicin was generally observed.[41] Activity against the relatively refractory Glasgow osteogenic sarcoma was somewhat superior to that of doxorubicin. The studies performed with the NCI tumor panel demonstrated the potential for variable non-cross-resistance with doxorubicin.[20] For example, a doxorubicin-resistant line of mammary carcinoma 16C remained fully sensitive to CI-942, although cross-resistance with the doxorubicin-resistant P388 leukemia line was observed. Diminished resistance against a similarly doxorubicin-resistant I6/C cell line was reported for CI-937 and CI-942.[42] Limited cross-resistance against a doxorubicin-resistant B16 melanoma subline (8.5- to 11.4-fold) was reported for the anthrapyrazoles CI-937 and CI-941.[43] Against a multidrug-resistant human small cell lung cancer line, H69AR, which does not express P-glycoprotein, CI-942 was relatively inactive but CI-937 and CI-941 both demonstrated potent cytotoxicity with resistance of only 11-fold relative to the parent cell line, compared with 54-fold for doxorubicin.[44] In a more detailed study using a multidrug-resistant P388 line, Klohs *et al.* showed that some members of the anthrapyrazole series showed significantly less resistance than doxorubicin.[45] They also demonstrated potentiation by verapamil proportional to the degree of resistance, consistent with resistance mediated by P-glycopro-

tein. Coley *et al.* demonstrated incomplete cross-resistance in a multidrug-resistant variant, EMT6/AR1.0, of the EMT6/P murine mammary tumor between doxorubicin, with a resistance factor of 34, and CI-941, with a factor of 9.[46] This cell line overexpresses P-glycoprotein and accumulates doxorubicin less than the parent line which suggests that CI-941 may be less susceptible to this efflux mechanism. In conclusion, the anthrapyrazoles differ in their cytotoxicity against resistant tumor cells and cross-resistance with doxorubicin may not always be observed.

Preclinical pharmacology of the development compounds

Toxicology

Detailed toxicology, assay methodology and rodent pharmacokinetics have been published for CI-941[31] and CI-942.[32] In both cases leukopenia was dose limiting. CI-941 produced dose-related leukopenia, weight loss, alopecia, diarrhea and also lethal convulsions were observed at the highest dose levels when the drug was administered by rapid i.v. bolus.[31] Neurotoxicity was not reported with any of the anthrapyrazoles tested by Leopold *et al.*[20] and the relevance of this problem to likely clinical toxicity was questioned, since it occurred at doses well above those resulting in antitumor activity. Furthermore, the drug was undetectable in the brain.[31] The LD_{10} for CI-941 in female BALB-c mice was 20 mg/kg and the LD_{50} was 22 mg/kg.

In the case of CI-942, values of 25 mg/kg (75 mg/m^2), 33 mg/kg (99 mg/m^2) and 44 mg/kg (132 mg/m^2) were reported for single dose LD_{10}, LD_{50} and LD_{90} in mice, respectively.[32] The mouse equivalent LD_{10} ($MELD_{10}$) in the dog was 3.81 mg/kg (76.2 mg/m^2). At the $MELD_{10}$ dose in dogs, reversible myelosuppression and testicular atrophy were observed. Again, seizures were observed in rats and dogs at high doses given by rapid i.v. bolus. This was prevented by reducing the rate of administration. At the highest doses, hepatic necrosis was observed.

Pharmacokinetics

HPLC methods for the measurement of CI-941[47] and CI-942[48] have been published. Graham *et al.* demonstrated that CI-941 is highly protein bound, 80–84% in mouse plasma, 92–96% in human plasma, and rapidly eliminated from the plasma following bolus i.v. administration in mice.[31] The kinetics of elimination conformed to a three-compartment model, with rapid initial clearance but a relatively long terminal half-life of 8–19 h. There was a linear relationship between dose and area under the concentration × time curve (AUC) over the dose range 1.5–15 mg/kg. However, at 20 mg/kg, the maximum tolerated dose, the pharmacokinetics became non-linear with a 2.5-fold increase in AUC compared with 15 mg/kg. Tissue distribution studies showed rapid tissue uptake, relatively low drug levels in liver and spleen, no detectable drug in the brain, comparable levels in pancreas, heart and lung and very high levels in the kidney. This was not associated with renal toxicity or a high rate of renal excretion, which was only 12–18% of the total. A starting dose of 5 mg/m^2 was recommended, with the intention being to use a pharmacokinetically guided dose escalation with reference to a target AUC of 110 μM.min (AUC at 15 mg/kg).[34] The AUC at the LD_{10} itself was not used owing to the non-linear increase in AUC at that dose.

CI-942, or piroxantrone, was shown to be susceptible to rapid oxidation in neutral and alkaline aqueous solutions[48] and also proved to be unstable in mouse and human plasma. A mixture of citrate and ascorbate was used to stabilize the drug following sample collection. The pharmacokinetics were studied following rapid i.v. bolus in the mouse. The drug was eliminated according to a two-compartment open model and metabolism to a glucoronide conjugate was demonstrated.[32] A similar pattern of elimination to that of CI-941 was observed with a short alpha half-life and prolonged terminal elimination, e.g. $t_{1/2\beta}$ of 5.5 h at 90 mg/m^2. The pharmacokinetic behavior of these agents, characterized by a large volume of distribution, relatively slow terminal elimination and low urinary recovery, is similar to that of the anthracyclines and anthracenediones. This is thought to be due to rapid tissue accumulation and slow release.[49,50] A target AUC of 40% that at the mouse LD_{10} (75 mg/m^2), i.e. 59 μg/ml.min, was chosen for dose escalation purposes.

Clinical studies

Phase I studies

Three anthrapyrazoles have completed phase I evaluation, CI-937,[33] CI-941[34–36] and CI-942.[37,38] In

each case the dose-limiting toxicity has proved to be myelosuppression. Problems have been encountered in the attempts to use pharmacokinetically guided dose escalation.

CI-937. In the National Cancer Institute of Canada study reported by Erlichman *et al.*,[33] CI-937 was given by i.v. bolus every 3 weeks. Granulocytopenia was found to be dose limiting with a maximum tolerated dose of 25.2 mg/m^2. At this dose 8/13 courses were associated with ≥grade 3 granulocytopenia. Other toxicities were mild and included nausea and vomiting, alopecia and a reversible rise in serum bilirubin. The significance of this is unclear since a number of the patients in whom hyperbilirubinemia was observed had metastatic liver disease. After one dose doubling the target AUC was reached, hence the dose escalation conformed to a modified Fibonacci schedule. A linear relationship between dose and AUC was observed. One response was seen in a patient with mesothelioma. A starting dose of 22 mg/m^2 has been recommended for phase II trials.

An alternative schedule of 3 weekly administrations repeated every 5 weeks is also being explored. Early indications are that this is well tolerated and the toxicities observed have been similar to those in the 3 week interval study.

CI-941. A phase I study of CI-941 was performed at the Royal Marsden Hospital using a single i.v. bolus every 3 weeks. As with CI-937, it was the intention to perform a pharmacokinetically guided dose escalation.[34] Unfortunately, interpatient variations in AUC at the starting doses were such that these data could not be used for dose escalation. In addition, the demonstration of non-linear kinetics in the mouse underlined the need for caution and a fixed increment schedule was adopted. Leukopenia proved dose limiting giving a maximum tolerated dose of 55 mg/m^2, other toxicities being mild.[35] There was no evidence of cumulative myelosuppression. A dose schedule of 50 mg/m^2 every 3 weeks was recommended for phase II evaluation. Responses were observed at that dose in patients with advanced breast cancer who had not been previously treated with either doxorubicin or mitoxantrone.[35]

A phase I study using a fractionated schedule was performed in Edinburgh. Patients were given 3 weekly i.v. injections of CI-941 separated by a 3-week interval between courses. The dose-limiting toxicity was leukopenia, but moderate nausea and vomiting and mild alopecia were also observed. The maximum dose level reached was 36 mg/m^2 and the recommended dose for phase II studies using this schedule was 24 mg/m^2. No responses were observed in this study.[36]

Pharmacokinetic studies were performed and again showed marked interpatient variability. Elimination from the plasma was triphasic with a long terminal half-life (mean 14.1 ± 7.8 h).

CI-942. A single i.v. infusion over 1 h every 3 weeks was studied at Johns Hopkins.[38] As with CI-941, problems were encountered with the attempt to escalate according to the AUC. In this case the sensitivity of the assay was insufficient to measure AUC at the first two dose levels. At 30 mg/m^2 AUCs of 130–560 μM.min were measured encompassing the target AUC. Further escalations therefore followed a modified Fibonacci schedule. Myelosuppression, predominantly granulocytopenia, was dose limiting with a maximum tolerated dose of 190 mg/m^2. In some cases recovery of the leukocyte count was delayed and there was evidence of cumulative myelosuppression.

Other toxicities were generally mild, e.g. nausea and vomiting, rare alopecia and some mucositis at the highest dose. However there was a significant incidence of thrombophlebitis. The majority of patients complained of burning or itching and developed local erythema. Sclerosis was observed in the vein used for administration in six patients. The vesicant nature of this agent was confirmed in rodent studies.[51] No evidence of cardiotoxicity was observed but few patients received a large cumulative dose of CI-942 so no conclusions can be drawn regarding the cardiotoxic potential of the drug.

Pharmacokinetic studies showed a linear relationship between dose and AUC and the AUC at the maximum tolerated dose was 435 μM.min, 40% higher than the AUC at the mouse LD_{10}. A dose of 150 mg/m^2 was recommended for phase II trials with the additional advice that the dose be modified on the basis of the initial myelosuppression observed.

A separate study was performed at the Mayo Clinic using the same starting dose of 7.5 mg/m^2 and identical schedule of single administration every 3 weeks. A similar attempt at pharmacokinetically guided dose escalation was made.[39] It was difficult to measure the drug at the first two dose levels, therefore two dose doublings were performed. Since one patient minimally exceeded the target AUC at 30 mg/m^2 the next dose was only increased by a factor of 1.5. At 45 mg/m^2 AUCs

were all below the target and a final dose doubling to 90 mg/m^2 brought every patient above the target AUC level. Further escalations to a maximum tolerated dose of 160 mg/m^2 were performed according to a modified Fibonacci scheme. This experience again underlines the difficulty of performing pharmacokinetically guided dose escalation since the assay sensitivity was insufficient to measure AUC at the starting dose and the large interpatient variability in drug concentrations made it difficult to extrapolate. Nevertheless, one could regard this trial as a qualified success for the approach. It was estimated that six to nine fewer patients were required than if pharmacokinetic information had not been used.

The dose-limiting toxicity was again leukopenia with other toxicities being mild. Local venous irritation was again observed leading to administration of the drug in a more dilute form. No cardiotoxicity was observed at cumulative doses up to 1280 mg/m^2. A dose schedule of 160 mg/m^2 was recommended for phase II evaluation by these authors.

The pharmacokinetics of CI-942 in humans showed a monoexponential fall in plasma concentration from the end of infusion with a $t_{1/2}$ of approximately 30 min. A more prolonged terminal elimination phase could not be excluded on the basis of the assay available. It is possible that the more rapid degradation of CI-942 in human than in mouse plasma contributes to the higher plasma clearance in man.

Responses were observed in two patients with metastatic breast cancer and malignant melanoma at doses of 140 and 160 mg/m^2. Phase II studies of piroxantrone are planned.

Phase II studies

CI-941. A phase II study of CI-941 in advanced breast cancer has been performed at the Royal Marsden Hospital.[51] A total of 31 patients with advanced breast cancer were treated with a dose of 50 mg/m^2 every 3 weeks, of whom 30 were evaluable for response. Patients who had been previously treated with doxorubicin or mitoxantrone were excluded. Two complete and 17 partial responses were documented giving a response rate of 63% (95% confidence interval of 46 to 81%). The median response duration was 28 weeks (range 4–70). There were four partial responses in six patients who had received adjuvant cyclophosphamide, methorexate, 5-fluororuracil (CMF) chemotherapy and two out of three patients who had failed to respond to CMF for advanced disease.

The main toxicity was granulocytopenia, which was WHO grade 4 in 54% of courses. However, recovery was rapid and there was no evidence of cumulative bone marrow toxicity. Few episodes of neutropenic infection were observed and there were no toxic deaths. Nausea and vomiting, alopecia and mucositis were mostly WHO grade 1–2. Patients were investigated for cardiotoxicity by serial isotope ventriculography. A small fall in left ventricular ejection fraction (LVEF) was observed during the course of treatment in a number of patients receiving ≥ 5 courses. The median fall in LVEF was 6%.[52] Long-term follow-up has subsequently shown that at 6 months following the end of CI-941 therapy there has generally been no further deterioration and in three cases a small degree of recovery has been observed.

The results in advanced breast cancer are certainly encouraging. If confirmed, these data show that CI-941 is probably as active a single agent as doxorubicin with significant reduction in certain toxicities such as nausea and vomiting, alopecia and mucositis. There may be some potential for cardiotoxicity but falls in LVEF in patients with advanced breast cancer have also been reported with other forms of chemotherapy, such as CMF[53] which is not associated with the formation of reactive oxygen species. It will require a randomized comparison between CI-941 and doxorubicin to determine whether by limiting the ability to form free radicals the development of this agent has genuinely resulted in reduced cardiotoxicity.

This phase II study was halted due to a problem with drug supply following the transfer of the patent rights from Warner-Lambert Health Care to Du Pont Pharmaceuticals. This has also delayed the start of randomized trials. Plans for a multi-centre randomized study are, however, well advanced.

A multi-centre phase II trial of CI-937 (now DuP-937) in a variety of solid tumors has begun in Europe and the USA.

Conclusions

The anthrapyrazoles appear to be an extremely exciting group of anticancer agents, characterized by a broad spectrum of activity against experimental tumors and much reduced potential for free radical generation. It is hoped that this will limit the likelihood of cardiotoxicity. In phase I clinical trials the dose-limiting toxicity for the three analogs

studied has been leukopenia. Other toxicities have been mild, in particular nausea and vomiting, mucositis and alopecia appear less than with equally myelosuppressive doses of doxorubicin. In a phase II study of CI-941 in advanced breast cancer a response rate of 63% has been observed which compares favorably with the single agent activity of doxorubicin and may be superior to that of mitoxantrone. Randomized trials are now indicated to compare the antitumor activity and cardiotoxicity of doxorubicin and the anthrapyrazoles.

There are some indications that the anthrapyrazoles may not be subject to precisely the same resistance mechanisms as the anthracyclines, including P-glycoprotein. This raises the possibility that they may have a different spectrum of activity and possibly retain some efficacy in doxorubicin-resistant disease.

Given that major toxicity is limited to granulocytopenia, there is clearly the potential for dose escalation using the usual supportive measures for high-dose chemotherapy with or without autologous bone marrow rescue or the use of colony stimulating factors. If significant antitumor activity is confirmed this is sure to be the focus of considerable research. The anthrapyrazoles would seem to be a group of new anticancer drugs with a very bright future.

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