Summary. In the past five years, several groups have reported acute myeloid leukemia (AML) often monoblastic, as a complication of chemotherapy regimens including the epipodophyllotoxins, etoposide and teniposide. This syndrome is distinct clinically, pathologically and cytogenetically from classical therapy-related myelodysplasia and AML. There is also evidence that other topoisomerase II inhibitors, such as the intercalating agents (including doxorubicin, mitoxantrone, and actinomycin D) may be leukemogenic. Furthermore, there may be further interactions from concomitant topoisomerase II inhibitors and alkylating agents. Topoisomerase II inhibitors induce DNA cleavage and other chromosomal aberrations, including sister chromatid exchanges. These clastogenic abnormalities are not fully understood, and may be specific for each cytotoxic agent. Work is in progress to clone breakpoints such as the t(9;11) and t(8;21) and the use of the resultant DNA probes will enhance our understanding of the leukemogenic process. Given the potential diversity in patients with secondary leukemia, cytogenetic studies should be mandatory for both enhancing our knowledge base and guiding treatment in individual patients. Clinicians must also be wary of the leukemogenic potential of 'dose-intense' regimens including agents such as etoposide and doxorubicin.

Key words: acute myeloid leukemia, secondary tumors, VP16, topoisomerase II inhibitors

Therapy-related myelodysplasia and acute myeloid leukemia (AML) are well-characterized complications of chemotherapy [1-6]. This syndrome often presents with pancytopenia and MDS after a median latency period of approximately four to five years. In most cases, the MDS will progress to overt AML, which is relatively refractory to chemotherapy [7], with a resulting short survival.

The vast majority of previously reported cases have been attributed to prior therapy with alkylating agents, including nitrogen mustard, procarbazine, cyclophosphamide, chlorambucil, and melphalan [8-14]. Furthermore, most large epidemiologic studies have demonstrated a relationship between cumulative alkylator dose and risk of MDS or AML. Cytogenetic studies of patients with therapy-related MDS or AML have most often demonstrated abnormalities of chromosomes 5 and/or 7 [1-6]. These abnormalities include whole chromosome losses, as well as deletions and unbalanced translocations involving the long arms of these chromosomes. Often, there are complex abnormalities involving multiple other chromosomes as well. In summary, therapy-related MDS/AML due to alkylating agents is a well-defined cytogenetic, pathologic, and clinical syndrome.

Therapy-related leukemia: A brief review

Therapy-related leukemia secondary to epipodophyllotoxins and related agents: A historical perspective of prior cases

Since most chemotherapy regimens include one or more alkylating agents, no data were available to refute a longstanding opinion that only alkylating agents were leukemogenic. Ratain et al. first suggested that etoposide was leukemogenic in a retrospective analysis of 119 patients with advanced non-small-cell lung cancer receiving cisplatin plus etoposide and/or vindesine [15]. Of the original 119 patients, only 24 survived longer than one year, of whom four eventually developed AML at 13, 19, 28, and 35 months from the start of treatment. This yielded a cumulative risk estimate of 15±11% and 44±24% at 2 and 2.5 years, respectively. There was strong evidence that the risk was related to etoposide dose, with a median dose in the first year of 6.8 g/m² in the four leukemia patients, versus 3.0 g/m² in the twenty nonleukemic one-year survivors. In addition, three cases had monoblastic features, two of which had balanced translocations involving 9p22 and 11q23. In summary, it appeared that etoposide, when given at high doses, could induce a secondary leukemia distinct from that caused by alkylating agents, because of its unusual morphologic and karyotypic pattern and short latent period.
Further evidence supporting our hypothesis was provided by two reports from St. Jude by Pui et al. [16, 17]. This group's initial report [16] suggested that patients with a T-cell acute lymphoid leukemia (who received teniposide) were at especially high risk, although both of their reports are consistent with the hypothesis that epipodophyllotoxins are leukemogenic. Eight of these thirteen cases included abnormalities, primarily balanced translocations, involving 11q23. Most of these cases had monoblastic features, and occurred with a relatively short latent period as described in our initial report.

Most recently, Pedersen-Bjergaard et al. reported five patients with AML after etoposide, cisplatin and bleomycin for germ-cell tumors [18]. The risk of leukemia was related to total etoposide dose. Two cases had monocytoid features, and one case had a typical t(9;11). Of interest, two cases had a t(8;21), an abnormality usually seen with de novo AML M2.

There have also been a number of case reports or series including one or more patients similar to our original description [19–28]. This body of literature convincingly demonstrates an association between prior epipodophyllotoxin therapy and subsequent development of a monoblastic leukemia with abnormalities of 11q23. There is also a suggestion that other intercalating agents acting via topoisomerase II inhibition may also be leukemogenic [24, 29], and that this class of drugs may induce other balanced translocations as well [17, 18, 30].

Pedersen-Bjergaard and Philip recently addressed this issue by reanalyzing 91 of their cases [29]. They correlated the prior treatment with the type of aberration involving 21q22 as well as 11q23, dividing patients into groups with balanced versus unbalanced translocations. All six t-AML cases with balanced 11q23 translocations had been previously treated with etoposide; four of them were also treated with multiple drugs including CCNU, cyclophosphamide, doxorubicin, and 5-fluorouracil. All five patients with 21q22 balanced translocations had also received topoisomerase II inhibitors. Of six patients with unbalanced translocations, none had received only topoisomerase II inhibitors. Although three patients had received these agents at some time, five of the six had also received alkylating agents. Among 56 patients who had not received topoisomerase II inhibitors, none had balanced translocations involving these two bands. Among our own series of more than 120 patients, eight of ten with balanced translocations had received topoisomerase II inhibitors (six of them in conjunction with alkylating agents) [R. A. Larson and J. D. Rowley, unpublished]. In contrast, only one of ten with unbalanced translocations had received a topoisomerase II inhibitor, thus confirming the observation of Pedersen-Bjergaard and Philip that topoisomerase II inhibitors are associated with balanced but not unbalanced translocations of these two chromosome bands.

It is appropriate to ask whether this is a new syndrome, or simply a newly recognized syndrome. The epipodophyllotoxins are relatively new agents, and probably account for most of the reported cases. However, it appears that other commonly used intercalating agents, such as doxorubicin and actinomycin D, are also leukemogenic. Doxorubicin is almost always given in combination with alkylating agents such as cyclophosphamide. Thus, it would be difficult to distinguish a doxorubicin-induced leukemia (with a balanced translocation) from cyclophosphamide-induced leukemia (with abnormalities of chromosome 5 and/or 7 or an unbalanced translocation) without careful cytogenetic analysis, often not available to most clinicians. In fact, there are examples in the literature of patients treated with alkylating agents plus other topoisomerase II inhibitors, such as actinomycin D [31, 32], amsacrine [31], doxorubicin [32, 33], and mitoxantrone [34], who developed secondary leukemias similar to those cases attributed to epipodophyllotoxins. Further indirect evidence is provided by Kaldor et al. [13] who noted that the risk of secondary leukemia after treatment for ovarian cancer with doxorubicin and cisplatin was comparable to that of cyclophosphamide at a high cumulative dose.

Clastogenic effects of chemotherapeutic agents

Carcinogenic effects of cytotoxic agents can be due to direct damage to DNA or to DNA cleavage in association with topoisomerase II inhibitors and induction of sister chromatid exchanges (SCE) and other chromosomal aberrations (clastogenesis). Whereas alkylating agents, by definition, bind directly to DNA, both intercalating agents and epipodophyllotoxins are clastogenic [35–41].

There is strong evidence to support the hypothesis that action of DNA topoisomerases, particularly topoisomerase II, is required for the development of SCE [42]. Topoisomerase I incises a single DNA strand, with resultant binding to the 3' end. In contrast, topoisomerase II, a dimeric protein, produces double-strand breaks with binding to each of the 3' ends, leaving the 5' ends free. These topoisomerases thus allow for the unwinding of DNA, and are thought to be important for efficient DNA replication.

Several models have been proposed for the formation of SCE [42]. All of these models require DNA incision, followed by DNA-topoisomerase binding. There is no clear conclusion as to the final event in SCE induction, with evidence for both exchange of topoisomerase II subunits [37] and strand switching during removal of parental helical turns by topoisomerases [43, 44].

Inhibitors of topoisomerase II include both the intercalators [45] and non-intercalating epipodophyllotoxins [46]. These agents inhibit the ligase activity of topoisomerase II, thereby resulting in protein-associated double-strand DNA breaks, as well as SCE. Of
particular note, DNA strand breaks induced by topoisomerase II inhibitors are relatively selective, and may be distinct for each agent [47–50]. In support of the clinical data, 11q is one known site of etoposide-induced aberrations [50].

Although cytogenetic analysis can localize sites of chromosomal abnormalities, it does not allow determination of the abnormal gene and leukemogenic process. Of significance to this subject, the breakpoint junctions in both the t(8;21) and t(9;11) are very near to being cloned [51, 52]. Once this is accomplished, the DNA probes may be used to compare the breakpoints in the balanced and unbalanced translocations. Thus, we will soon be able to determine whether or not the same genes are affected, and subsequently, the mechanisms of involvement of inhibitors of topoisomerase II in these translocations. Although part of the 11q23 region has been mapped [53–58], it appears that all of the known genes are quite some distance from the breakpoint, with the CD3 complex at least 100 kb centromeric and the CBL-THY1 gene cluster being several hundred kb telomeric [52].

Implications and future directions

We have attempted to review the literature from a variety of disciplines relevant to the primary hypothesis that inhibitors of topoisomerase II are leukemogenic in man. This is supported both by epidemiologic data and studies of the molecular pharmacology and clastogenic properties of this class of agents. As a corollary, the likelihood of such clastogenic events would be increased under conditions which enhance topoisomerase II activity, such as schedules which utilize frequent dosing. One must be especially concerned about the development of ‘dose-intense’ regimens using epipodophyllotoxins and related agents, especially in patients treated with curative intent or for adjuvant therapy. Concomitant alkylating agents might also increase the risk of SCE, since alkylating agents produce lesions which might be repaired by topoisomerase II.

Given that secondary leukemias will occur, what can be done to minimize unnecessary risk and maximize outcome? Clearly, maintenance therapy using such agents should be minimized. The usual practice of continuing therapy in the absence of progressive disease may have been a factor in at least one series of patients [15]. Most importantly, cytogenetic studies should be obtained on all patients with secondary leukemia. This will allow both the expansion of our current knowledge base, as well as potentially guiding therapy for individual patients. Patients with alkylator-induced leukemia (with abnormalities of chromosomes 5 and/or 7) generally respond poorly to most antileukemic regimens [7], whereas topoisomerase II inhibitor-related leukemias, with t(9;11) or other balanced translocations, may be responsive to chemotherapy, based on anecdotal reports [15, 27, 33]. Furthermore, it has been reported that patients without antecedent MDS, a common finding in topoisomerase II inhibitor-related leukemia, have a better outcome with chemotherapy [6].

These hypotheses may be directly tested in several ways. It might be possible to induce topoisomerase II inhibitor-related leukemia in an animal model. This would be direct proof, as well as providing a model for development of new therapies. Clastogenic studies in vitro would also be useful, for assessing modulation of topoisomerase II-induced SCE by other agents in the context of combination chemotherapy. Finally, work in progress to clone breakpoints such as 11q23 and 22q11, will allow eventual elucidation of the specific genes that are susceptible to the leukemogenic process. Analysis of the DNA surrounding these genes will yield valuable information regarding chromosome structures that may be unusually vulnerable to the actions of topoisomerase II inhibitors.

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