Transplacental Chemical Exposure and Risk of Infant Leukemia with MLL Gene Fusion

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ABSTRACT

Infant acute leukemia (IAL) frequently involves breakdown and recombination of the MLL gene with one of several potential partner genes. These gene fusions arise in utero and are similar to those found in leukemias secondary to chemotherapy with inhibitors of topoisomerase II (topo-II). This has led to the hypothesis that in utero exposures to chemicals may cause IAL via an effect on topo-II. We report a pilot case-control study of IAL across different countries and ethnic groups. Cases (n = 136) were population-based in most centers. Controls (n = 266) were selected from inpatients and outpatients at hospitals serving the same populations. MLL rearrangement status was derived by Southern blot analysis, and maternal exposure data were obtained by interviews using a structured questionnaire. Apart from the use of cigarettes and alcohol, very few mothers reported exposure to known topo-II inhibitors. Significant case-control differences were apparent for ingestion of several groups of drugs, including herbal medicines and drugs classified as “DNA-damaging,” and for exposure to pesticides with the last two being largely attributable, respectively, to one nonsteroidal anti-inflammatory drug, dipryrone, and mosquitocidals (including Baygon). Elevated odds ratios were observed for MLL<sup>++</sup>ve (but not MLL<sup>++</sup>ve) leukemias (2.31 for DNA-damaging drugs, P = 0.03; 5.84 for dipryrone, P = 0.001; and 9.68 for mosquitocidals, P = 0.003). Although it is unclear at present whether these particular exposures operate via an effect on topo-II, the data suggest that specific chemical exposures of the fetus during pregnancy may cause MLL gene fusions. Given the widespread use of dipryrone, Baygon, and other carbamate-based insecticides in certain settings, confirmation of these apparent associations is urgently required.

INTRODUCTION

In most cases of IAL, both ALL and AML have rearrangements of the MLL gene at 11q23, which are referred to as MLL<sup>++</sup>ve (1–4).

Received 4/24/00; accepted 1/24/01.

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1 Supported by the Kay Kendall Leukaemia Fund. Additional local funding came from the Associazione Italiana per la Lotta contro le Leucemie-Sezione di Roma (ROMAIL), the Japan Leukemia Research Fund, the Japan Children’s Cancer Association, a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan, the University of Athens Medical School, and the Harvard School of Public Health. The support of The Hong Kong Paediatric and Haematology Study Group, the Japan Infant Leukemia Study Group, and the Hematology and Oncology Group of Greece is acknowledged.

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3 The abbreviations used are: IAL, infant acute leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; topo-II, topoisomerase II; NQ01, NADPH: quinone oxidoreductase; OR, odds ratio; CI, confidence interval.

4 M. Smith, personal communication.

Investigation of identical twin pairs, concordant for infant leukemia (5), has shown that this genetic lesion was acquired in utero by one twin and transferred to the other, and in utero origin has been confirmed by retrospective analyses of neonatal blood spots (Guthrie cards) of affected infants (6). The high concordance rate for leukemia in monozygotic twins of this age and the very short latency suggests that an MLL fusion in the appropriate fetal hematopoietic stem cell is sufficient to cause leukemia (7). MLL rearrangements are rare in older cases of de novo leukemia but frequent in cases arising subsequent to chemotherapy with inhibitors of topo-II. This suggested that similar exposures in utero might be relevant to infant leukemia (5) and has led to the specific biologically based hypothesis that MLL<sup>++</sup>ve infant leukemia is caused by transplacental exposure to substances that form cleavable complexes with topo-II (8, 9).

Although the hypothesis is very precise in terms of case biological subgroups, timing of exposure, and biological mechanism, it is difficult to test because many inhibitors of topo-II remain unidentified. An additional problem is that the magnitude of the effect of different substances is unknown, so that computation of total exposure is not possible.

topo-II inhibitors include chemotherapeutic agents (10), benzene metabolites (such as benzoinone and, hence, smoking; Refs. 11, 12), isoflavones, flavonoids, lignans (e.g., Ref. 13), some herbal medicines (14), anthraquinone laxatives such as senna (15), podophyllin resin, quinolone antibiotics, some pesticides including certain fungicidals and mosquitocidals (16, 17), some culinary herbs (e.g., turmeric; Ref. 18), and many, though not all, phenolic substances or their metabolites (19). Herbal medicines are frequently potent (20, 21), and an association with MLL<sup>++</sup>ve AIL was a prior hypothesis.

Limited epidemiological support is available. Maternal dietary consumption of topo-II inhibitors has been associated specifically with infant acute myeloid leukemia in one United States case-control study (n = 84 cases; Ref. 22). The metabolism of quinones, as exemplified by benzene detoxification, is critically controlled by the enzyme NQ01 (19). Two polymorphic variants of NQ01 have been identified (23). The first effectively inactivates the enzyme (24). This has been associated with chemotherapy-related leukemia (25) and MLL<sup>++</sup>ve infant leukemia (26).

The objectives of the present study were: (a) to test the hypothesis that exposure in utero known to inhibitors of topo-II increased risk of MLL<sup>++</sup>ve infant leukemia; (b) to conduct a preliminary evaluation of
the more general hypothesis that exposures in utero to pharmaceutical drugs, solvents, pesticides, and herbal medicines were associated with IAL and specifically MLL $^{+ve}$ disease; and (c) to generate hypotheses that specific substances were associated with MLL $^{+ve}$ infant leukemia.

**MATERIALS AND METHODS**

**Cases and Controls.** An international collaboration has permitted a pilot case-control study of IAL to be conducted in regions within countries in Europe and the Middle East (Italy, Greece, and Egypt), South America (Brazil and Chile), and Asia (mainland China, Hong Kong, and Japan). $^5$ Case ascertainment was based on referrals to treating hospitals but was population based $^6$ (i.e., included all of or almost all of the cases of acute leukemia in infants <18 months of age diagnosed in defined time periods and geographical areas). Informed consent was requested from mothers, and 136 were recruited. Controls ($n = 266$) were selected from inpatients and outpatients at the same or similar hospitals serving the same populations. Appropriate diagnostic inclusion and/or exclusion criteria were applied to the controls.$^7$ The case:control ratio was approximately 1:2 in each center, and to give similar distributions of the time elapsed from the birth index to the mother’s interview, two controls were selected with the same gender and similar dates of birth (normally, ± 1 month) as soon as each case mother had been interviewed (normally shortly after diagnosis). Details provided by some centers (believed to be representative) indicate high percentages of mothers agreeing to be interviewed (cases, >98%; controls, >90%).

**MLL Gene Rearrangement Status.** A number of methods are available for evaluation of MLL gene rearrangement including Southern blot, reverse transcription-PCR, and fluorescence in situ hybridization. At the time of the initiation of this study, 4 years ago, we considered Southern blot to be the most practical. The procedure was as described previously (27) using a PCR-generated cDNA probe (exons 5 to 7 of MLL) and BamHI digest. A standardized protocol was established, and individual centers either performed their own tests (Italy, China, Japan, and Hong Kong) or referred extracted DNA samples to the reference laboratory (M. F. G.) in London. In some instances (China, Japan, and Hong Kong), confirmation of the result was carried out in the reference laboratory. DNA from a MLL-AF4 positive cell line (RS4:11) and a leukemic cell line (KG1) without a MLL gene rearrangement was available as a control. A germ-line (nonrearranged) MLL gene allele served as an internal control for the quality of DNA preparation. Either blood (with adequate blast cell counts) or bone marrow was used. For a proportion of cases, no suitable diagnostic specimen was available for analysis, or referred DNA samples (e.g., on slides) were degraded and, in consequence, their MLL status is unknown. This information is not from the cases from Greece. This limitation restricted the power of the study but does not introduce any bias. In subsequent studies, we would recommend using fluorescence in situ hybridization methods on stored (frozen) unstained bone marrow smears ($^8$).

**Exposure Classification.** Maternal exposure data were obtained by interviews using a structured questionnaire. This was translated into local languages, and the validity of the translations was checked by dual Interviewing of bilingual mothers. Questions focused on lifestyle (alcohol and smoking use), medical drug ingestion (prescription and other), and occupational exposures. Medication use was obtained from questions concerning the use of certain types of drugs (e.g., antibiotics and herbal medicines), infectious illnesses, and medical consultations. A pharmacist (blind to numbers of mothers and status) reviewed the list of medications reported by the mothers, but none were recognized as known topo-II inhibitors. Analysis categories for drugs were determined after examination of overall frequencies blind to case-control status. The first type of category was based on the medical problem (e.g., urinary tract infection); the second was based on pharmacological action (e.g., nonsteroidal anti-inflammatory drugs); the third considered “DNA-damaging” drugs (i.e., those for whom genotoxic effects had been reported for some organ in some species).$^9$ Finally, individual drugs with frequent reported use were to be analyzed if members of the groups associated with the case status and/or harmful effects were reported by the pharmacist. Occupational exposure of the mother to pesticides, solvents, organic dusts, and ionizing radiation was also sought in the interview.$^9$ Interviewers in some countries, notably Brazil, included nonoccupational exposures of mothers in the interview. Because these were qualitatively similar to many occupational exposures (e.g., housewife/housemaid), they were combined for analysis. A policy for selection of specific substances for analyses similar to that for drugs was formulated.

**Statistical Analysis.** Exposures of case and control mothers during the year before the birth of index child have been compared using logistic regression (29) and exact methods (30) implemented by the packages SAS and EGRET. All of the analyses have adjusted for sex and region of residence (South America, Asia and Europe and the Middle East), and important results have been checked with further adjustment by country. All of the controls have been included in each analysis.$^{10}$ Results are expressed as ORs with 95% CIs. All of the $P$ are two-sided.

**RESULTS**

Details of cases are shown in Table 1. Apart from the use of cigarettes and alcohol and a few instances where solvent exposure was specified as benzene or gasoline, no mothers reported exposure to known topo-II inhibitors. The ORs for “ever” compared with “never” for alcohol consumption and cigarette use were slightly elevated in the total series but $<1$ for MLL $^{+ve}$ cases (Table 2), although they are consistent with approximately 2-fold elevation of risk. Several drug groups determined by reason for use (vaginal infection, anemia or anemia prophylaxis, pain, and digestive problems) showed statistically significant (0.01 < $P$ < 0.05) positive associations with infant leukemia in the total series but reduced or similar ORs when MLL $^{+ve}$ cases and MLL $^{−ve}$ cases were compared with the controls. Only four mothers (three cases with two MLL $^{−ve}$ and one MLL $^{+ve}$ and one

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$^5$ The whole of Greece, Chile, and Hong Kong but parts of other countries.  
$^6$ Other than mainland China. Exceptions include a small number of children treated in private hospitals.  
$^7$ Exclusion criteria for children in general pediatric wards as reasons for hospitalization were: leukemia, lymphoma or other cancer, Fanconi anemia, Bloom’s syndrome, ataxia telangiectasia, neurofibromatosis, major congenital abnormalities (including Down’s syndrome), immune deficiency, congenital infection acquired in utero, and hematological conditions (except nutritional anemia, thalassemia minor, hemophilia, and hemoglobinopathy). Japan derived controls from hematological wards so that the hematological conditions listed above became control inclusion criteria. In Hong Kong, controls were selected from neighborhood child health clinic attendees.

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Table 1 Cases included in the study

<table>
<thead>
<tr>
<th>Region</th>
<th>Cases (MLL $^{+ve}$)</th>
<th>Controls (MLL $^{−ve}$)</th>
<th>Total</th>
<th>MLL status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>19 (53%)</td>
<td>17</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>South America</td>
<td>29 (66%)</td>
<td>13</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Asia (mainland China, Hong Kong, and Japan)</td>
<td>2 (40%)</td>
<td>3</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>0–5 mo</td>
<td>18 (72%)</td>
<td>15</td>
<td>130</td>
</tr>
<tr>
<td>6–11 mo</td>
<td>14 (58%)</td>
<td>10</td>
<td>18</td>
<td>42</td>
</tr>
<tr>
<td>12–17 mo</td>
<td>18 (50%)</td>
<td>18</td>
<td>25</td>
<td>87</td>
</tr>
</tbody>
</table>

$^a$ Percentage of MLL $^{+ve}$ of those cases with known MLL status.  
$^b$ NK: no diagnostic sample available or sample degraded.  
$^c$ Includes biphenotypic.

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$^8$ This category was derived by one of us (F. E. A.) by record linkage searching abstracts of papers published in English from 1995–1999 and using the following key words: genotoxic, carcinogenic, highly reactive, single strand breaks, and sister chromatid. Any evidence of genotoxic behavior of the drug and/or a metabolite in any organ of any species was taken as a defining entry to this category. Frequently, this was based on the Comet or similar assays. This process was conducted blind to the numbers and the status of mothers reporting drug use, and it was suggested by the observation of Prof. Martyn Smith (University of Berkeley, CA) that many of the drugs in the list were capable of causing DNA damage.

$^9$ Except in Greece.

$^{10}$ Except when, for analyses of case subgroups, those in countries with no cases in the subgroup have been excluded. Specifically, controls in Egypt and Greece were excluded for analyses of MLL $^{+ve}$ and $^{−ve}$ subgroups.
control) reported exposure to gasoline or benzene. More convincing evidence, including significant associations for both the total series and MLL \textsuperscript{ve} cases compared to controls, was found for DNA-damaging drugs, herbal medicines, and maternal pesticide exposure (Table 2).

Two specific drugs were identified by the pharmacist as being potentially harmful (dipyrone, a nonsteroidal anti-inflammatory drug, and metronidazole, an antibiotic used for vaginal infections). These are members of the group of DNA-damaging drugs, and numbers of reports were adequate for analyses. Only one specific pesticide was used by more than two mothers (Baygon, reported by seven mothers; this has been combined with mosquitocidals for analysis). Analyses of these exposure subgroups show highly significant and selective associations with MLL \textsuperscript{ve} leukemia for dipyrone and for Baygon/mosquitocidals. There is a suggestion of a stronger association with MLL \textsuperscript{ve} leukemia for dipyrone and for Baygon/mosquitocidals. The present data suggest that previous results may not have been attributable to exposures of this. The best argument against recall and interviewer bias is when positive results relate selectively to MLL \textsuperscript{ve} disease, because interviewers and mothers of cases were unaware of MLL status. Case selection bias is unlikely but impossible to quantify because cancer registries do not operate in the study regions. Any such bias, either at ascertainment or at recruitment, would be independent of MLL status. Because the same control set was used for analyses of MLL \textsuperscript{ve} and MLL \textsuperscript{ve} cases, the selective associations that we have observed are very unlikely to be attributable to confounding by deprivation or other features that may have influenced control selection. Those associations, which we have found to be of similar strength across case subgroups (e.g., that for herbal medicines), must be treated cautiously.

Testing the hypothesis of exposure to known topo-II inhibitors was not possible because few case and control mothers reported their use. The weakness of the association of infant leukemia with maternal alcohol use is surprising, because most previous studies (31) have reported significant positive associations. The present data suggest that previous results may not have been attributable to exposures of MLL \textsuperscript{ve} cases. Previous studies (31) of tobacco use have been inconsistent and included negative results similar to ours.

However, this study has found evidence that \textit{in utero} exposure to chemicals is associated with risk of IAL, in general, and MLL \textsuperscript{ve} disease in particular. The “DNA-damaging drug” category effect will be diluted by the inclusion of drugs that could not harm human hematopoietic cells and the exclusion of others that could do so. The significant association in the presence of the diluting effect would be consistent with a much stronger association with a more restricted
class of drugs. A similar comment applies to pesticides and herbal medicines. Pesticides have frequently been associated with childhood leukemia and specifically with infants (either as occupational or home exposure and involving both parents and children; see Ref. 32 for review). The literature shows the same concentration of risk in AML cases as we have found. The reason for use was not often provided for herbal medicines, and substances were frequently “herbal teas.” Camomile and many other herbal teas contain polyphenols (33). Three subjects who used herbal teas (all of them mothers of MLL<sup>1</sup> cases) indicated weight loss as their objective. Many herbal teas used to reduce weight contain a topo-II inhibitor, senna, in pharmacologically important quantities (34).

We found two statistically highly significant associations that may be of etiological importance. These are for dipyrone and Baygon (or another usually unnamed substance used against mosquitoes). Dipyrone (or “Mexican aspirin”) was widely used as an analgesic before it was hypothesized that exposures on which we have focused have a distinct etiology, but the exposures on which we have focused are extremely small.

The present data support the hypothesis that MLL<sup>1</sup> leukemia would also be likely to cause adverse pregnancy outcome. It is known that some mosquitocidas inhibit topo-II. Propoxur is a phenol-liberating substance (40) and mosquitocidal. Because some mosquitocidas and many phenolics inhibit topo-II (see “Introduction”), it is plausible that any leukemogenic action of Baygon operates via topo-II.

Ross (4) has identified two important questions concerning the etiology of infant leukemia: (a) can topo-II inhibitors cause other in utero genetic damage and, hence, MLL<sup>1</sup> leukemia; and (b) for MLL<sup>1</sup> infant leukemia, is the etiology of ALL and AML the same?

The present data support the hypothesis that MLL<sup>1</sup> infant leukemia has a distinct etiology, but the exposures on which we have focused appear to have stronger associations with AML; however, the numbers are extremely small.

This pilot study evaluated a broad range of exposures in diverse settings. We did not consider diet, although this is likely to be important (4, 41). Our study has supported the hypothesis that in utero exposure to chemicals causes MLL<sup>1</sup> infant leukemia and has generated specific hypotheses that require further testing. Exposure to dipyrone is widespread, particularly in Central and South America where it is available as an inexpensive, nonprescription drug. Mosquitocidas are similarly in general use in these same settings. Propoxur is also widely used against cockroaches, fleas, and similar pests. Therefore, it is important that the associations observed in this study are reevaluated in an extended case-control study. In view of the fact that we have reported recently (26) between MLL<sup>1</sup> infant leukemia and lack of function NQO1 alleles, screening for metabolic polymorphisms involved in carcinogen metabolism should also be included in subsequent studies.

ACKNOWLEDGMENTS

We thank Prof. Tim Eden and Prof. Martyn Smith for viewing the list of drugs used by mothers and Dr. Tasneem Khalid for going through this list in detail. Assistance from Fani Athanasiadou-Piperopoulou, Maria Kalmanti, Dimitrios Koliouskas, Helen Kosmidis, John Panagiotou, Fotini Tzortzatou-Stathouloupoulou, Kapellaki Rania, Pourtzidis Apostolos, Tsampira Panagiota, and Dogia Ky ratso in Greece and Terezinha Salles, Regina Ferreira, Rita Bayington, and J. Dobin in Brazil is also acknowledged. Dr. Gayle Sherman (University of Witwatersrand, Johannesburg, South Africa) is thanked for comments and discussions on the conduct of the study. Annette Durant is thanked for inputting the data and assisting with drug coding. Patricia Bisset and Ella Chatterjee (University of Edinburgh, UK) are thanked for typing the text and providing secretarial assistance.

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*Cancer Res* 2001;61:2542-2546.

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