EDITORIAL

Infantile leukemia and soybeans – a hypothesis

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Recent molecular–genetic studies have revealed that in the majority of patients with secondary leukemia induced by topoisomerase II (topo II) inhibitors and also with infantile acute leukemia (IAL), the breakpoints are clustered within scaffold attachment regions (SARs) of 3′-MLL-bcr near exon 9. Genistein, abundant in soybeans, is reported to be a potent nonintercalative topo II inhibitor. It interferes with the break–reseal reaction of topo II by stabilizing a cleavable complex, which in the presence of detergents, results in DNA strand breaks. The present study revealed that genistein induced chromatid-type aberrations, in which chromatid exchanges are often observed. Genistein seems to act in a manner very similar to that of VP-16, although the latter is reported to produce both chromatid- and chromosome-type aberrations. In view of this pharmacological similarity between genistein and VP-16, and also the similarity of breakpoint clustering regions within the MLL gene in reported cases with secondary leukemia and IAL, genistein may be largely responsible for the development of IAL.
Keywords: infantile leukemia; genistein; topoisomerase II inhibitors; scaffold attachment regions (SARs); chromatid exchanges

Infantile acute leukemia (IAL) which usually occurs within the first 12 months of life is characterized by a high leukocyte count at diagnosis, often exceeding 100×10^9/l, the presence of a variety of reciprocal translocations with 11q23 and extremely poor prognosis, regardless of whether the IAL is myeloblastic, monoblastic or lymphoblastic.[1] It seems reasonable to assume that leukemic clones of IAL develop *in utero*. With evidence obtained by long-range PCR using primers spanning the *MLL-AF4* genomic junction applied to Guthrie cards, Gale *et al*[2] have shown that leukemic cells were present at birth in three patients who were diagnosed at 5, 6 and 24 months of age, and presented unequivocal evidence of the prenatal initiation of acute leukemia. We should therefore take a closer look at environmental factors during pregnancy to ascertain the cause of IAL.

Recent studies have revealed that in the majority of patients with secondary leukemia induced by topoisomerase II (topo II) inhibitors and also in those with IAL, the breakpoints are clustered within the scaffold attachment regions (SARs) of 3′-MLL-bcr near exon 9,[3,4] which contains several topo II consensus sites for cutting.[3] These findings suggest that similar pathogenetic mechanisms are involved in these two diseases. On the basis of an epidemiological study, Ross *et al*[5] suggested that an association exists between IAL and dietary intake of naturally occurring topo II inhibitors, as well as of marijuana, pesticides, and alcohol during the time of pregnancy.

In the 1970s and 1980s, Sugimura *et al*[6] identified many naturally occurring, potentially carcinogenic mutagens in the environment, as well as many naturally occurring substances that may act to prevent the development of cancer. The former include bracken, royal fern, butterbur flower stalk, pyrolysates, and chemicals made by modern industries, and an example of the latter latter is flavonoids. According to their findings, flavonoids are potentially mutagenic, but they may suppress the progression in carcinogenesis.

Recent studies have shed further light on flavonoids, by identifying a certain degree of chemoprevention by flavonoids of certain classes of cancer. It was found that rapidly proliferating cancer cells at a very early stage could theoretically be killed in the presence of topo II inhibitors, and that thus their growth might be effectively suppressed. On the other hand, it has been suggested that isoflavone phytoestrogens represent a potential hazard for infants, especially for their reproductive organs, as their steric structure closely resembles that of estrogens and they bind weakly to steroidal receptors.[7,8] Daidzein and genistein, aglycones of isoflavones, are found abundantly in soybeans, and their placental transfer has been demonstrated in cord blood analysis.[7] For these reasons, and also because the concentrations of phytoestrogens in soy-based infant formulas are estimated to be several hundred times higher than those in breast milk, these formulas are now the subject of extensive study for elucidation of their potential adverse effects on humans.[8]

Specifically, genistein, known as an undesirable and bitter component of soybeans, is reported to inhibit the activity of tyrosine kinase, p56/p53[lyn], but also to rank as a potent topo II inhibitor among the many flavonoid compounds.[9] It interferes with the break–reseal reaction of topo II by stabilizing a cleavable complex, which in the presence of detergents, results in DNA strand breaks. Unlike adriamycin, actinomycin D, or amsacrine, genistein is classified as a nonintercalative topo II inhibitor, as are VP-16 and VM-26, whereas daidzein possesses no topo II inhibitory action.[10] Thus, genistein seems to act in a manner very similar to that of VP-16 and VM-26,[9] although the mechanism of the action of VP-16 is as yet not fully understood.[11] Nevertheless, in view of this pharmacological similarity between genistein and VP-16, and also the similarity of breakpoint clustering regions within the *MLL* gene in reported cases with secondary leukemia[3] and IAL,[3,4] genistein may be largely responsible for the development of IAL.

Soybeans are a very popular natural food in Japan. Large amounts of soybeans are ingested through a variety of food, and a total amount of 5 million tons is imported from the USA and other countries every year. Therefore, it needs to be recognized that genistein derived from soybeans ingested by a pregnant woman may occasionally and potentially be related to the development of acute leukemia in her baby. According to a report of Shimada *et al*,[12] who analyzed the effect of soybean lipoxygenase on the perceived taste of Tofu (bean curd), the major component of isoflavone occurs in a malonyl form, daidzin, in both soybeans and their dietary product Tofu. The amount of genistein in three different (a wild

and two lipoxygenase deficient) kinds of soybeans and their Tofu products ranged from 0.9 to 1.1 mg/100 g for dried soybeans and from 0.7 to 0.9 mg/100 g in fresh Tofu, indicating that there is no significant loss of genistein during the manufacturing process. Toda et al[13] reported that Miso (bean paste) is the richest in genistein of eight typical Japanese soyfoods. This may be due to the long-term exposure to β-glucosidase during the fermentation process.

The estimated daily intake of genistein by Japanese people from five very common soybean foods is about 1200 μg per person per day. This figure was calculated on the basis of daily consumption of soybeans in Japan (data not shown). Therefore, it is probable that the daily amount may be no more than 20 mg (about 20 times higher than average) even for individuals who are very fond of soybean-based foods. Whether this amount of genistein poses a potential risk for the development of IAL cannot be assessed at present. It seems to be extremely low, however, when compared to the reported median cumulative dose of 6795 mg/m^2 of VP-16 in patients with secondary leukemia.[14] On the other hand, Pui et al[15] suggested that in children treated for acute lymphoblastic leukemia (ALL), the subsequent development of secondary leukemia depends on the frequency of treatment rather than the total received dose. If the Japanese daily intake of soy products were considerably greater than in other countries, the incidence of patients with IAL in Japan should be much higher than in other countries. However, hypothesis is contradicted by the findings of three leading pediatric hematologists, who are also engaged in either epidemiology or molecular cytogenetics.[16] They found that the incidence of IAL in Japan is comparable to that in North America and Europe.

To explain how a common food can sometimes be a contributory cause of a certain disease state, we refer to favism here, because it is a good example of gene–environment interaction resulting in the development of a common disease. Vicia faba only affects individuals with G6PD deficiency, especially those with the Mediterranean phenotype, and often results in severe hemolytic anemia with sudden onset. Since only 10 to 20% of all individuals with G6PD deficiency are affected, however, additional factors have been suspected,[17] including vicine and convicine, or rather their pyrimidine aglycones, divicine and isouramil.[18] On the other hand, Bottini et al[19] suggested recently an association between favism and erythrocyte acid phosphatase polymorphism. These observations seem to imply that individual genetic variations, perhaps in conjunction with other genes as well, make their possessors more susceptible to the potentially adverse effects of certain dietary components.

The pathogenesis of IAL differs considerably from that of favism in that the afflicted infants are born to an apparently normal mother and their incidence is estimated to be extremely low. At present no information is available to provide a deep insight into in utero leukemogenesis, but it is possible to visualize a scenario of how it could develop. Maraschin et al[20] reported that VP-16-induced non-random chromosome-type and chromatid-type aberrations in vitro and chromosomes 1, 11 and 17 were most frequently involved.

We examined the chromosome aberration formations induced by genistein in human cultured lymphocytes. Peripheral blood samples obtained from a healthy donor was cultured conventionally by using PHA in RPMI-1640 supplemented with 10% FCS. Genistein (derived from soybeans; Sigma G6776, St Louis, MO, USA) was added to each culture bottle 15 h after the start of culture at final concentrations of 10, 50, and 100 μM. The samples were then cultured for an

additional 55 h. G-banded (also non-banded) metaphase cells were obtained with a conventional technique. A total of 296 metaphase cells were photographed, 28 of which were karyotyped. The yield and the types of chromosome abnormalities induced by genistein are shown in Table 1. Two typical G-banded metaphase cells obtained after exposure to 50 μM of genistein are shown in Figure 1. Genistein at the concentration of 100 μM was too toxic for cells, so that the mitotic index was greatly reduced. As shown in Figure 1, many chromatid breaks (small arrows), as well as chromatid exchanges (large arrows) were observed within cells, exposed to 50 μM of genistein. In addition, attenuated (elongated) achromatic regions (solid stars) and also an increase in satellite associations (not shown) were often observed. Some chromosomes showed a dicentric morphology in non-banded metaphases, but karyotype analysis revealed that it was also due to attenuation. Thus, detailed karyotypic examination revealed that the abnormalities induced by genistein were exclusively chromatid-type, in sharp contrast to the findings for VP-16,[20] in which abnormalities observed were predominantly chromosome-type. It is noteworthy that a high frequency of chromatid exchanges (cte's) was observed in the present experiment (Table 1). Dicentric, quadriradial and complete cte's[21] were often observed (data not shown). It is well known that cte's appear as 'derived' chromosome-type abberation at the second mitosis following the induction.[22] The adjacent segregation of symmetrical chromatid exchanges may produce a balanced reciprocal translocation in the second mitosis after exposure to clastogens to produce long-lived lesions in DNA.[22]

Studies to determine whether targeted reciprocal translocations, that is, those with 11q23, are produced in subsequent mitoses after exposure to genistein are being carried out with the aid of sophisticated methods. As an adequate latent period is a prerequisite for the clonal growth of leukemic cells in utero, exposure to such substances in the early stage of pregnancy may be crucial, but the majority of fetuses with 11q rearrangements or with leukemic phenotype may be spontaneously aborted, so that only a few would survive as afflicted live-born infants. This situation is similar to that of the genesis of de novo types of chromosome abnormalities, except that in the latter a clastogenic/leukemogenic agent is directed exclusively at hematopoietic stem cells, as indicated by the examination of phenotypes of the live-born infants with leukemia.

It is well known that the chromosome constitution of approximately 50% of fetuses spontaneously aborted in the first trimester is abnormal. Tsuda and Sugiura[23] described chromosome abnormalities in 149 (50.3%) of 296 instances, and of the 149 cytogenetically abnormal fetuses, 86 (58%) were trisomies. Balanced and unbalanced structural chromosome aberrations resulting in fetal wastage accounted for 11 cases (3.7%), including one with a 46,XX,del(11)(q23) karyotype. Unlike trisomy or monosomy, structural aberrations may be caused by a variety of environmental factors, including certain classes of teratomutagenic agents.[24] A healthy woman is said to experience one miscarriage in every five to six pregnancies without being aware of having been sick either physically or mentally, or of having been exposed to obviously hazardous agents. The reason for this is not clear. However, persons continuously exposed to genistein in soy-based foods may be at risk, but to prove it conclusively is difficult. It should be pointed out again that IAL may develop in extremely susceptible fetuses that have a genetic/enzymatic defect(s) in the metabolism of isoflavones, including genistein.

Table 1 Chromosome abnormalities induced by genistein in human cultured lymphocytes from a normal subject

Genistein Conc (μM)	Mitotic index (%)	No. of cells examined	No. of abn cells (%)	Total No. of abn chromosomes and their types			
				ctb	csb	cte	cse
10	4.2	151	10 (6.6)	10	0	0	0
50	0.4	141	70 (49.6)	102	0	19	0
100	0.005	4	4 (100.0)	26	0	5	0

Abn, abnormal; ctb, chromatid break; csb, chromosome break; cte, chromatid exchange; cse, chromosome exchange.
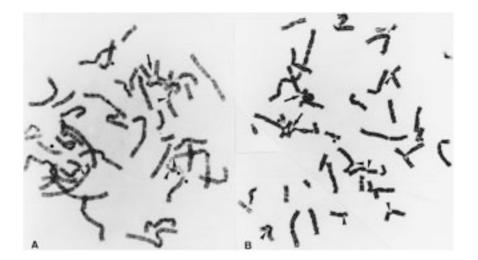

Figure 1 Two G-banded metaphase cells showing chromatid breaks (small arrows), chromatid exchanges (large arrows), and attenuated achromatic regions (stars).

This does not exclude the possibility that chemicals other than isoflavones may play a role in this development.

I should like to stress that leukemogens are not restricted to ionizing radiation, viruses, and chemicals, especially those that pollute the environment and are therefore hazardous to humans, because there are also many natural substances that act as topo II inhibitors.[25,26] It is important to remember that common diseases always occur as a result of interactions between environmental factors and individual genetic variations. Every effort should therefore be made to identify susceptibility genes or linked polymorphic markers as a contribution to investigations of such environmental factors.[27]

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