EXCLI Journal 2002;1:8-14 (ISSN 1611-2156)
received: 19. December 2002, accepted: 29. December 2002, published: 30. December 2002

Review:

Dietary topoisomerase II-poisons: contribution of soy products to infant leukemia?

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ABSTRACT

DNA topoisomerases are nuclear enzymes inducing transient breaks in the DNA allowing DNA strands or double helices to pass through each other. The clinically used DNA topoisomerase II-poison etoposide is known to induce DNA double strand breaks leading to chromosomal aberrations and leukemias. Recently, some alarming studies have been published, suggesting that maternal exposure to low doses of dietary topoisomerase II-poisons, including bioflavonoids such as genistein or quercetin, may contribute to the development of infant leukemia: approximately 80% of infants with acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL) have chromosome translocations involving the MLL (mixed lineage leukemia) gene. It has been shown that antineoplastic chemotherapy with the leukemogenic topoisomerase II-poison etoposide induced identical chromosomal aberrations involving the MLL gene compared to children with infant leukemia. Interestingly, the MLL cleavage sites induced by etoposide colocalized with the cleavage sites observed in infant leukemia. In addition, an almost 10-fold higher risk of infant AML has been reported for mothers consuming relatively high levels of topoisomerase II-poison containing foods. These observations are relevant, since many foods contain topoisomerase II-poisons, predominantly soy and soy products, but also coffee, wine, tea, cocao, as well as some fruits and vegetables. Further studies on the role of dietary topoisomerase II-poisons are urgently required. If the causal relationship between dietary exposure to topoisomerase II-poisons and infant leukemia will be confirmed, care should be taken to reduce exposure to critical foods during pregnancy.

Keywords: Topoisomerase II-inhibitors, soy, infant leukemia, cleavable complex, chromosomal aberrations, carcinogenicity

INTRODUCTION

Due to the periods of rapid cell turnover it is likely that fetal tissues or tissues of children are more susceptible to some environmental or dietary genotoxic agents (Hengstler et al., 1998, 1999a; von Mach, 2002). Recently, some studies suggested a causal relationship between infant leukemia induced *in utero* and maternal exposure to dietary

bioflavonoids - that besides many other effects, especially those as endocrine disruptors (Degen and Bolt, 2000), also act as topoisomerase II-poisons (Ross, 2000; Strick et al., 2000; Alexander et al., 2001; McDonald et al., 2001; Abe, 1999). In contrast to infant leukemia no role of dietary topoisomerase II-poisons has been observed in pathogenesis of adult leukemia. The majority of studies reported beneficial effects of dietary bioflavonoids for adult individuals. Several epidemiological studies observed a decreased risk for prostate, breast, uterus, colon and lung cancer for adults consuming high levels of bioflavonoids. Numerous mechanisms have been reported that may explain these protective effects including inhibition of tyrosine kinases, anti-estrogenic effects, release of transforming growth factor beta, induction of apoptosis and antioxidative effects. One of the main sources of dietary flavonoids are soy beans and soy products (**Fig. 1; Table 1**). Soy products, including for instance soy burgers, soy hot digs, soy cheese, etc., have proliferated due to the labelling of soy as a food that reduces the risk of some tumor types and also the risk of heart disease. Exposures to dietary isoflavones have been reported to range between 50 and 100 mg/day in East Asian populations resulting in plasma concentrations of 40-240 ng/ml daidzein and genistein (combined) (Bolt et al., 2000). In European populations dietary exposures to isoflavones are much lower usually not exceeding 1 mg/day. However, the consequences of exposure to bioflavonoids may differ between adults and transplacentally exposed embryos. Due to the rapid cell proliferation the aspect of topoisomerase II inhibition by bioflavonoids may be much more critical for embryos than for adults.

In this review, we report about the mechanisms of action of topoisomerase II-poisons, carcinogenic effects of the latter and discuss some recent studies suggesting that dietary exposure to topoisomerase II-poisons may have a causative role in infant leukemia.

MECHANISM OF ACTION OF TOPOISOMERASE II AND INHIBITORS

DNA topoisomerases are nuclear enzymes, which induce transient breaks in the DNA allowing DNA strands or double helices to pass through each other (Toonen and Hande, 2001; Wang, 2002; Hengstler et al., 1999b). By this action topoisomerases solve topological problems of DNA in replication, transcription, recombination and chromosome condensation as well as decondensation. DNA topoisomerases fall into two major classes: the type I enzymes that induce single stranded cuts in DNA, and the type II enzymes that cut and pass double stranded DNA.

The mechanism of action of topoisomerase II can be dissected into a series of steps initiated by the binding of DNA to both subunits (S1 and S2) of the enzyme (**Fig. 2A**). In the next step, topoisomerase II cleaves the DNA, forming a phosphotyrosine linkage between each DNA single strand break product and the catalytic tyrosines of both topoisomerase II subunits (**Fig 2B**). The latter step depends on the presence of magnesium. In a next ATP-dependent step, topoisomerase II traps a second DNA double strand (**Fig. 2C**). Trapping of the second DNA douplex is achieved by a conformational change of the N-terminal domains of both subunits, leading to a closed clamp. After trapping of the second DNA double strand (termed trans- or T-strand) it will pass the gap of the first DNA double strand (termed gap- or G-strand) (**Fig. 2D**). As soon as the T-strand has passed the G-strand the carboxy terminal portion of the enzyme undergoes a conformational change (**Fig. 2E**) that allows the exit of the T-strand (**Fig. 2F**). In a next step topoisomerase II reverses the cleavage reaction of the G-strand (**Fig. 2G**). After religation of the G-strand hydrolysis of ATP leads to a conformational change of the N-terminal part of both subunits of topoisomerase II that allows dissociation of the G-strand (**Fig. 2H**).

Fig. 1: Soy products: as a rich source of isoflavones soy has been reported to reduce coronary heart disease and cancer risk in exposed adults. However, some recent studies provide evidence that transplacental exposure of embryos to isoflavones may contribute to infant leukemia.

The "classical" topoisomerase II targeting substances act by trapping the cleaved G-strand-enzyme intermediate, thus, blocking religation and enzyme release, leaving the DNA with a permanent double strand break. These substances that lead to higher levels of covalent topoisomerase II-DNA complexes have been termed topoisomerase II-"poisons", whereas substances that inhibit the enzyme during other steps are referred to as "catalytic inhibitors" (Walker and Nitiss, 2002). The best known topoisomerase II-poisons belong to two classes of antineoplastic agents, the epipodophyllotoxins (e.g. etoposide and teniposide) and the anthracyclines (e.g. doxorubicin). Catalytic inhibitors include for instance derivatives of coumarin antibiotics, such as the coumermycins and novobiocin, the thiobarbiturate merbarone and the bisdioxopiperazines (Walker and Nitiss, 2002). However, besides the mentioned drugs there is a relatively large number of natural and synthetic products present in environment and food that act as topoisomerase II-poisons and/or catalytic inhibitors (**Table 1**).

CARCINOGENICITY OF TOPOISOMERASE II-POISONS

DNA double strand breaks induced by topoisomerase II-poisons can induce apoptosis of tumor cells contributing to the therapeutic effects of epipodophyllotoxins and anthracyclins (Walker and Nitiss, 2002). In addition, the presence of covalent topoisomerase II-DNA complexes arrests the replication fork, which also contributes to the antineoplastic effects. However, if healthy cells survive exposure to topoisomerase II-poisons DNA double strands may lead to chromosomal aberrations. The best studied chromosomal aberrations induced by topoisomerase II-inhibitors in humans are translocations involving the MLL gene located at chromosome 11q23. After exposure to etoposide or doxorubicin MLL is rearranged with partner genes in at least 40 different translocations the most common being translocations with chromosomes 4, 6 and 9 (Strick et al., 2000). The latter translocations are frequently observed in patients that have been treated with etoposide and teniposide and developed therapy-related acute myeloid leukemias (AML). There is no doubt that the topoisomerase II-poison etoposide is carcinogenic in humans. AML develops relatively early after etoposide therapy (2-3 years). Cumulative etoposide doses of 2 to 3 g/m^2 body surface are associated with a cumulated 6-year risk for development of secondary leukemia of 2.2 % (Toonen and Hande, 2001). For patients receiving less than 2 g/m^2 etoposide a 0.4-0.6 % risk was found.

Table 1: Topoisomerase II-poisons and/or catalytic inhibitors in environment and food.

Substance	Sources of human exposure
Flavonoids	
Quercetin	Fruits and vegetables
Genistein	Soy
TTIG-2535	Investigational soy drug product
Catechins	Tea, wine, chocolate
Caffeine	Coffee, tea
Thiram	Agricultural fungicide
Benzene metabolites	Gasoline stations
p-Benzoquinone	
Hydroquinone	
Catechol	
4,4'-Biphenol	
Senna	Anthraquinone laxative
Dipyrone, Baygon, Thiram	Insecticides

In conclusion, exposures to high doses of topoisomerase II-poisons in cancer therapy have clearly shown that topoisomerase II-poisons induce chromosomal translocations and leukemia.

DIETARY EXPOSURE TO TOPOISOMERASE II-POISONS: ROLE IN INFANT LEUKEMIA

The carcinogenicity of high doses of etoposide and the fact that humans are exposed to low doses of several topoisomerase II-poisons in food (**Table 1**) led to concern that exposure to these substances may contribute to carcinogenesis in the general population. In this context the question whether carcinogenic topoisomerase II-poisons act by threshold or non-threshold mechanisms is of high relevance (Hengstler et al., 2003). At a first glance one might expect that carcinogens that act by inhibition of enzymes have thresholds (von Mach et al., 2002; von Mach et al., 2003). This may be true for inhibitors of detoxifying enzymes or some DNA repair enzymes, where low doses of inhibitors will be without consequences as long as they do not reduce enzyme activity to a relevant extent. However, the situation for topoisomerase II can be expected to be more critical: in principle a single molecule of a topoisomerase II-poison may trap a topoisomerase II protein at a step where DNA strands have been cleaved. This may lead to a single DNA double strand break, induce a single chromosome translocation and initiate a series of steps finally leading to neoplastic transformation. Of course the probability for neoplastic transformation resulting from a single DNA double strand break is extremely low, but it can be expected to be higher than zero. Thus, from a theoretical point of view topoisomerase II-poisons can be expected to act by a non-threshold mechanism.

Recently, some alarming studies have been published, suggesting that exposure to low doses of topoisomerase II-poisons may indeed be relevant (Ross, 2000; Strick et al., 2000; Alexander et al., 2001; McDonald et al., 2001): Approximately 80% of infants (younger than 1 year of age) with acute

myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL) have chromosome translocations involving the MLL gene. The above mentioned leukemias (AML) associated with etoposide chemotherapy manifested identical chromosomal translocations involving the MLL gene (Ross, 1998). The MLL cleavage site induced by etoposide colocalized with those observed in infant leukemia (Strick et al., 2000). In addition, molecular studies have suggested that leukemias in the first postnatal year have been initiated *in utero*. This led to the hypothesis that maternal exposure during pregnancy to environmental substances that inhibit topoisomerase II may cause development of leukemia in infants. This hypothesis has been examined in several studies (Ross, 2000; Strick et al., 2000; Alexander et al., 2001). Since topoisomerase II-inhibitors have been found in soy, wine, coffee, tea, cocao, pesticides, medications, specific fruits and vegetables, mothers of infant cases and matched controls were interviewed for potential exposure to topoisomerase II-inhibitors (Ross, 1998). An approximately 10-fold higher risk of infant AML with increasing maternal consumption of topoisomerase II-poison containing foods was reported in this study. Based on 20 bioflavonoids tested, Strick et al. (2000) identified a common structure essential for topoisomerase II-induced DNA cleavage, suggesting that maternal ingestion of bioflavonoids induce MLL breaks leading to infant leukemia in utero. In addition, at least some bioflavonoids that act as topoisomerase II-poisons have been reported to be able to cross the rat placenta and reach fetal tissues (Schroder-van der Elst et al., 1998; Degen et al., 2002). In addition, the incidence of infant leukemia is almost 2-fold higher in several Asian cities, for example Hong Kong or Osaka, than in Western countries. This difference might be explained by the high food intake of bioflavonoids in many regions of Asia, especially by consumption of soybeans and soybean products (Strick et al., 2000).

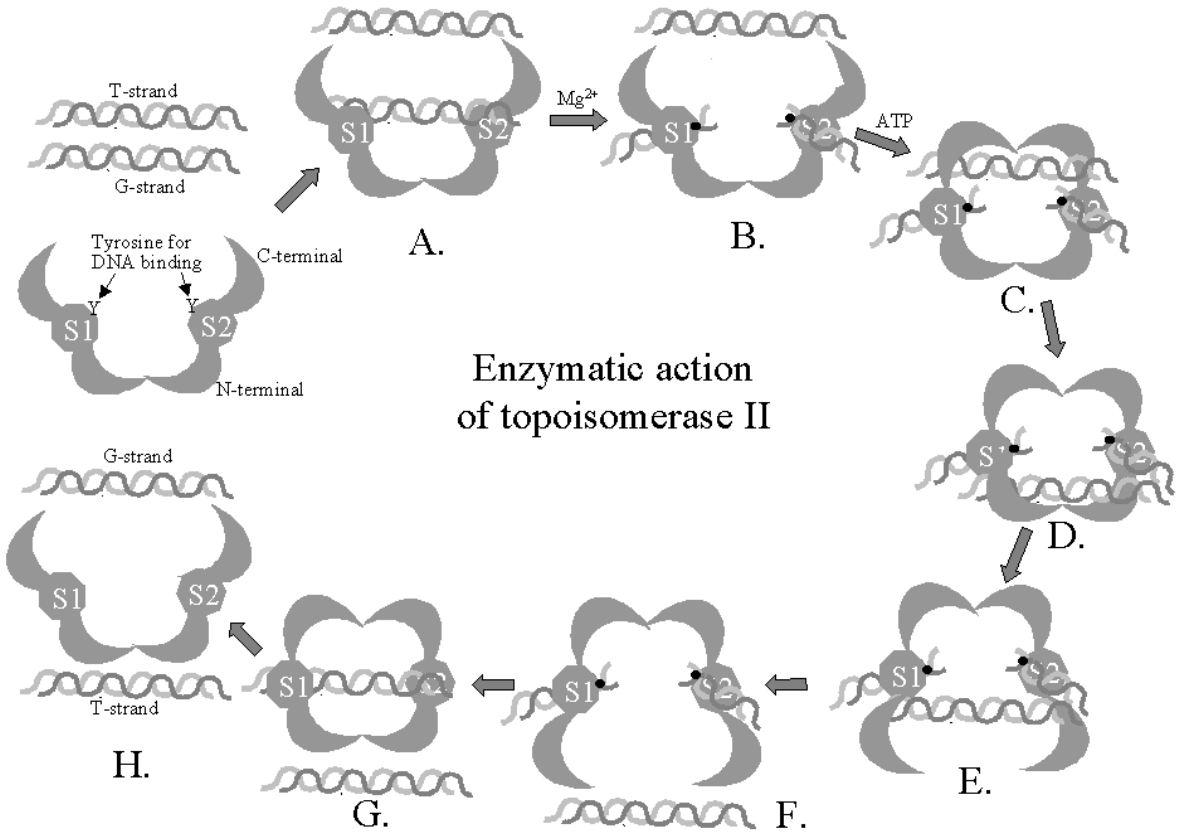

Fig. 2: Enzymatic action of topoisomerase II. The "classical" topoisomerase II-poisons act by trapping the G-strand-enzyme intermediate (step F. in this figure), thus, blocking religation and enzyme release, leaving the DNA with a permanent double strand break.

The observation that topoisomerase II associated chromosomal aberrations occur in infant leukemia leads to the question whether MLL-breaks induced by topoisomerase II-poisons are important also for leukemias of adults. However, MLL abnormalities in adult leukemia that is not associated with topoisomerase II-therapy are less frequent than 5 % in contrast to approximately 80 % in infant leukemia. In addition no epidemiological data support a central role of diet in pathogenesis of adult leukemia. An explanation might be differences in the number of proliferating cells between adult and fetal bone marrow. It is known that the alpha isoform of topoisomerase II is expressed at higher levels in proliferation compared to quiescent cells. Due to the periods of rapid cell turnover it is likely that fetal tissues may be more susceptible to dietary topoisomerase II-poisons than adult tissues.

It should be considered that a large number of studies has reported health benefits of bioflavonoids. Several epidemiological studies of human cancers, including prostate, colon and lung, reported a decreased risk associated with consumption of foods containing high levels of flavonoids. It has been hypothesized that high plasma concentrations of isoflavones could act like natural chemotherapeutic agents (Strick et al., 2000; Record et al., 1997). The example of topoisomerase II-poisons shows that the same substances may be beneficial or harmful probably depending on target cell types and their proliferation status during exposure.

In conclusion, strong evidence has been presented that dietary topoisomerase II-poisons may contribute to infant leukemia. If these observations can be confirmed by independent groups, soy products and other foods containing high levels of topoisomerase II-poisons should not be consumed during pregnancy.

REFERENCES

Abe T. Infantile leukemia and soybeans--a hypothesis. Leukemia 1999;13:317-20.

Alexander FE, Patheal SL, Biondi A, Brandalise S, Cabrera ME, Chan LC, Chen Z, Cimino G, Cordoba JC, Gu LJ, Hussein H, Ishii E, Kamel AM, Labra S, Magalhaes IQ, Mizutani S, Petridou E, de Oliveira MP, Yuen P, Wiemels JL, Greaves MF. Transplacental chemical exposure and risk of infant leukemia with MLL gene fusion. *Cancer Res* 2001;61:2542-6

Bolt HM, Janning P, Michna H, Degen GH. Comparative assessment of endocrine modulators with oestrogenic activity: I. Definition of a hygiene-based margin of safety (HBMOS) for xeno-oestrogens against the background of European developments. *Arch Toxicol* 2001;74:649-62

Degen GH, Janning P, Diel P, Michna H, Bolt HM. Transplacental transfer of the phytoestrogen daidzein in DA/Han rats. *Arch Toxicol* 2002;76:23-9

Degen GH, Bolt HM. Endocrine disruptors: update on xenoestrogens.
Int Arch Occup Environ Health 2000;73:433-41

Hengstler JG, Arand M, Herrero ME, Oesch F. Polymorphisms of N-acetyltransferases, glutathione S-transferases, microsomal epoxide hydrolase and sulfotransferases: influence on cancer susceptibility. *Recent Results Cancer Res* 1998;154:47-85

Hengstler JG, Van der Burg B, Steinberg P, Oesch F. Interspecies differences in cancer susceptibility and toxicity, *Drug Metab Rev* 1999a;31:917-970

Hengstler JG, Lange J, Kett A, Dornhöfer N, Meinert R, Arand M, Knapstein PG, Becker R, Oesch F, Tanner B. Contribution of *c-erbB-2* and topoisomerase IIα to chemoresistance in ovarian cancer, Cancer Res. 1999b:59:3206-3214

Hengstler J, Bogdanffy MS, Bolt HM, Oesch F. Challenging dogma: thresholds for genotoxic chemicals? The case of vinyl acetate. Annu Rev Pharmacol Toxicol 2003;43, epub ahead of print

Leone G, Voso MT, Sica S, Morosetti R, Pagano L. Therapy related leukemias: susceptibility, prevention and treatment. *Leuk Lymphoma* 2001;41:255-76

McDonald TA, Holland NT, Skibola C, Duramad P, Smith MT. Hypothesis: phenol and hydroquinone derived mainly from diet and gastrointestinal flora activity are causal factors in leukemia. *Leukemia* 2001;15:10-20

Record IR, Broadbent JL, King RA, Dreosti IE, Head RJ, Tonkin AL. Genistein inhibits growth of B16 melanoma cells in vivo and in vitro and promotes differentiation in vitro. *Int J Cancer* 1997;72:860-4

Ross JA. Dietary flavonoids and the MLL gene: A pathway to infant leukemia? *Proc Natl Acad Sci U S A* 2000;97:4411-3

Ross JA., Maternal diet and infant leukemia: a role for DNA topoisomerase II inhibitors? *Int J Cancer* (Suppl) 1998;11:26-8

Schroder-van der Elst JP, van der Heide D, Rokos H, Morreale de Escobar G, Kohrle J. Synthetic flavonoids cross the placenta in the rat and are found in fetal brain. *Am J Physiol* 1998, 274:E253-6

Strick R, Strissel PL, Borgers S, Smith SL, Rowley JD. Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukemia. *Proc Natl Acad Sci U S A* 2000;97:4790-5

Toonen TR, Hande KR. Topoisomerase II inhibitors. *Cancer Chemother Biol Response Modif* 2001;19:129-47

von Mach MA, Weilemann LS: Zunehmende Bedeutung von Antidepressiva bei suizidalen und parasuizidalen Intoxikationen. *Dtsch Med Wochenschr* 2002;127:2053-6

von Mach MA. Primary biliary cirrhosis in classmates: coincidence or enigmatic environmental influence? *EXCLI J* 2002;1:1-7

von Mach MA, Lauterbach M, Kaes J, Hengstler JG, Weilemann JG: Suizidale und parasuizidale Intoxikationen mit Paracetamol: Eine Analyse von 1995 bis 2002 *Dtsch Med Wochenschr* 2003;128:15-9

Walker JV, Nitiss JL. DNA topoisomerase II as a target for cancer chemotherapy. *Cancer Invest* 2002;20:570-89

Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. *Nat Rev Mol Cell Biol* 2002;3:430-40