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Original Contribution

RADIATION-INDUCED CLASTOGENIC FACTORS: ANTICLASTOGENIC EFFECT OF GINKGO BILOBA EXTRACT

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Abstract—Clastogenic factors (CFs) were first described in the blood of persons irradiated accidentally or for therapeutic reasons. Work of our laboratory has shown that they occur also under other circumstances, which are characterized by oxidative stress, and that CF-induced chromosome damage is regularly prevented by superoxide dismutase (SOD). Recently we found CFs in a high percentage of salvage personnel of the Chernobyl reactor accident. These liquidators represent a high-risk population and might benefit from cancer chemoprevention by antioxidants. SOD would have to be injected and is not appropriate for long-term prophylactic treatment. In the present study, we therefore evaluated the anticlastogenic effect of the Ginkgo biloba extract EGb 761, which is known for its superoxide scavenging properties. EGb 761 was tested on CF-treated blood cultures of healthy donors. After establishing the optimal protective EGb concentration, using CFs produced by irradiation of whole blood from healthy volunteers, the extract was tested on cultures exposed to CFs from plasma of persons irradiated as liquidators. The anticlastogenic effect could be confirmed for a final concentration of 100 μg/ml. In 12 consecutive experiments, CFs induced an average of 18.00 ± 4.41 aberrations/100 cells. This was reduced to 7.33 ± 3.08 in the parallel cultures receiving 100 μg/ml EGb 761 ($p < .001$). SOD was anticlastogenic in the same system at concentrations of 30 cytochrome C units/ml (approximately 10 μg/ml). Preliminary results obtained in a small series of liquidators showed regression or complete disappearance of CFs in the plasma after 2 months of treatment with EGb 761 (3 × 40 mg/d).

Keywords—Clastogenic factors, Anticlastogens, Ginkgo biloba, Superoxide dismutase, Chernobyl liquidators, Radiation protection, Free radicals

INTRODUCTION

Clastogenic factors (CFs) were first described by radiobiologists in plasma from persons irradiated accidentally or for therapeutic reasons.[1,2] Further reports of the chromosome-damaging effects of plasma from irradiated individuals came from Japan, where heavily exposed A-bomb survivors were studied. Clastogenic activity persisted up to 31 years after radiation exposure.[3]

Work of our laboratory has shown that CFs are also observed in patients with chronic inflammatory diseases, HIV-infected persons, asbestos workers, and in the congenital breakage syndromes ataxia telangiectasia, Bloom's syndrome, and Fanconi anemia.[4] The strongest argument for the implication of superoxide radicals in CF formation and CF action came from in vitro models, in which cell cultures were exposed to a source of superoxide. CF formation in these cultures, as well as the clastogenic action of preformed CFs in other cell cultures, could be prevented by superoxide dismutase (SOD).[5,6] Using the cytochrome C assay, it could be shown that CFs stimulate superoxide production by neutrophils and monocytes.[7]

The CFs isolated to date from patient sera or from cell culture supernatants share certain characteristics, including low molecular weight, and are able to pass through a 10,000-dalton filter yet are retained by a 1000-dalton molecular weight cutoff filter.[8] The cellular origin of the factors is indicated by the fact that formation is only demonstrable in the presence of cells (i.e., not in a cell-free medium). The kinetics of formation reveal a 16- to 18-hour delay for activity.[5,6] Thio-

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barbituric acid reactive substances and conjugated dienes have been isolated in CF preparations, suggesting an association between CF formation and membrane lipid peroxidation. In addition, inosine nucleotides (ITP and IDP), which are primers of superoxide production by phagocytes and also compete with the adenosine 5′-triphosphate (ATP) binding sites of DNA topoisomerases, have been detected in CF preparations of scleroderma patients.[9] Tumor necrosis factor alpha has been identified as a component of the CFs isolated from Fanconi anemia[10] and from HIV-infected persons (Emerit and Fuchs, in preparation). These data from our previous work may be seen in more detail in a recent review article in this journal.[4]

Formed via the intermediacy of superoxide and generating superoxide, CFs are self-sustaining and therefore responsible for a long-lasting clastogenic process, which may correlate with the high risk of cancer and leukemia, encountered in the aforementioned diseases.[4] CFs have been supposed to be risk factors for late effects of ionizing radiation.[11] On the other hand, given the superoxide-mediated mechanisms of CF-induced chromosome damage, antioxidants act as anticlastogens and possibly as anticarcinogens. In conditions accompanied by CF formation, disappearance of this oxidative stress factor may be used as an intermediate endpoint for the evaluation of promising drugs in cancer chemoprevention trials.

Recently we reported that CFs were found in the plasma of 33 out of 47 accident recovery workers, who were sent to Chernobyl between 1986 and 1989 for various tasks.[12] These liquidators may be considered a high-risk population for the development of cancer and leukemia. According to registries of Russia, Belarus, and the Ukraine, they represent about 800,000 persons,[13] who might benefit from antioxidant treatment or disease prevention. SOD has been consistently protective against CF-induced chromosome damage.[4,8,14,15] However, this enzyme would have to be injected and is not appropriate for long-term prophylactic use. It is therefore our aim to test other antioxidants in comparison to the well-known anticlastogenic effect of SOD. In the present study, we evaluate the anticlastogenic effect of an extract from Ginkgo biloba leaves, whose superoxide scavenging properties are established by previous studies.[16]

MATERIALS AND METHODS

CF preparation

CFs were isolated from the plasma of liquidators from Armenia, who were engaged in emergency interventions around the Chernobyl atomic power station in the summer and autumn of 1986. They had been examined at the Institute for Medical Radiology in Yerevan before they left for Chernobyl and are now under regular checkup at that institute for various health problems.

The blood samples were collected in Yerevan and brought frozen to Paris, where they were handled according to an established technique for the isolation of CFs.[8] Briefly, this consists of an ultrafiltration procedure, using filters with a cutoff between 10 and 30 kd, to remove all high molecular weight plasma components. Aliquots of these ultrafiltrates (0.25 ml) are added to test cultures set up with blood from healthy donors.

For CF production by cells in vitro, blood was exposed to gamma radiation delivered by a Cs 132 source at a dose rate of 0.46 Gy/min. The total dose of 10 Gy was chosen on the basis of data in the literature[17] and previous experiments in this laboratory.[12] After irradiation, the blood was centrifuged and the plasma ultrafiltered. Aliquots of 0.5 ml were added to the test cultures.

Cytogenetic assay

The test cultures were set up with 0.5 ml of whole blood from healthy donors in 5 ml TCM 199 (Flow Lab, Paris) and 1 ml fetal calf serum (Gibco, France). Lymphocyte proliferation was stimulated by addition of phytohemagglutinin (PHA, Wellcome Diagnostics, Dartford, UK). After 72 h of incubation at 37°C, the mitoses were arrested in metaphase by addition of colchicine 2 h before harvesting. Microscopic slides for chromosomal analysis were prepared according to standard procedures and stained with Giemsa.[8]

The chromosomes were examined on coded slides for the presence of gaps, breaks, fragments, exchanges, rings, and dicentrics. A minimum of 50 mitoses was studied. The aberration rates were compared for parallel cultures exposed to CFs in the presence or absence of one of the antioxidants. Untreated cultures served for evaluation of the spontaneous aberration rate of the donor.

Antioxidants

Antioxidants were added to the cultures 30 min before addition of CFs and remained in the culture system over the entire cultivation period. Cu, Zn-SOD from bovine erythrocytes (Palleau Production, Chateau-Landon, France) was added at a final concentration of 30 cytochrome C units/ml).

EGb 761

The Ginkgo biloba extract EGb 761 was provided by IPSEN, France. EGb 761 is extracted from the green leaves of the Ginkgo biloba tree growing in plantations in South Korea, Japan, and France. The mode of culture, harvesting, and extraction is perfectly standardized and submitted to rigorous analytical control, measuring the amount of active components and confirming absence of undesirable contaminants.[16] The dried leaves are first subjected to an extraction procedure using a mixture of acetone and water. A further series of treatments (about 18 steps) is applied both to eliminate unwanted substances and to enrich the extract in active substances. The extract designated EGb 761 contains 24% Ginkgo flavone glycosides and 6% Ginkgolides-bilobalides. The detailed analysis of the extract and comparison with other extracts from Ginkgo biloba leaves may be seen in ref. 16, pages 10–11. The extract had been co-developed by IPSEN (France) and Dr. Willmar Schwabe (Germany) and obtained the code name EGb 761 when other Ginkgo extracts, different from EGb 761 in the composition of their active ingredients, came onto the market. At present, EGb 761 preparations are on the market in more than 30 countries under different trade names (e.g., Tebonin in Germany, Tanakan in France). For in vivo application in this study, EGb 761 was kindly provided by IPSEN (France) as the drug version Tanakan, which contains 40 mg of the standardized abstract in sugar-coated tablets.

Participants

Ten persons were chosen among the liquidators of our previous study and gave their written consent for participation in this trial, which was also authorized by the Armenian Ministry of Health. The presence of CFs before treatment was confirmed by a blood sample taken immediately before start of the treatment. After 2 months, another blood sample was taken the day after the last intake of the drug. One of the 10 persons did not come for the posttreatment control. The plasma was sent frozen to Paris, where it was ultrafiltrated and tested for its clastogenic effects. Aliquots of 0.25 ml were added to the cultures. Statistical analysis was done with the Student's t test.

RESULTS

A correct evaluation of anticlastogenic effects is only possible if the agent to be tested does not reduce the mitotic index by more than the acceptable limit of 50%. Because CFs slightly reduce the mitotic index, as already known from previous work,[18] we tested the influence of EGb 761 on the mitotic index in presence of CFs (Table 1). Concentrations between 10 and 100 μg/ml reduced only slightly the number of dividing cells in the cytogenetic preparations, whereas the 50% limit was reached with concentrations of 200 μg/ml. SOD was not tested because similar concentrations did not affect the mitotic index in previous experiments.[18]

Because only small amounts of CFs were available from liquidators, CFs were produced in vitro by irradiation of blood from healthy donors. These CFs obtained by a radiation dose of 10 Gy induced 21.80 ± 7.95 structural chromosome aberrations per 100 cells, in agreement with our previous experiments, wherein similar conditions induced 21.0 ± 7.6 aberrations.[12] Compared to untreated control cultures, this represents a threefold increase ($p < .01$). EGb 761 reduced the CF-induced aberration rates at concentrations higher than 50 μg/ml (Table 2). The differences became statistically significant for a dose of 100 μg/ml, which was used for the following experiments with CF from liquidators' plasma. In three of five experiments with a dose of 200 μg/ml, no culture growth was obtained, as expected from the results obtained by the study of

Table 1. Mitotic Index in CF-Treated Cultures in Presence of Increasing Doses of EGB 761

No. Exp.	Test Culture −CF	Test[a] Culture +CF	Test Culture + CF + EGB 761 (in μg/ml) 10	50	100	200	500
1	5.05	2.90	4.39	—	4.44	—	—
2	4.90	2.60	3.00	1.50[b]	3.85	—	0.50
3	7.50	6.40	—	3.35	4.85	2.45	1.45
4	7.25	5.75	—	4.50	2.50	3.80	1.25
5	4.20	3.20	3.20	5.65	4.20	2.30	—
$\bar{x}$ ± SD	5.78 ± 1.49	4.17 ± 1.77	3.53 ± 0.75	3.75 ± 1.77	3.97 ± 0.90	2.85 ± 0.83	1.07 ± 0.50

[a] Obtained by irradiation of blood (10 Gy).
[b] Exceptionally low value, probably due to technical reasons.

Table 2. Reduction of CF-Induced Chromosome Breakage in the Test Cultures by EGb 761

Experiment	−CF	+CF[a]	Test Cultures Receiving CF + Various Concentrations of EGb 761, μg/ml				
			10	50	100	150	200
1	10	28	24	—	14	—	No growth
2	8	30	26	20	14	—	No growth
3	10	19	—	8	8	—	18
4	6	22	18	18	10	—	12
5	2	10	—	—	6	—	No growth
6	4	22	22	4	10	No growth	—
7	10	20	—	16	—	No growth	—
8	10	20	—	16	10	4	—
$\bar{x} \pm$ SD	7.50 ± 3.16	21.34 ± 6.07	22.50 ± 3.42	13.67 ± 6.25	10.29 ± 2.93		
		$p < .01$		Not significant	$p < .05$		

[a] CF obtained by irradiation of blood from healthy donors (10 Gy). The figures represent chromosome aberrations per 100 cells.

the mitotic index. This was the case also for two of the three experiments performed with 150 μg/ml EGb.

The CF preparations from liquidators' plasma induced a mean aberration rate of 18.00 ± 4.41% in the test culture system. Only 7.33 ± 3.08 aberrations per 100 cells were found when cells were exposed to CFs in the presence of 100 μg/ml EGb. Individual results varied between 14 and 28 aberrations per 100 cells (Table 3). In addition, SOD was regularly protective. The mean aberration rate in the seven cultures receiving 30 U/ml SOD was 6.28 ± 3.72 compared to 19.14 ± 5.52 for the parallel unprotected CF-treated cultures. In four experiments, in which EGb 761 and SOD were studied on CFs from the same person, the mean aberration rates were 19.0 ± 4.8 for CF-treated cultures and 5.8 ± 3.3 and 6.6 ± 5.0, respectively, for the EGb- and SOD-protected cultures. These values were not significantly different from the background level of aberrations in the untreated cultures of the same donors.

The satisfactory anticlastogenic effect of EGb 761 in vitro prompted us to study its effect in vivo in a limited number of liquidators. The mean aberration rate induced in the test cultures with ultrafiltrates from plasma taken before treatment was 21.33 ± 6.00. The clastogenic activity was also increased in all participants at the earlier analysis 11 months before (Table 4). No clastogenic activity was detectable in the samples collected immediately after arrest of the treatment. The aberration rates induced in the test cultures with these samples (6.22 ± 3.92) were not significantly different from the baseline level of aberrations in the untreated blood cultures of the same donors (5.50 ± 1.91). Six of the nine persons who had a second control after an interval of 3 months still exhibited low CF activity. Four persons could be studied after 7 months, and their plasma ultrafiltrates did not contain significant amounts of CFs.

The types of chromosome aberrations induced by the ultrafiltrates from liquidators' blood before and after EGb treatment may be seen in Table 5. They were principally of the chromatid type, as already claimed by the early work of Scott.[17]

DISCUSSION

Chromosome aberrations observed in irradiated persons many years afterward are generally thought to be the consequence of persisting lesions in the DNA of stem cells. Our work, and that of the others before us,[1-3,11] demonstrates the role of CFs at the origin of continuously produced chromosome damage. At least part of the chromosome damage observed in lymphocytes of liquidators is due to the action of CFs. As

Table 3. EGb 761 Reduces CF-Induced Chromosome Breakage in Test Cultures Exposed to Ultrafiltrates From Liquidators Plasma; Comparison with SOD

Exp.	Test Culture −CF	Test Culture +CF	Test Culture + CF + EGb	Test Culture + CF + SOD
1	4	16	2	4
2	6	28	No growth	8
3	2	16	No growth	4
4	4	17	9	—
5	4	14	No growth	6
6	2	26	6	4
7	4	18	5	4
8	6	18	8	—
9	6	17	9	—
10	8	14	12	—
11	4	16	5	—
12	10	16	10	14
$\bar{x} \pm$ SD	5.00 ± 2.33	18.00 ± 4.41	7.33 ± 3.08	6.28 ± 3.72
			$p < .001$	$p < .001$

The figures represent the total number of aberrations observed in 100 cells. Final concentrations of EGb and SOD are 100 μg/ml and 30 cytochrome C U/ml.

Table 4. Clastogenic Activity in Liquidators' Plasma Before and After 2 Months of Treatment With EGb 761 (Tanakan)

Before Treatment October 2, 1992	Treatment		After Arrest of Treatment	
	August 30, 1993	October 26, 1993	January 25, 1994	May 31, 1994
22	20	8	4	6
20	16	4	2	—
14	18	2	—	—
26	12	0	4	—
20	30	8	6	10
16	20	4	—	—
16	30	12	4	10
26	24	10	4	—
16	22	10	8	8
Mean ± SD				
19.46 ± 4.45	21.33 ± 6.00	6.22 ± 3.92	4.0 ± 1.26	8.50 ± 1.90

The figures represent the number of aberrations per 100 cells in the test cultures exposed to plasma ultrafiltrates collected either before treatment or after various follow-up after treatments (first day, 3 and 7 months). Untreated test cultures showed 5.50 ± 1.91 aberrations/100 cells.

pointed out earlier, CFs are autosustaining because they are formed via superoxide and contain components such as ITP and TNF alpha, which are able to stimulate further superoxide production by phagocytes. The various components of CF, released by cells or formed in free radical chain reactions, act either as direct clastogens or as repair inhibitors, as does the aldehyde 4-hydroxynonenal derived from membrane lipid peroxidation (for review, see ref. 4). CF-induced chromosome damage is comparable to chemically induced chromosome damage, except that the clastogens are not xenobiotics but are released from the body's own cells as a consequence of exposure to oxyradicals. SOD's role in preventing clastogenesis lies in the interruption of the vicious circle of superoxide production by CFs and more CF formation by superoxide. In the present study, we show that this can also be achieved with other antioxidants acting as superoxide scavengers.

Key concepts for disease preventive agents include the need for oral route of administration and the matching of toxic side-effects to degree of disease risk.[19] The Ginkgo biloba extract chosen for the study meets both criteria. Adverse drug events were observed only in 51 out of 9772 patients participating in 44 clinical trials during the years 1982–1988.[16] They consisted of gastrointestinal symptoms, headache, or dizziness. The major clinical indications since the first registration of the drug in 1965 had been insufficiency of the cerebral and peripheral circulation as well as psychobehavioral disturbances of the elderly.[16] In recent years, Ginkgo biloba extract has been evaluated with respect to free-radical-related disease states after demonstration of its free radical scavenging properties in various in vitro and in vivo models. Beneficial effects of Ginkgo biloba could be demonstrated in cerebral ischemia of gerbils.[20] Comparative studies using Ginkgo, SOD, and vitamin E were conducted in ischemia reperfusion of rat retina.[21] Protective effects of EGb 761 could be shown by hemodynamic and electron spin resonance

Table 5. Nature of Chromosomal Aberrations Induced in the Test Cultures With Plasma Ultrafiltrates From Liquidators, Collected Before and After EGb 761 Treatment for 2 Months

Gaps of one or both chromatids	11 (2.45%)	5 (1.11%)	4 (1.33%)	4 (2.0%)
Chromatid breaks	67 (14.89%)	18 (4.00%)	7 (2.33%)	6 (3.0%)
Isochromatid breaks	7 (1.56%)	2 (0.44%)	1 (0.33%)	4 (2.0%)
Fragments	6 (1.33%)	0		3 (1.5%)
Dicentrics	1 (0.22%)	1 (0.22%)		
Ring chromosomes	1 (0.22%)	0		
Translocations	1 (0.22%)	0		
Pulverization	2 (0.44%)	2 (0.44%)		
Total of aberrations (%)	96 (21.33%)	28 (6.22%)	12 (4.0%)	17 (8.5%)
Total of cells studied	450	450	300	200

studies in free-radical-induced ischemia reperfusion injury of isolated rat hearts[22] and during open-heart surgery in humans.[23] Superoxide production by neutrophils was inhibited by EGb 761.[24]

The Ginkgo biloba extract contains two major classes of constituents: flavonoids and terpenoids.[16] The flavonoid substances, particularly the flavonol glycosides and the proanthocyanidins, were considered to be responsible for the free radical scavenging properties of EGb 761 (ref. 16, page 15). Superoxide scavenging properties, in addition to the scavenging of hydroxyl radicals, were also reported by others studying the radical chemistry of flavonoid antioxidants by pulse radiolysis or photolysis.[24-28] The terpenoids comprise ginkgolides, a group of diterpenes, and bilobalide, a sesquiterpene. Pulse radiolysis studies of two subfractions of the EGb 761 extract, differing with respect to terpenes, concluded that the $O_2^{\cdot -}$ scavenging properties were correlated with the presence of terpenes.[29]

The superoxide scavenging properties of EGb 761, with its well-known good tolerance in long-term treatment, were the main reasons for choosing this drug for prevention of CF-induced chromosome damage. The results show that EGb 761 is not only anticlastogenic in vitro but also in vivo. The protective effects were observed with the usual dose (3×40 mg/day) recommended for the marketed drug Tanakan. CF activity did not reappear during several months after arrest of the treatment. Further follow-up will show whether this biomarker of oxidative stress disappears definitely or whether, after some time, the treatment must be repeated.

The liquidators never returned to Chernobyl and are now living outside the contaminated areas. CF formation is therefore not due to continuous exposure to radioactivity but is probably comparable to CF formation in patients with chronic inflammatory diseases (in particular those with autoimmune reactions), whereby CF formation is related to increased oxyradical production by inflammatory cells.[4] Subclinical inflammatory changes, including neutrophilia, increased levels of acute phase proteins, and accelerated erythrocyte sedimentation rates, have been reported in A-bomb survivors.[30] These consequences of radiation deserve further study.

The present study is encouraging for the use of antioxidants for therapy and disease prevention, in particular because their intake need not be continuous, as indicated by these first results.

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REFERENCES

1. Goh, K. O.; Sumner, H. Breaks in normal human chromosomes: Are they induced by a transferable substance in the plasma of irradiated persons exposed to total body irradiation? *Radiation Res.* **6**:51–60; 1968.
2. Hollowell, J. G.; Littlefield, L. G. Chromosome damage induced by plasma from irradiated patients. An indirect effect of X-ray. *Proc. Soc. Exp. Biol. Med.* **129**:240–244; 1968.
3. Pant, G. S.; Kamada, N. Chromosome aberrations in normal leukocytes induced by the plasma of exposed individuals. *Hiroshima Med. J. Sci.* **26**:149–154; 1977.
4. Emerit, I. Reactive oxygen species, chromosome mutation, and cancer: Possible role of clastogenic factors in carcinogenesis. *Free Radic. Biol. Med.* **16**:99–109; 1994.
5. Emerit, I.; Khan, SH.; Cerutti, P. Treatment of lymphocyte cultures with a hypoxanthine-xanthine oxidase system induces the formation of transferable clastogenic material. *Free Radic. Biol. Med.* **1**:51–57; 1985.
6. Khan, SH.,; Emerit, I. Lipid peroxidation products and clastogenic material in culture media of human leukocytes exposed to the tumor promoter phorbol-myristate-acetate. *Free Radic. Biol. Med.* **1**:443–449; 1985.
7. Emerit, I. Superoxide production by clastogenic factors. In: Crastes de Paulet, A. et al., eds. *Free Radicals, Lipoproteins and Membrane Lipids.* New York: Plenum Press; 1990:99–104.
8. Emerit, I. Clastogenic factors: Detection and assay. In: Packer, L.; Glazer, A. N., eds. *Methods in Enzymology.* New York: Academic Press; 1990:555–564.
9. Auclair, C.; Gouyette, A.; Levy, A.; Emerit, I. Clastogenic inosine nucleotides as components of the chromosome breakage factor in scleroderma patients. *Arch. Biochem. Biophys.* **278**:238–244; 1990.
10. Emerit, I.; Levy, A.; Pagano, G.; Pinto, L.; Amato, M.; Zatterale, A. Transferable clastogenic activity in plasma from patients with Fanconi anemia. *Human Genetics.* In press.
11. Faguet, G. G.; Reichard, S. M.; Welter, D. A. Radiation-induced, clastogenic plasma factors. *Cancer Genet. Cytogenet.* **12**:73–83; 1984.
12. Emerit, I.; Levy, A.; Cernjavski L.; Arutyunyan, R.; Oganesian, N.; Pogosian, A.; Mejlumian, H.; Sarkisian, T.; Gulkandanian, M.; Quastel, M.; Goldsmith, J.; Riklis, E.; Kordysh, E.; Poliak, S.; Merklin, L. Transferable clastogenic activity in plasma from persons exposed as salvage personnel at the Chernobyl reactor. *J. Cancer Res. Clin. Oncol.* **120**:558–561; 1994.
13. WHO/EOS 94/24. Report of the Management Committee Meeting on International Programme on the Health Effects of the Chernobyl Accident (IPHECA), Geneva March 16–17, 1994.
14. Emerit, I.; Michelson, A. M. Chromosomal instability in human and murine autoimmune disease: Anticlastogenic effect of superoxide dismutase. *Acta Physiol. Scand. Suppl.* **492**:59–65; 1980.
15. Emerit, I. Chromosome breakage factors: Origin and possible significance. *Progress Mutat. Res.* **4**:61–74; 1982.
16. DeFreudis, F. V. *Ginkgo Biloba Extract (EGb 761): Pharmacological Activities and Clinical Applications.* Amsterdam: Elsevier; 1991.
17. Scott, D. The effect of irradiated plasma on normal chromosomes and its relevance to the longlived lymphocyte hypothesis. *Cell Tissue Kinet.* **2**:295–305; 1969.
18. Emerit, I.; Cerutti, P. Tumor promoter phorbol-myristate acetate induces chromosome damage via indirect action. *Nature* **293**:144–146; 1981.
19. Kelloff, G.; Boone, C. W.; Malone, W. F.; Steele, V. E. Chemoprevention clinical trials. *Mutat. Res.* **267**:291–295; 1992.

20. Spinnewyn, B.; Blavet, N.; Clostre, F. Effects de l'extrait de Ginkgo biloba sur un modèle d'ischémie cérébrale chez la gerbille. *Presse Médicale* **15:**1511–1515; 1986.
21. Szabo, M. E.; Droy-Lefaix, M. T.; Doly, M. Modification of reperfusion-induced ionic imbalance by free radical scavengers in spontaneously hypertensive rat retina. *Free Radic. Biol. Med.* **13:**609–620; 1992.
22. Pietri, S.; Culcasi, M.; Vernière, E.; d'Arbigny, P.; Drieu, K. Effect of Ginkgo biloba extract (EGb 761) on free radical-induced ischemia-reperfusion injury in isolated rat hearts: A hemodynamic and electron spin resonance investigation. In: Ferradini, C.; Droy-Lefaix, M. T.; Christen, Y., eds. *Ginkgo Biloba Extract (EGb 761) as a Free Radical Scavenger.* Amsterdam: Elsevier; 1993:163–172.
23. Culcasi, M.; Pietri, S.; Carrière, I.; d'Arbigny, P.; Drieu, K. Electron spin resonance study of the protective effect of Gingko biloba extract (EGb 761) on reperfusion-induced free radical generation associated with plasma ascorbate consumption during open heart surgery in man. In: Ferradini, C.; Droy-Lefaix, M. T.; Christen, Y., eds. *Ginkgo Biloba Extract EGb 761 as a Free Radical Scavenger.* Amsterdam: Elsevier; 1993:153–162.
24. Baumann, J.; Wurm, G.; von Bruchhausen, F. Hemmung der Prostaglandinsynthese durch Flavonoide und Phenolderivate im Vergleich mit deren Superoxide-Radikalfangereigenschaften. *Arch. Pharm.* **313:**330–341; 1980.
25. Takahama, U. Oxidation products of kaempferol by superoxide anion radical. *Plant Cell Physiol.* **28:**953–957; 1987.
26. Bors, W.; Saran, M. Radical scavenging by flavonoid antioxidants. *Free Radic. Res. Comm.* **2:**289–295; 1987.
27. Robak, J.; Gryglewski, R. J. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmcol.* **37:**837–842; 1988.
28. Bors, W.; Heller, W.; Michel, C.; Saran, M. Radical chemistry of flavonoid antioxidants. In: Emerit, I.; Packer, L.; Auclair, C., eds. *Antioxidants in Therapy and Preventive Medicine.* New York: Plenum; 1990.
29. Gardes-Albert, M.; Abdelhafid, S.; Ferradini, C.; Droy-Lefaix, M. T. EGb 761 scavenger effect against OH and $O2^-$ radicals. A gamma pulse radiolysis study. *Free Radic. Biol. Med.* **9**(Suppl. 1):190; 1990.
30. Neriishi, K. Possible involvement of free radical mechanisms in late effects of A-bomb. *Fifth International Congress on Oxygen Radicals,* Kyoto, 1999 (abstract).