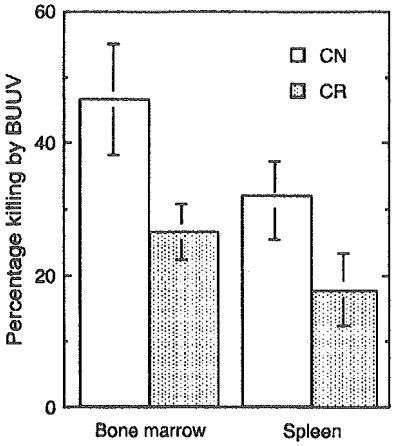

Figure 6. Percent cycling fraction (percentage killing by bromodeoxyuridine ultraviolet [BUUV] method) of hematopoietic progenitor cells, splenic colony-forming units (CFU-S) in bone marrow and spleen of mice with or without caloric restriction. BUUV assay was utilized [16], see Materials and Methods section in text. Cells from three mice each from the 0Gy-CalR(–) and CalR(through) groups were pooled and examined at 50 weeks old, that is, 44 weeks after caloric restriction. Data shown are the mean of three experiments for the spleen and of four experiments for the bone marrow. 0Gy-CalR(–) = mice fed a 95-kcal diet from 6 weeks old. 0Gy-CalR(thru) [CalR(through) in the text] = mice fed a 65-kcal diet from 6 weeks old; after 10 weeks old, the mice were fed a different diet to maintain the body weight of each mouse within 25–27 g ($p < 0.01$, the bone marrow; $p < 0.01$, the spleen).

death. In the nonrestricted dietary groups, the percentage of tumor-free mice decreased from 10.1 for the 0Gy-CalR(–) group to 7.4 for the 3Gy-CalR(–) group, following 3-Gy irradiation. When caloric intake was restricted from 6 weeks until death [0Gy-CalR(through)], the percentage of tumor-free deaths increased to 46.4%, the highest, and when it was restricted from 10 weeks (0Gy-CalR(post), the percentage of tumor-free deaths was 35.7%, the second highest, among the nonirradiated diet-restricted groups [0Gy-CalR(through) and 0Gy-CalR(post); Table 1]. Following irradiation (3 Gy), the percentage of tumor-free deaths in the 3Gy-CalR(through) group was 20.0% and that in the 3Gy-CalR(post) group was 17.5%.

Although the 0Gy-CalR(through) group was expected to show the longest survival period, the median survival period and maximum lifespan in this group was limited to 874 days and 1115 days, respectively [vs 896 days and 1145 days, respectively, for the 0Gy-CalR(post) group], the reason for this is as yet unknown; presumably, CalR in the developmental stage of life may not be completely beneficial for health, but it may be beneficial for extending lifespan. Extension of lifespan was caused by changes in the spectrum of diseases attributable to tumor-free death. Cause of tumor-free death is either glomerulosclerotic renal failure, subsequent auricular thromboses with or without pulmonary edemas and increased pulmonary effusions, or cardiac failure due to progressive myocardial fibrosis and calcinosis associated or not associated with coronary sclerosis.

Discussion

Dietary restriction, particularly caloric restriction, is a major carcinogenic modifier observed during experimental carcinogenesis and significantly decreases incidence of spontaneous tumors and tumors induced by chemical and radiation exposure [18–23]. In our previous study, incidence of myeloid leukemia was experimentally decreased by caloric restriction [4]. In the present study, particular attention was paid to the timing of caloric restriction, that is, pre- or postirradiation, to determine which would be more effective in preventing development of radiation-induced leukemias, and to determine the underlying mechanism that would play a role in this prevention by caloric restriction. In addition, we speculated that decreased incidence of leukemias may correlate with number of hematopoietic stem/progenitor cells as target cells for leukemic transformation, because caloric restriction in our previous preliminary study decreases the number of HPCs when the restriction was implemented throughout the experimental period [9]. When caloric restriction was implemented from 6 weeks to 10 weeks old only before 3-Gy irradiation [3Gy-CalR(pre)], the incidence of radiation-induced myeloid leukemias was lower than that in the nonrestricted control-diet group [3Gy-CalR(–)] (22.2% vs 16.3% in Table 1). However, there were no statistically significant differences in the incidence of leukemias (Table 1) and in the number of progenitor cells between the 0Gy-CalR(pre) and control diet [0Gy-CalR(–)] groups, evaluated 4 weeks after the dietary change (Fig. 5). On the other hand, the incidence of myeloid leukemias significantly decreased (9.5%) in mice when caloric restriction was started after irradiation and continued throughout their lifespan [3Gy-CalR(post), in Table 1]. This is essentially the same result as that obtained in our previous study [4], although the number of progenitor cells in the 3Gy-CalR(post) group at the time of irradiation was expected to be the same as those in the nonrestricted control diet 0Gy-CalR(–) and 3Gy-CalR(–) groups. On the other hand, 4 weeks of caloric restriction after the age of 10 weeks decreased the number of progenitor cells in the 0Gy-CalR(post) group, which is close to that of hemopoietic progenitor cells in the 0Gy-CalR(through) group [see 0Gy-CalR(through) and 0Gy-CalR(post) groups for reference in Fig. 5].

In the case of preirradiation CalR, 3Gy-CalR(pre) mice were returned to the nonrestricted diet immediately after irradiation. Thus, the body weight of these mice increased rapidly to the non-CalR level after irradiation as compared with that of mice in the restricted diet group [CalR(post) group]. Moreover, the number of progenitor cells in the CalR(pre) group was approximately the same as that in the control diet [0Gy-CalR(–)] group 4 weeks after the dietary change (Fig. 5). After return to the regular non-CalR

diet, the HPCs, with or without potential lethal damage caused by the 3-Gy irradiation, may have received strong growth stimulation signals. Consequently, despite return to non-CalR level of HPCs and the negated prevention of myeloid leukemogenesis, the results imply that preirradiation CalR potentially prevented leukemia, which was negated by return to the regular non-CalR diet. Thus, the results also imply that the effect of CalR during the initiation stage of leukemogenesis may be canceled out by return to non-CalR during the promotion stage of leukemogenesis. Presumably due to the characteristics of the bone marrow function in mice, it was noted that there was no significant difference in the number of CFU-S observed in the femoral bone marrow of mice among experimental treatments in any experimental treatment [24,25]. The reason spleen colonies were assayed was that the contribution of the spleen to radiation-induced leukemias was reported to be more highly significant than that of the bone marrow [24–29]. The bone marrow showed no significant difference in number of progenitor cells (Fig. 5D–F), because external impacts (caloric restriction in the present study) on mice generally cannot change the function of bone marrow significantly, as bone marrow maintains the minimal essential steady-state hematopoiesis fully functional [29]. Our present study of nonlymphoid leukemias specifically focused on myeloid leukemias, which have been presumed to be hemopoietic stem cell diseases [30].

Tessitore and colleagues reported that complete fasting followed by refeeding is responsible for induction of hepatocarcinogenesis in rats by a subnecrogenic dose of carcinogen, possibly due to an enhancement of the rat's growth and that of the growth of aberrant crypt foci in the rat colon and rectum by the carcinogen [31,32]. Repeated fasting/refeeding and caloric restriction in the present study may not have induced comparable growth stimulation; however, caloric restriction and fasting may share possible biological effects on the cell-cycling rate [33–36].

In the present study, the number of target progenitor cells for leukemic transformation may have been lower in the CalR groups than in the non-CalR groups at the time of irradiation. However, the number of such progenitor cells with potential lethal damage may have increased afterward, when the dietary regimen was changed. Consequently, the number of target progenitor cells may have decreased, followed by a rapid increase as observed by Tessitore and colleagues [31,32], which may explain why the 3Gy-CalR(pre) group did not exhibit any significant decrease in the incidence of leukemias. Target cells for radiation-induced leukemogenesis may not be identical to conventionally assayable progenitor cells or CFU-S. However, the number of such target cells may be proportional to the number of progenitor cells and/or CFU-S [37,38]. Thus, we conclude that caloric restriction contributes to the decrease in the incidence of radiation-induced leukemias on the basis of two mechanisms. First, suppression during the initiation stage of direct genotoxic leukemogenesis, i.e., caloric restriction started before irradiation and continued until irradiation. Second, suppression during the promotion stage of indirect epigenetic leukemogenesis, i.e., restriction started after irradiation and continued until death. Furthermore, cell-cycle kinetics in progenitor cells, CFU-S, evaluated by BUUV assay showed a qualitative suppression during the promotion stage, during which CFU-S in the CalR groups were more quiescent than those in the non-CalR control (74.0% vs 54.0% in quiescence in the bone marrow, and 82.3% vs 68.6% in quiescence in the spleen; opposite to the case of percentage killing), resulting in a lower risk of epigenetic leukemogenesis for these cells. The 0Gy-CalR(–) and CalR(through) groups were evaluated at 50 weeks old, which is the time leukemogenesis generally becomes overt. These findings are compatible with those of Hursting et al. [35], who described that caloric restriction delays spontaneous tumorigenesis in p53 knockout mice due to a decreased amount of cell-cycling fractions in splenocytes and their precursors.

Our results demonstrate that caloric restriction is effective in suppressing the nongenotoxic promotion stage as well as the genotoxic initiation stage of radiation-induced leukemogenesis. Furthermore, from the results of the present study, the mechanisms underlying effects of caloric restriction on suppression of leukemogenesis, can be speculated to have many different aspects. Our present study focused on the number and cell cycling of hemopoietic stem/progenitor cells, despite other preventive factors that may also contribute, such as oncogene expression [39–41], DNA methylation [41], free-radical formation [42] , induction of apoptosis [43–46] , and activation of immunity [47–51], among others. Several studies of the pathway of insulin signaling and the mechanism underlying the effects of caloric restriction on suppression of tumorigenesis have been reported. Dunn et al. reported that dietary restriction decreased the level of insulin-like growth factor 1 (IGF-1) and the extents of apoptosis, cell proliferation, and tumor progression in p53-deficient mice [52]. The administration of recombinant IGF-1 to CalR mice led to improvement of physiological factors in mice fed a nonrestricted diet [52]. In *Caenorhabditis elegans*, mutation of the *daf-2* gene, in association with that of the *daf-16* gene encoding a member of the insulin receptor signaling molecules, extends the lifespan of the microorganism and confers oxidative stress resistance [53,54]. The *daf-2* gene network also controls longevity by regulating the Mn-SOD (superoxide dismutase)-associated antioxidant defense system [55]. The signal transduction of IGF in *C. elegans* is homologous to that of IGF-1/insulin in mammals. Therefore, the signal-transduction pathway of IGF-1/insulin may be partly involved in the decrease in the incidence of radiation-induced myeloid leukemias following caloric restriction.

In the present study, the incidence of tumors other than myeloid leukemias also decreased statistically significantly

in the group in which caloric restriction was started at post-irradiation (Table 1). Caloric restriction limited to the pre-irradiation period, from 6 to 10 weeks old caused a limited decrease in the incidence of myeloid leukemogenesis, implying that for other tumors, caloric restriction suppresses the promotion stage rather than the initiation stage of radiation-induced tumorigenesis. Results of the present study may contribute to identifying the potential preventive factor comparable to the epidemiological relevancy in atomic bomb survivors. A significant increase in the incidence of tumor-free death to 46.4%, the highest, in the nonirradiated restricted group was observed when caloric intake was restricted from 6 weeks until death, which is the first evidence of an increase in the incidence of tumor-free death with an increase in that of cardiovascular diseases by caloric restriction after irradiation, as confirmed by laborious observations of mice throughout their lifetime.

Uncited References

The following references were uncited: [56,57]

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Original

Electron Microscopical Evidence of the Protective Function of Thioredoxin (TRX/ADF) Transgene against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced Cellular Toxicity in the Liver and Brain

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Abstract: The present study was performed to assess the protective role of thioredoxin/adult T-cell leukemia-derived factor (TRX/ADF) on the liver and brain cell damages induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in ADF wild-type (WT) and transgenic (Tg) mice. The ADF WT and Tg mice were intraperitoneally injected with a single dose of TCDD (150 μg/kg body weight). One day after the treatment, the liver and brain tissues were examined electron microscopically to evaluate the cellular toxicity. In the ADF WT mice, marked reduction of subcellular components, such as mitochondria, rough endoplasmic reticula, and glycogen granules, as well as swelling of the remaining mitochondria, were evident in the liver cells. However, attenuation of these changes was evident in TCDD-treated TRX/ADF mice. Similar subcellular changes noted in the neuronal cells of TCDD-treated WT mice were also attenuated in Tg mice. The results suggest that oxidative cellular damage contributes to the acute toxicity induced by TCDD and that TRX/ADF protects against it. (J Toxicol Pathol 2005; **18**: 41–46)

Key words: Ah receptor, brain, liver, 2,3,7,8-tetrachlrodibenzo-*p*-dioxin (TCDD), thioredoxin/adult T-cell leukemia-derived factor (TRX/ADF), transgenic (Tg) mouse

Introduction

As one of the aromatic hydrocarbons, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a widely spread environmental pollutant that has a broad spectrum of toxic effects on a variety of tissues such as the thymus, liver, testes and central nervous system in mammals[1–6]. Although a number of studies have shown that the toxic effects of TCDD are mediated by intracytoplasmic aromatic hydrocarbon receptor (AhR)[7–9], the toxic mechanism of TCDD on the target organs is still not fully understood. Among the toxic events, oxidative stress is considered to play a major role in the toxic mechanism of TCDD, as characterized by marked increases of lipid peroxidation, the formation of reactive oxygen species, and DNA single-strand break[9–14].

Exogenous xenobiotics, such as aromatic hydrocarbons, result in profound induction of cytochrome P450 enzymes in the liver, resulting in the generation of reactive oxygen species[15,16]. On the other hand, the brain is rich in peroxidizable fatty acids and has relatively low catalase activity[17]. Therefore, these organs are considered to be highly susceptible to oxidative stresses[18]. In fact, the contribution of oxidative stress in TCDD-induced cellular damage of the liver and brain has been suggested in previous studies[13,18–22].

Adult T-cell leukemia-derived factor (ADF) is a human thioredoxin (TRX) associated with the reduction/oxidation (redox) regulation of the cellular environment[23]. TRX/ADF is a stress-inducible protein and its expression is up-regulated after viral infection as well as in cellular stress conditions induced by oxidative agents such as hydrogen peroxide or diamide, irradiation with X-rays and ultraviolet

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light, or ischemic reperfusion[23]. Previous studies have shown that TRX/ADF plays a role in the cellular defense mechanism against oxidative cellular damage via the regulation of intracellular redox status, since exogenously administered TRX/ADF protected cells from oxidative cellular injury[24,25].

We recently reported for the first time the protective function of TRX/ADF against TCDD-induced hematotoxicity in ADF transgenic (Tg) mice, indicating oxidative stress contributes to the hematotoxic mechanism of TCDD[26]. We hypothesized in the present study that overexpression of TRX/ADF might also be effective for protection against the toxic effects of TCDD on the liver and brain tissues in which oxidative stress has also been implicated in the toxic mechanism. For this purpose, we injected TCDD with a dosage capable of inducing oxidative stress in the liver following acute exposure[21], to ADF wild-type (WT) and transgenic (Tg) mice, and then compared subcellular changes electron microscopically in the liver and brain tissues.

Materials and Methods

Animals

TRX/ADF overexpressed mice (ADF Tg mice), originally produced by Dr. A. Mitsui[27], were maintained in a laboratory facility with a 12:12-hour light-dark cycle at an ambient temperature of 21 ± 2°C at the National Institute of Health Sciences (NIHS) of Japan by breeding ADF WT and Tg mice. Animals were screened by PCR of their tail DNA to determine their genotypes. At 8 weeks of age, male ADF WT and Tg mice (23.5–24.8 g) were transferred to a vinyl isolator established in a hazard room designed to prevent contamination from the outside environment and randomly allocated within the same genotype to housing with 6 animals per cage. A pelleted basal diet (CRF-1; Funabashi Farm, Funabashi, Japan) and tap water were provided *ad libitum* throughout the study.

Chemical

TCDD was obtained from Radian International, Cambridge Isotope Laboratories, Inc. (Andover, MA, USA; purity: 98 %). TCDD was initially dissolved in a small volume of acetone and subsequently adjusted to the concentration of 10 μg/ml in olive oil.

Experimental design

ADF WT and Tg mice were divided into vehicle controls and TCDD treatment groups, each consisting of 6 animals. After one week of acclimation, TCDD at 150 μg/kg was intraperitoneally injected once to animals of treatment groups, and the corresponding volume of olive oil was similarly injected to vehicle controls. The dosage of TCDD was selected based on previous study results that showed oxidative stress in the liver was induced by a single bolus injection to mice[21]. One day after the treatment, the animals were sacrificed by decapitation and then examined grossly. The liver and brain were then excised and their weights were measured.

The animal protocol was reviewed and approved by the Animal Care and Use Committee of the NIHS, Japan.

Morphological assessment

For histological examination, liver tissues in all animals were fixed in 10% neutral buffered formalin (pH 7.4). After routine processing, the paraffin-embedded sections were stained with hematoxylin and eosin and then examined histopathologically under a light microscope.

For electron microscopical examination, tissue specimens from the liver and cerebral cortex were respectively prepared from three animals each of the control and treatment groups of ADF WT and Tg mice. Small tissue blocks, sized 1 mm^3, were fixed with 2.5% glutaraldehyde in 0.2 M Sorenson's sodium phosphate buffer, pH 7.2, for 8 hours at 4°C. After washing with 0.1 M PBS (pH 7.4), the tissues were post-fixed with 1% osmium tetroxide for 90 minutes. After washing in 0.1 M PBS, the tissues were dehydrated with ethanol and propylene oxide and then embedded in Epon 812. Ultrathin sections were double-stained with uranyl acetate and lead citrate. The sections were examined with JEOL-1200 EX II electron microscope (JEOL, Tokyo, Japan).

Results

After one day of TCDD treatment, absolute liver weight had decreased to 71.4% of the vehicle control group in ADF WT mice and 83.2% in ADF Tg mice (data not shown).

Histologically, apoptotic liver cell debris and also focal liver cell necrosis were sparsely observed in the centrilobular areas of both TCDD-treated WT and ADF Tg mice, without showing apparent difference in the severity between genotypes (data not shown). Vehicle control animals did not show such liver cell changes in either genotype.

Electron microscopically, liver cells of the WT mice treated with TCDD exhibited a prominent decrease of cytoplasmic glycogen granules and rough endoplasmic reticula (RERs) and an increase of smooth endoplasmic reticula (SERs) (Fig. 1B). The number of mitochondria was also decreased and the remaining mitochondria showed swelling with disorganized cristae and lucent matrix. Increased fat droplets were also evident in the cytoplasm of less affected hepatocytes. On the other hand, transgene of Trx/ADF notably attenuated these morphological changes following TCDD treatment (Fig. 1C). In the cerebral cortex, neuronal cells showed a decrease in the number of RERs, ribosomes and mitochondria in WT mice treated with TCDD (Fig. 2B) but not in ADF Tg mice treated similarly with TCDD (Fig. 2C). Vehicle control animals did not show such neuronal cell changes in either genotype.

Discussion

In the present study, acute treatment with TCDD

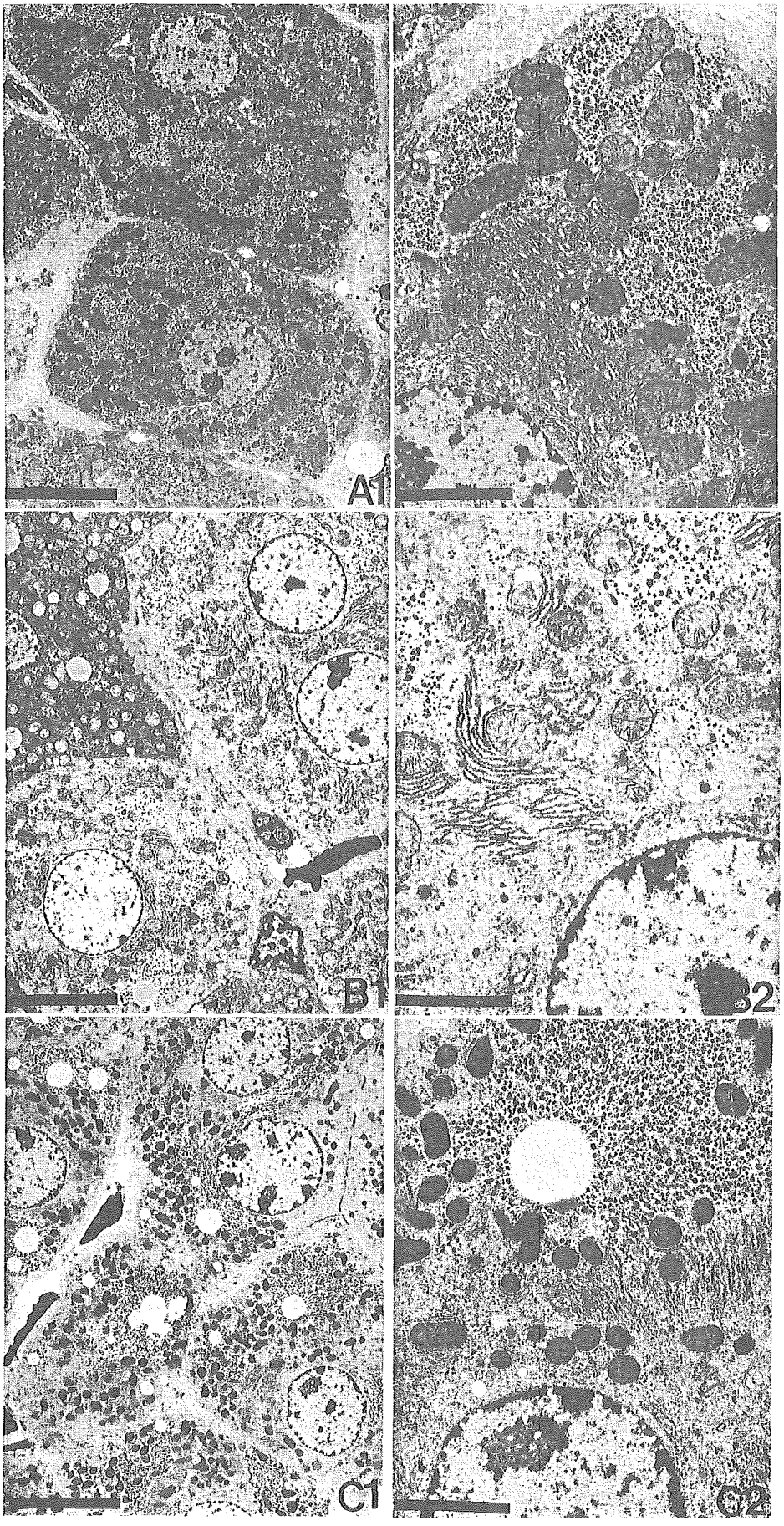

Fig. 1. Electron micrographs of liver cells from ADF WT and Tg mice treated with vehicle or TCDD. (A) Vehicle-treated ADF WT mouse, (B) TCDD-treated ADF WT mouse, and (C) TCDD-treated ADF Tg mouse. Note cytoplasmic swelling associated with a profound decrease of glycogen granules, RERs and mitochondria in the liver cells of the TCDD-treated ADF WT mouse (B). Swelling of the remaining mitochondria with disorganized cristae and lucent matrix is also evident (B). Attenuation of these morphological changes is evident in the TCDD-treated ADF Tg mouse (C). Uranyl acetate and lead citrate. Bar=10 μm (A1, B1, C1), Bar=3 μm (A2, B2, C2).

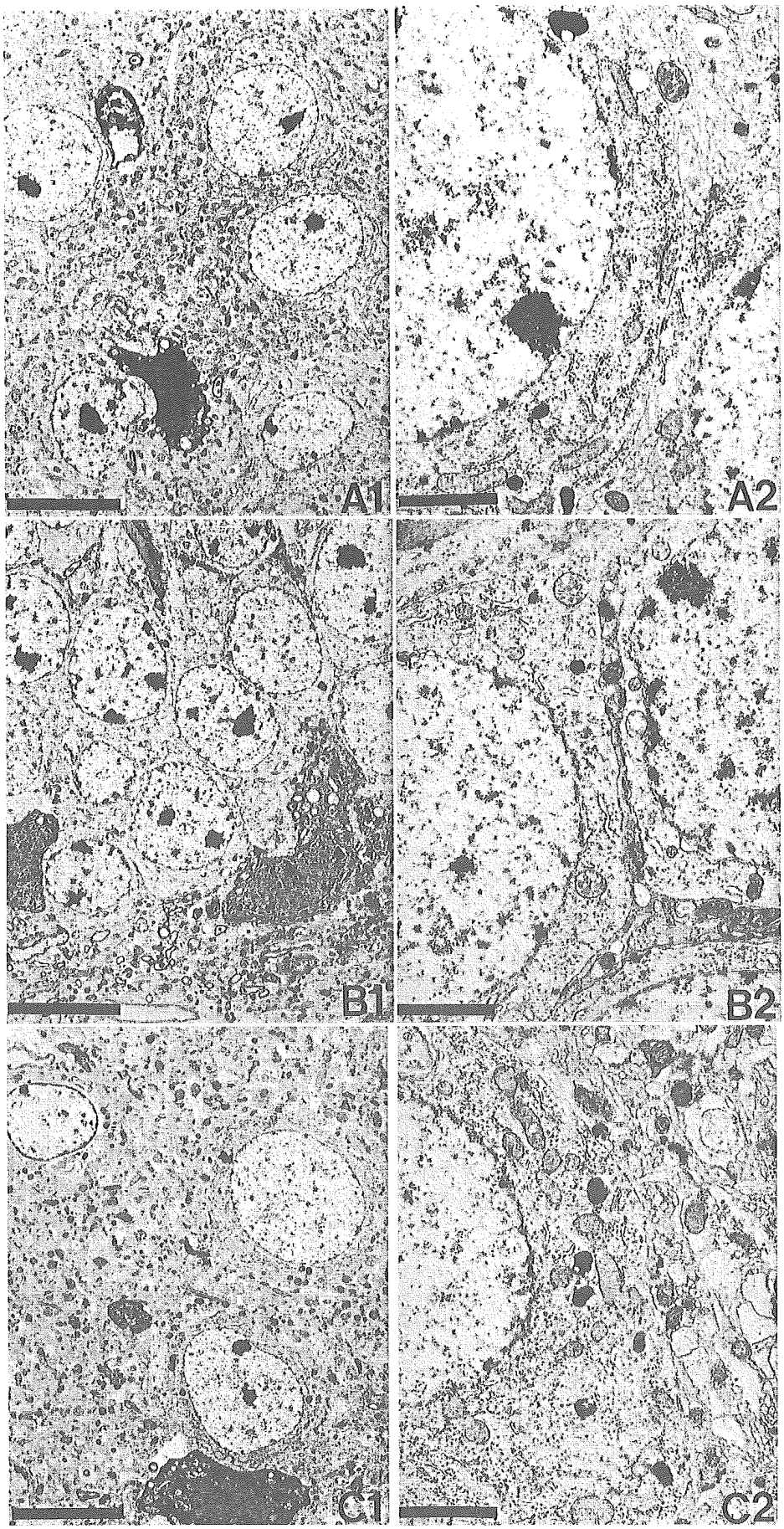

Fig. 2. Electron micrographs of neuronal cells in the cerebral cortex from ADF WT and Tg mice treated with vehicle or TCDD. (A) Vehicle-treated ADF WT mouse, (B) TCDD-treated ADF WT mouse, and (C) TCDD-treated ADF Tg mouse. Note the decrease of RER, ribosome and mitochondria in the cytoplasm of neuronal cells of the TCDD-treated ADF WT mouse (B). In the TCDD-treated ADF Tg mouse, mitochondrial swelling is also evident, but attenuation of the morphological changes can be seen, too. (C). Uranyl acetate and lead citrate. Bar=10 μm (A1, B1, C1), Bar=2 μm (A2, B2, C2).

induced ultrastructural alterations in the cytoplasmic components of liver cells characterized by prominent decrease of glycogen granules and RERs, proliferation of SERs, decrease and degradation of mitochondria, and increase of lipid droplets. These subcellular alterations were mostly consistent with those noted in the guinea pig liver following TCDD treatment[28], but concentric membrane arrays in the liver cells were not evident in the present study, presumably due to the different experimental protocol or the different species used in the studies. In the cerebral neuronal cells in the present study, alterations in subcellular components by TCDD were also evident, despite the changes being less profound than those in the liver cells. These subcellular changes in the liver and neuronal cells may represent the cytotoxic outcome of TCDD due to oxidative cellular damage and also cellular adaptation including detoxification.

Effective prevention of TCDD-induced toxicity by administration of antioxidants such as oltipraz[5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione] or butylated hydroxyanisole, or by pretreatment with vitamins A and E further supports the hypothesis that oxidative processes are involved in TCDD-induced toxicity[29,30]. Attenuation of subcellular changes in the liver and neuronal cells by transgene of TRX/ADF in the present study indicates the critical role of oxidative stress in the toxic events induced by TCDD, and also the protective function of ADF/TRX in these organs, as in our previous study of TCDD-induced bone marrow toxicity[26]. The protective effect of TRX/ADF against oxidative cellular damage is believed to be achieved by free radical scavengers[31], activation of DNA repair enzymes, such as activator protein endonuclease (Ref-1; redox factor-1)[32], and activation of nuclear factor-kappa B (NF-kB)[33].

Taken together, the results of our present study strongly suggest that the acute toxic effect induced in the liver and brain by a single large dose of TCDD is due to oxidative cellular damage, and that TRX/ADF plays a role in protection against TCDD-induced acute toxicity. Considering the routes and concentrations of TCDD exposed to humans, research on the effect of extremely low doses of TCDD by oral ingestion on the oxidative cellular damage of target organs is clearly warranted.

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BENZENE-INDUCED HEMATOPOIETIC TOXICITY TRANSMITTED BY AHR IN THE WILD-TYPE MOUSE WAS NEGATED BY REPOPULATION OF AHR DEFICIENT BONE MARROW CELLS.

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Introduction

Recent studies have shown that the aryl hydrocarbon receptor (AhR) in primitive cells transmits negative signals for the proliferation of such cells[1, 2]. As we previously reported, primitive hemopoietic progenitor cells increases in number in AhR-knockout (KO) mice; on the other hand, relatively mature progenitor cells on the other hand, decreases in number in a homeostatic manner[1].

We have reported that benzene-induced hemopoietic toxicity is transmitted by AhR[3]. We also found that cytochrome P450 2E1 (CYP2E1) related to benzene metabolism is also up regulated in the bone marrow by benzene exposure in the bone marrow[4]. Therefore, it is of interest to hypothesize a greater role of bone marrow cells in hemopoietic toxicities rather than the hepatic metabolism. Accordingly, in the present study, benzene-induced hemopoietic toxicity was evaluated in wild type (Wt) mice after a lethal dose of whole-body irradiation followed by repopulation of bone marrow cells that lack AhR or, *vice versa,* in AhR KO mice after repopulation of Wt bone marrow cells.

As results, benzene-induced hemopoietic toxicity seems to have been transmitted through AhR, and benzene was transformed by *de novo* metabolism with CYP2E1 in the bone marrow.

Materials and Methods

Animals. The establishment of homozygous AhR KO ($AhR^{-/-}$) mice, the 129/SvJ strain, is described elsewhere[3, 5]. The breeding of heterozygous AhR KO ($AhR^{+/-}$) males with $AhR^{+/-}$ females generated wild-type ($AhR^{+/+}$), $AhR^{+/-}$, and $AhR^{-/-}$mice. The neonates were genotyped by PCR screening of DNA from the tail. Female mice (12 weeks old) were used in the study. Eight-week-old C57BL/6 male mice from Japan SLC (Shizuoka, Japan) were used as recipients for the repopulation assay and the assay of CFU in the spleen. All the mice were housed under specific pathogen-free conditions at 24 ± 1°C and 55 ± 10%, using a 12-hr light-dark cycle. Autoclaved tap water and food pellets were provided *ad libitum.*

Blood and bone marrow (BM) parameters. Peripheral blood was collected from the orbital sinus. Peripheral blood leukocyte (WBC), red blood cell (RBC) and platelet (PLT) counts were determined using a blood cell counter (Sysmex M-2000, Sysmex Co., Kobe, Japan). Bone marrow (BM) cellularity was evaluated by harvesting BM cells from the femurs of each mouse[6]. The animals were sacrificed. Then a 27-gauge needle was inserted into the femoral bone cavity through the proximal and distal edges of the bone shafts, and BM cells were flushed out under pressure by injecting 2 ml of a-MEM. A single-cell suspension was obtained by gently triturating the BM cells through the 27-gauge needle, and cells were counted using Sysmex M-2000.

Irradiation. Recipient mice were exposed to a lethal radiation of 800.1 cGy, at a dose rate of 124 cGy/min, using a ^{137}Cs-gamma irradiator (Gamma Cell 40, CSR, Toronto, Canada) with a 0.5-mm aluminum-copper filter.

CFU-S Assay. The Till and McCulloch method[7]was used to determine the number of colony-forming units in the

spleen (CFU-S). Aliquots of BM cell suspensions were used to evaluate the number of CFU-S. The number of BM cells was adjusted to that appropriate for producing nonconfluent spleen colonies, and the cells were then transplanted into lethally irradiated mice by injection through the tail vein. Spleens were harvested 9 and 13 days after the injection, and fixed in Bouin's solution. Macroscopic spleen colonies were counted under an inversion microscope at a magnification of x 5.6.

CFU-GM and CFU-E Assay. *in vitro* colony formation was assayed in semisolid methylcellulose culture[6, 8]. Briefly, 8 x 10^4 BM cells suspended in 100 ml of medium were added to 3.9 ml of a culture medium containing 0.8% methyl cellulose, 30% fetal calf serum, 1% bovine serum albumin, 10^{-4} M 2-mercaptoethanol, with 10 ng/ml murine granulocyte-macrophage colony-stimulating factor (GM-CSF) for CFU-GM or 1ng/ml murine Interleukin-3 and 2 U/ml erythro-poietin for erythroid CFU (CFU-E). One-ml aliquots containing 2 x 10^4 BM cells were plated in triplicate in a 35-mm tissue-culture plate, and incubated for six days in a completely humidified incubator at 37 °C with 5% CO_2 in air. Colonies were counted under an inverted microscope at magnifications of x 40 for CFU-GM after 6-day culture and x100 for CFU-E after 3-day culture.

BM repopulation assay[9]. The BM repopulation assay was performed similarly to the assay of CFU-S, except that 10^6 BM cells were injected into lethally irradiated mice. One month after the transfusion of BM cells, the repopulated mice were used in the experiment.

Results and Discussion

As previously reported, AhR-KO mouse showed a significant increase in WBC counts (**Figure 1** A). This was also consistent with the high number of myeloid progenitor cells, *i.e.*, CFU-S-9 and CFU-S-13, observed in the AhR-KO mice (**Figure 1**, B). Thus, steady-state hemopoiesis is presumed to be suppressed by AhR signaling due to the possible presence of a physiological ligand, which is not readily observed in AhR-KO mice. In response to such an AhR-null effect, the AhR-KO mouse reversely shows extensive hemopoiesis in the spleen, although this hemopoietic enhancement is also reflected in another negative hemopoietic regulation in the BM. Accordingly, in the present study, benzene-induced hematotoxicity was evaluated in the Wt mice after a lethal dose of whole-body irradiation followed by the repopulation of BM cells that lack AhR.

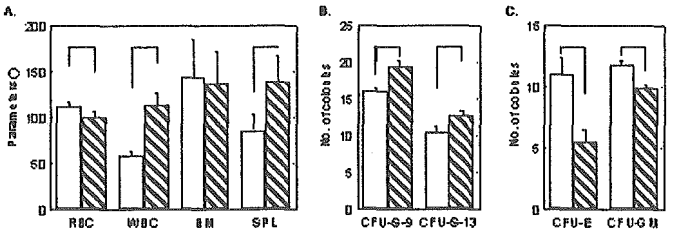


Figure 1: Comparison of various blood parameters between Wt mice (open columns) and AhR-KO mice (shaded columns) [1]; A. Peripheral blood, bone marrow and spleen weight. * Parameters indicate the counts of peripheral red blood cells (RBCs, $\times 10^8$/ml) and white blood cells (WBCs, $\times 10^6$/ml), bone marrow cellularity (BM, $\times 10^5$/femur), and weight of spleen (SPL, mg). B. Number of colony-forming units in spleen (CFU-S/1$\times 10^5$ BM cells) observed on days 9 (CFU-S-9) and 13 (CFU-S-13). C. Numbers of *in vitro* granulocyte-macrophage *CFUs (CFU-GM/5*$\times 10^3$ *BM cells)* and erythroid *CFU (CFU-E/1*$\times 10^4$ BM cells). †: Significant difference between Wt and AhR-KO mice determined by *t*-test at $p<0.05$.

Figures 2, A-C, show the RBC (A), WBC (B), and platelet (PLT: C) counts (per mL) in the peripheral blood after repopulation of the BM. In each figure, in the Wt mice repopulated with Wt BM cells (two columns on the left), the groups subjected to intraperitoneal benzene exposure (second from the left) show significant decreases in RBC and PLT counts (92% and 69%; p=0.010 and 0.016, respectively) compared with the sham exposure groups (farthest left in each figure), except 2B, i.e., WBC counts (96%). When the mice repopulated with AhR-KO BM cells (two columns on the right) are exposed to benzene, there are no significant differences between the sham exposure groups (second from the right) and the benzene exposed groups (farthest right) in A through C. Significant decreases

observed in the Wt mice repopulated with Wt BM cells were negated when the Wt mice were repopulated with AhR-KO BM cells; thus, the reduction in the number of peripheral blood cells observed in the Wt mice after benzene exposure is assumed to be responsible for the AhR expression in BM cells. Although the two sham exposures (i.e., Wt mice, open column; and AhR-KO mice, solid column) are essentially identical in A and C, there seems to be insufficient recovery of the BM in transplantation in Figure 2B, and the solid column is significantly reduced (see, Figure 3 on CFU-GM).

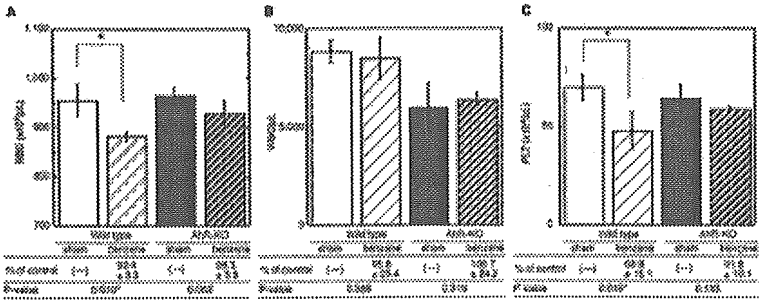


Figure 2: Comparison of various blood parameters in peripheral blood; A, RBC, B, WBC, and C, platelets (open bars vs lightly shaded bars in Wt mice repopulated with Wt BM cells; solid bars vs heavily shaded bars in Wt mice repopulated with AhR BM cells).

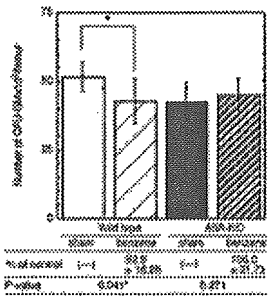
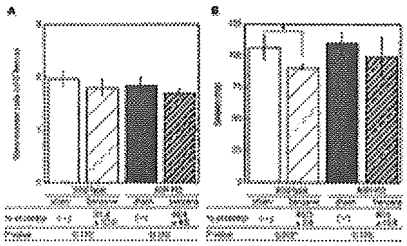


Figure 3: *: Significant difference between sham and exposed determined by *t*-test at $p<0.05$.

In Figure 3, again, the significant decrease in the number of granulocyte-macrophage colony-forming units *in vitro* (CFU- GM/5 x10^3 BM cells) in the BM cells from the Wt mice repopulated with Wt BM cells (82.9% in benzene exposure, lightly shaded column to the right of the sham exposure, open leftmost column; p=0.041) is negated in the BM cells from mice repopulated with AhR-KO BM cells (sham exposure, solid column; and benzene exposure, heavily shaded column, respectively). In this figure, the efficiency of repopulation with AhR- KO BM cells (solid column) seems to be insufficient, since the solid column is smaller than the open column (p=0.025). The mechanism underling the incomplete recovery of AhR-KO BM cells, is still unknown; however, the sublethal irradiation of the recipient mice may be the case, where suppressive intrinsic factors may have been released from tissues given the lethal dose of irradiation received by the host animals.

Despite the insufficient recovery of the number of GM-CFU in mice repopulated with AhR-KO BM cells, number of BM cells, regardless of repopulated cell type (either Wt or AhR-KO BM cells) and type of exposure (either benzene or sham exposure), there were no significant differences in number of BM cells among the groups in a homeostatic manner (**Figure 4**, A; 91.4% and 92.4%, respectively, p>0.1). However, after benzene exposure, a significant decrease in splenic weight was observed in the Wt→Wt group (85.0%, p=0.022), but not in the AhR-KO-BM→Wt group (90.0%, p=0.173). This supports the notion that AhR-KO negates the suppressive effect on splenic weight after benzene exposure.

Figure 4: Comparison of number of BM cells (A) or weight of spleen (B) with or without benzene exposure, in mice repopulated with Wt BM cells or in mice repopulated with AhR-KO BM cells. (Wt mice repopulated with Wt BM cells, lightly shaded columns, second from left; or without benzene exposure, open columns, farthest left, benzene exposure vs Wt mice repopulated with AhR-KO BM cells, each of the two right columns).



Conclusions

The up-regulation of CYP2E1 after benzene exposure was specifically observed in our previous microarray study of the bone marrow tissue[4]. The analysis of the gene expression specifically derived from the hematopoietic stem cell compartment[10], and the evaluation of the toxicological alteration of such an expression as a measure of stem cell specific toxicological biomarkers are hot issues in the current hematotoxicology[11]. Mice that have been lethally irradiated and repopulated with BM cells from AhR-KO mice essentially did not shown any benzene-induced hematotoxicity, implying that such toxicity is derived from *de novo* metabolisms with CYP2E1 in the BM other than hepatic metabolism. The present study raises two questions on AhR-mediated TCDD-induced hematotoxicity: Do Wt mice repopulated with AhR-KO BM cells show hematotoxicity by TCDD unlike in the case of benzene exposure? If such is the case, what would be the transmitter from the site of xenobiotic metabolic activation to the bone marrow?

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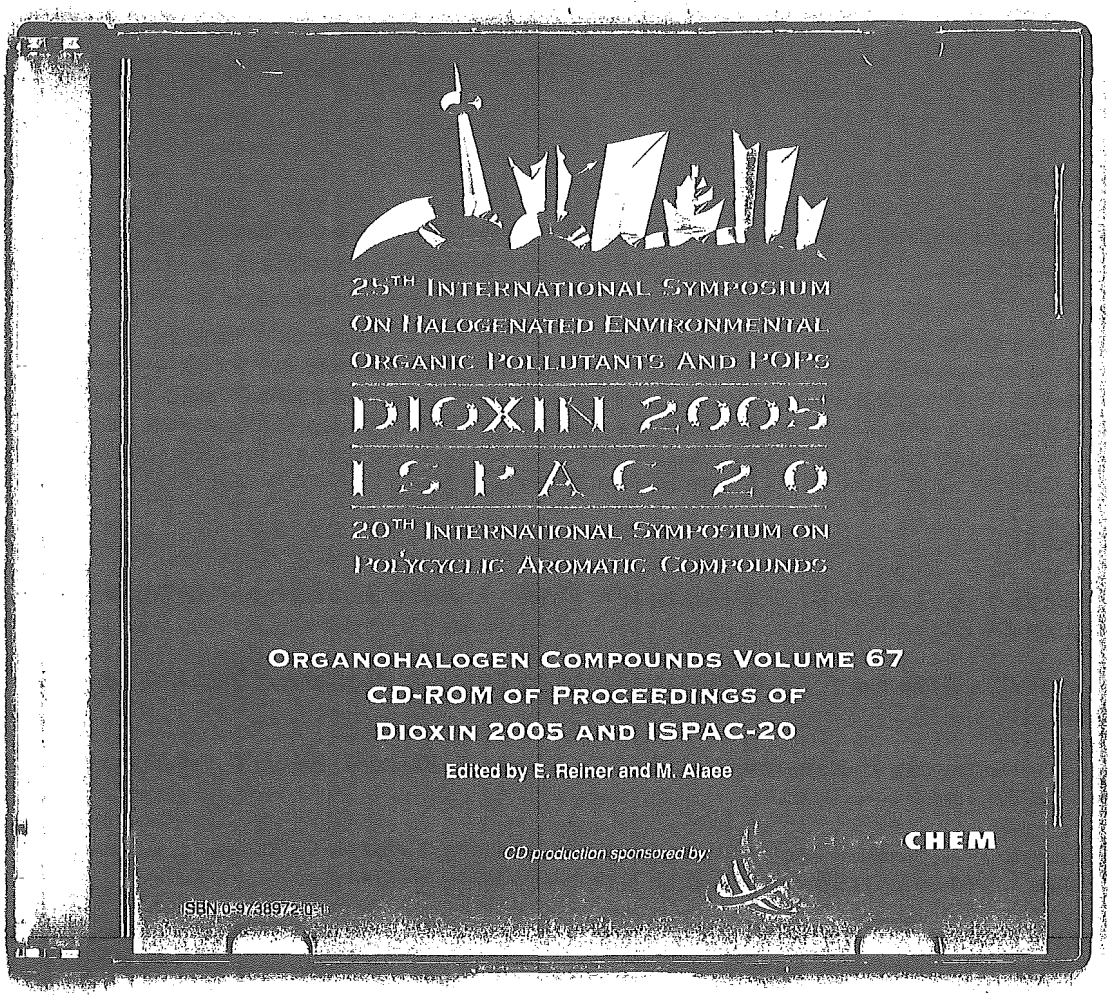
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環境ストレス応答と生体ホメオスターシス

―総　論―

井上　達

◎生体は，内外の環境中に漂ううたかたのようなものである．生体の外部環境が外気や水環境によることはもちろんのことであるが，内部環境についてもその主たる部分は，それら外部環境中の物質を食物もしくはその混入物として消化管内に取り込んだ状態にほかならないから，消化管壁には種々の防御機構が備わっているとはいえ対外環境の延長線上で理解されうるものである(図1)．環境と食品を同列で取り扱う所以もこの点にある．ちなみに外気を取り込む呼吸器も消化器を発生原基としているので，消化器の機能を考える際に，そうした生体内腔を貫通する外部環境としての共通性を呼吸器を含めて念頭におくことは無駄にはならない．本章における生体と内外環境物質についての生体の異物ストレス応答の各論に入る前に，環境・食品などによるレドックス制御の逸脱と，環境・食品中の成分によるそうした酸化ストレスの消去の例の概略を紹介する．

Key word：環境ストレス応答，チオレドキシン，放射線，臭素酸カリウム，食品化学発癌

各環境ストレス応答と生命系

内外の環境と生体の相互作用の中で，レドックス平衡機能に働くチオレドキシン(TRX)などの分子種は，①原核生物から真核生物まで普遍的に備わっている酸素種による傷害の防御機構としての役割から，②有酸素下でのミトコンドリアを利用した好気的生命活動レベルで発揮される諸機能，さらに，③そうしたレドックスを生体の調節機構として利用する機能系にいたるまで，それぞれ異なったレベルでの環境応答機構に役割を果たしていることが明らかになっている[1]．放射線や紫外線に対する物理・化学的で直接的に作用する強い活性酸素やラジカル生成に対する還元蛋白としての役割を①とすれば，水溶性分子として排出する代謝過程での水解酵素チトクローム P450 の転写活性化など異物代謝の制御に働く機構は②に該当し，また NF-κB のような転写因子の遺伝子発現制御を通じた免疫系のレドックス調節などは③の一例として理解される．本書では，それらレドックス機構の基礎については第1章，関連する病態については第2章に紹介されており，その破綻・修復と予防のためのメカニズムを研究して健康医学への方策を考えることを，本章の目的としてい

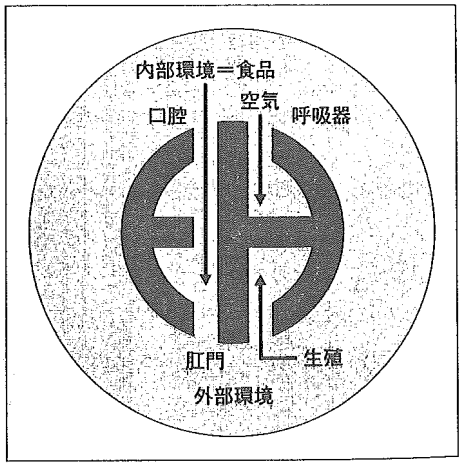

図1　生体内外環境の概念図
消化管は，外部環境の陥入組織である．食品を環境成分としてとらえる意義はこの点にある．ちなみに呼吸器は，消化器を原基として発達する組織であり同様に理解すべきものと考えられる．

Redox regulation in xenobiotic response
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る.

たとえば,先のTRX遺伝子をノックアウトしてさまざまのレベルで働いているTRXを消去すると,ホモ欠失では胚細胞は胎生致死をきたし[2],また,その過剰発現系を作製すると,種々のレベルでのレドックスの平衡状態の破綻に対して顕著な抵抗性をみることが浮かび上がってくる(本書,平林「ダイオキシンの生体影響と防御機構」の項参照)[3,4].ここでトランスジェニックマウスの作製に導入されたTRX遺伝子は,ヒトのそれなので,この分子種の機能の普遍性が窺われよう.酸化ストレスに対する還元蛋白の誘導が原核生物からヒトまでよく保存されていることからも想定されるように,抗酸化ストレス機構については,種間の相同性がよく保存されており,本章で取り扱われる種々の環境・食品などの物質の作用も,それらの種を超えて理解することが可能と考えられる.

第4章では,まず内外環境物質として生体に異物ストレス応答を迫ってくる物質が取り上げられ,生体におけるレドックス制御の逸脱の機構が紹介されており,ついでレドックス制御の積極的な維持が健康医学の中心課題にあるものとの考え方から予防的見地にたった諸研究が紹介されている.それら個別の各論に入る前に,環境・食品などによるレドックス制御の逸脱の例と,反対に環境・食品中の成分によるそうした酸化ストレスの消去を企図する例を取り上げて,総論としてのつとめを果たしたい.

環境要因によるレドックス制御からの逸脱

放射線や紫外線あるいは,種々の食品や食品添加物は,環境要因として,レドックス平衡を酸化傾向に傾け,酸化ストレス状態を引き起こすことがある.酸化ストレスが生体に対して引き起こす傷害は,第1章で解説されているようなさまざまの細胞機能の不全状態を引き起こす.それらには細胞のプログラム死,蛋白のミス・フォールディング,ミトコンドリアの機能不全,プロテアソームの機能障害などがあげられている[5]が,とりわけその直接的なDNA傷害に基づく修復エラーは発癌を引き起こす要因となる[6].レドックス制御が抗酸素種傷害防御機構のみならず,レドックスを生体の調節機構として利用する機能系に広くかかわっていることからするならば,その平衡状態の逸脱の影響も,さらに広範な生物機能の障害として理解されるはずである.しかしそれらの背景は,TRX遺伝子のノックアウトのような系で観察するときその子細が初めて明らかになるものの,通常の動物実験などによる観察ではその認識は困難であることが少なくない.裏返すならば,そうした背景にこそ,環境医学・健康医学の新しい領域としてのおもしろさが潜んでいることが理解されよう.

ここでは,電離放射線,臭素酸カリウム($KBrO_3$),ヘテロサイクリックアミンの3つの例をとりあげて,それぞれの発癌性に介在する酸化ストレスの関与の機構を簡単に紹介する.

1. 電離放射線

放射線は,その物理化学的過程で活性酸素をつくりだし,生体に酸化ストレス状態を惹起する.その詳細は,すぐれた総説[7,8]にゆずるが,放射線の発癌機構としては,DNAや染色体に直接傷害を引き起こす一次発癌機構と,細胞増殖の亢進やアポトーシスの抑制などのエピジェネティックな機構に基づく二次発癌機構との双方が考えられる.放射線が引き起こすDNA傷害の発見は古く[9],前者の主要因と考えられ,酸化ストレスによるものと理解される.しかしながら放射線の引き起こすDNA傷害は,放射線発癌や放射線白血病の成因を,それらの頻度などとの相関性においてかならずしもよく説明しない.放射線照射後の造血幹細胞の機能変化などが詳細に探求されたが,この問題は解決していない.実験的に放射線照射を行った後に骨髄移植を行うと,骨髄は波をうって幾度かのオーバーシュートを繰り返しつつ回復に向かうが最終的には完全な回復をみることはなく,たとえば造血前駆細胞のコロニー形成能を指標にみる限り80～85%程度の回復にとどまる[10].これは骨髄の造血支持細胞の放射線障害の修復不全によるものと考えられ,骨髄は,たとえば*myc*の過剰発現状態をもって幹細胞のS期分画を高目に維持し,末梢血が無処置の状態に匹敵するまでの回復を下支えしている[11].こうした骨髄の造血支持細胞の造血にあたえる持続的なストレス[10]や,放射

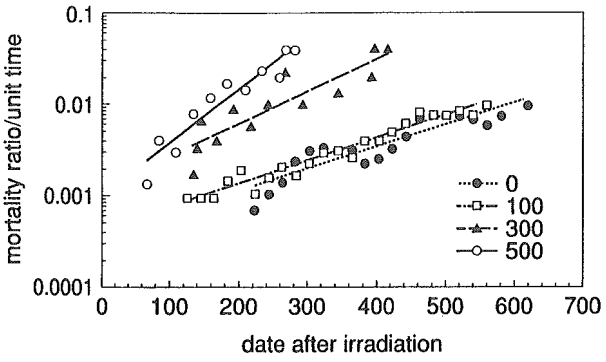

図 2　ゴンペルツ表現による照射線量に応じたマウスの死亡曲線の変化

横軸に年齢をとり，縦軸に累積死亡率を対数でとると，マウスは，指数関数的に直線的に死亡する(非照射対照群参照)．照射線量を増すごとに，死亡率は傾きが急峻化し，促進老化の形をとって早期に白血病死する(照射線量，cGy)．

線によって引き起こされる遺伝子の不安定性につながる要因が，エピジェネティックな白血病の発症機構の一翼となっているものと考えられる(図2)．図に見られるように放射線量を増すに従って死亡直線は傾きが急峻化する．反対に TRX トランスジェニックマウスによる放射線白血病の頻度低下と死亡の遅延の観察はまだできていないが，造血幹細胞などの放射線感受性や紫外線感受性が TRX トランスジェニックマウスで寛恕になることが観察されている．

2. 臭素酸カリウム($KBrO_3$)

臭素酸カリウム($KBrO_3$)は，製パンの過程でソフターとしてドー(dough)に混入させるものである．かつて黒川ら[12]は，ラットでこのものに腎腫瘍誘発性があることを見出した．実験は数次にわたって行われているが，F344 系雌雄のラットを用いた 104 週の飲水投与実験では，500, 250, 125, 60, 30, 15, および 0ppm の投与に対して，シグモイド状の発生頻度の腎細胞癌が観察され，閾値の設定が困難な結果であった[13]．実際に製パン業に使用されていたことと相まって，$KBrO_3$はサルモネラ菌に対する復帰突然変異を引き起こさないので，その発癌機構が関心を集めた．その後の研究で，これは，$KBrO_3$が放出する活性酸素が DNA 付加体，8-hydroxydeoxyguanosine を形成することによる一次発癌と，遺伝子傷害性化学物質 *N-ethyl-N*-hydroxyethylnitrosourea の前処置後の投与によって発癌性の亢進を示す二次発癌との双方の性質を有する，弱いながらも完全発癌物質であることが明らかになった[14](脚注*1)．昨今は，食品もしくは食品添加物の生体影響，発癌性のいかんに対する関心が高まっているが，この臭素酸カリのそれはひと昔前のことでもあり，歴史的に特筆される事件として記憶されることとなった．

3. ヘテロサイクリックアミン(MeIQX)による発癌

MeIQX は，魚の焼けこげから抽出された物質のひとつであり，その後高熱で焼いた肉類でも形成されることの知られている癌原物質である[15]．この MeIQX による発癌は，抗酸化物質によって抑制されることが知られている(後述)ので，酸化的 DNA 傷害に起因するものと想定されていたがその直接的証明はなかった．これに対して，あらかじめ遺伝子傷害性化学物質の diethyl nitrosamine (DEN)を投与しておいて，これに MeIQX と種々の合成抗酸化剤を併用投与し，前腫瘍性病変と考えられている胎盤型グルタチオン *S*-トランスフェラーゼ(GST-P)陽性巣を指標に抗酸化剤の効果をみた実験がある[16]．それによれば，1-*O*-hexyl-2,3,5-trimethylhydroquinone(HTHQ)などの合成抗酸化剤は，GST-P 陽性巣の抑制が顕著であったに

*1：アメリカをはじめとする各国は，このものの発癌性が軽微であったので，$KBrO_3$を製パン過程で十分加熱することによって残留量を低く抑えるなどの注意喚起をもって移行措置とし，後に使用中止とした．

もかかわらず，また同時に行った茶カテキンでは顕著なGST-P巣形成の抑制が認められたにもかかわらず，DNA付加体8-OHdGの形成の抑制は認められなかった．そしてHTHQの抑制効果は，MelQXの代謝活性化の抑制によるものであったものと結論している．MelQXの発癌性には，同時にレドックスを介したnicotinamide adenine dinucleotide(NADH)の関与を経て酸化的DNA傷害が介在するものとの報告[17]もみられるので，双方の過程が存在するものと想定され，酸化ストレスによるレドックス制御の破綻がむしろ双方の過程にかかわっているものとも考えられる．

環境制御によるレドックス制御逸脱の修復

酸化ストレスを低く抑制することによって，レドックス平衡が逸脱するのを予防する試みが健康医学の立場から活発に進められている．筆者のこれまでにかかわってきた関連する研究の中から，カロリー制限による放射線白血病の抑制，茶カテキン類やTRX過剰発現マウスを用いた実験癌の予防などについて紹介し，最後に，アリールヒドロカーボン受容体結合食品に関する松井らの研究について簡単に解説して，本章の総論としての務めを果たしたい．

1. カロリー制限

カロリー制限の歴史は古く，1935年のMcCayの実験的試みにまでさかのぼる[18]．Yuらは，ラットを用いたカロリー制限実験で，寿命の延長にリンクした腫瘍死の遅延を観察した．興味深いことに彼らはこの実験でサテライト群をもうけて経時的に屠殺し，先の腫瘍死の遅延が腫瘍発生の遅延ではなく腫瘍の成長の緩徐化にあることを明らかにした[19]．このようにカロリー制限による腫瘍死の遅延・抑制のメカニズムが，腫瘍の発生そのもの，すなわち一次発癌に影響するのか，腫瘍の増生，すなわち二次発癌としてのプロモーションに影響するのかを明らかにするため，放医研の吉田らは，興味深いデザインの実験を行った[20-22]．放射線白血病誘発のための放射線1回照射(生後8週に施行)の前と後に分けて，カロリー制限を5週齢で開始し，照射後，通常食にもどす照射前カロリー制限群(①)，反対に照射までは通常食で飼育し，照射直後から制限する照射後カロリー制限群(②)，そして，照射前後を通して生涯カロリー制限を行う群(③)の各群を設けた．①はカロリー制限により，白血病の潜在的標的細胞である造血幹細胞の数量が減ることが知られているので，いわばカロリー制限のイニシエータに対する修飾因子としての役割に注目した群と考えられるのに対して，②は，照射後にカロリー制限を行うもので，いわばカロリーというプロモータ作用を抑制した状態に注目した群と考えることができる．結果としてカロリー制限は，③の生涯カロリー制限群でもっとも白血病の発症が抑制されたことはともかくとして，②でも発症が抑制されることがわかり，イニシエータ作用の方も，プロモータ作用の方にも，抑制効果があるものと考えられた(脚注*2)．意外にも①群は，照射後，通常食に戻すことにより急速に体重は増加し，カロリー制限を行わなかった通常食群における放射線白血病頻度と有意差はみられず，抑制効果はなかったので，ここで想定したカロリーの“プロモーション効果”の方が大きいことが示される結果となった．ここでカロリー制限は，エネルギー消費の抑制として酸化の防止に役割を果たしているわけであるが，そのことを実験的に明快に示した例として，Merryの行ったマイクロアレイによる解析[23]がある．その結果によれば，組織におけるレドックス状態は，蛋白や脂質，そしてDNAの酸化産物の蓄積が顕著であることはもちろんであるが，カロリー制限などと比較して明らかになる点は，むしろ，レドックス制御による転写活性化による細胞機能により大きな差が見出されるとして，これが，加齢過程のメカニズムを解き明かすことにつながる，としている．

2. 緑茶成分

緑茶成分の抗酸化作用は，今日では知らない人がないほどであるが，緑茶のどの成分が活性酸素の吸収にどのように作用するかの詳細や，その有効量と実際の飲茶量の関係などの細かい点にはつ

*2：ところで，この③の生涯カロリー制限群の白血病頻度は抑制されたものの，この群の寿命は，最長ではなかった．発育早期の過度なカロリー制限は，健全な老年期の維持に対して負の影響を及ぼすもののようである．

きない興味がある．ちなみに緑茶は，2-nitropropane(2-NP)のような一次発癌剤[24]に対しても，pentachlorophenol(PCP)のような二次発癌剤[25-27]に対しても発癌の抑制効果が観察されている(脚注*3)．発癌用量の2-NP投与群に緑茶成分として通常の飲茶の要領で熱湯抽出した茶を飲水として飲ませた群では，顕著なトリグリセリドの低下，8-OHdG付加体の無処置群レベルまでの低下，肝臓のBrdUrd標識率の同じく無処置対照群レベルまでの低下をみている．このときの飲茶に含まれていた緑茶成分は，epigallocatechin gallate(EGCG)で822 μg/ml，総カテキン量として1,606 μg/mlであったが，その総飲水量は，1日当りEGCG換算で118 ml，カテキン換算で106 mlのお茶に相当し，通常のヒトの日常摂取量に近い値であった．発癌機構のどの部分にEGCGが関与するのかを考えてみると，これらの抗酸化成分がプレイオトロピックに発癌のさまざまな部分に関与していることがわかる[27]．

3. 実験発癌予防

魚の焼けこげから抽出された変異原物質ヘテロサイクリックアミン類と酸化ストレスの関連については先に述べた．これは，チトクローム P450 の1A2による*N*-hydroxylationと一次酵素による，*N*-acetyl transferaseによってDNA傷害物質が生成され，これがDNA-付加体の形成に関与しているものと考えられている．そこで，抗酸化物質との併用による癌予防を念頭においた精力的な実験が行われている[17]．さまざまの合成抗酸化剤のうち，この報告では，HTHQにもっとも強い癌抑制が観察されているが，興味深いことに，これらの抗酸化物質がその抗酸化作用よりも，MeIQXの代謝活性化を傷害することによって発癌抑制に作用していることがわかったという．この解釈の正否は，代謝活性化による癌原性物質の生成機構をよく研究しないとわからない点があるが，前項の茶カテキンの作用機序のところでも述べたように，抗酸化物質の癌抑制機構は，多分にプレイオトロピックに働いていることが知られており，単一のメカニズムによるとは限らない点で，研究では注意して吟味する必要がある．実験癌予防としては，TRXの過剰発現マウスによる，酸化ストレス消去モデルがある．このものは，急性の酸化ストレス障害のみならず，ベンゼンによる白血病発症を抑制し，さらに寿命の延長をももたらした(本書，平林「ダイオキシンの生体影響と防御機構」の稿参照)．

4. アリールヒドロカーボン受容体(AhR)と結合する食品

シトクローム P450の分子種1A1の発現を引き起こす転写因子としてHoffmannらおよび藤井らのグループによってそれぞれクローニングされたAhRはダイオキシン受容体とも呼ばれ，このものはダイオキシン類(TCDD)と結合するとArntとヘテロ二量体をつくり，DNAの特定領域に結合して，CYP1A1の転写の活性化を惹起する．この応答領域はXenobiotic Response Element(XRE)といい，TRXの発現調節領域にもこのXRE配列が見出されている[28]．松田らは，このAhRが，結合する生体内天然リガンドの未知なオーファン受容体であることに注目し，これを探索していく過程で，天然の生体物質としては尿中に排泄されるインディルビンがリガンドとなることを見出し[29]，さらに食品中にも多数のAhRリガンドがあることを見出した[30]．それらの中には，緑茶，ウーロン茶，コーヒー，リンゴジュース，などの飲料中に含まれているものもある．AhRの異物代謝機能は，TRXの制御を受けることが知られており[31]，これらの食品は，なんらかの形で異物代謝に関与するものと考えられるが，それが生体にとって吉の役割を持つのか，潜在的な凶の役割を持つのか，わかっていない．

おわりに

以上，レドックス制御にかかわる生体の反応機構について通覧した．各論では，いくつかの代表的なテーマに沿って，より詳細なメカニズムについて解説される．

*3：一次発癌，二次発癌については，本稿の「環境要因によるレドックス制御からの逸脱，1．電離放射線」の項参照．

文献

1) Packer, L. and Yodoi, J.(eds.)：Redox Regulation of