Review

Application of regulation of reactive oxygen species and lipid peroxidation to disease treatment

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Although many diseases in which reactive oxygen species (ROS) and free radicals are involved in their pathogenesis are known, and antioxidants that effectively capture ROS have been identified and developed, there are only a few diseases for which antioxidants have been used for treatment. Here, we discuss on the following four concepts regarding the development of applications for disease treatment by regulating ROS, free radicals, and lipid oxidation with the findings of our research and previous reports. Concept 1) Utilization of antioxidants for disease treatment. In particular, the importance of the timing of starting antioxidant will be discussed. Concept 2) Therapeutic strategies using ROS and free radicals. Methods of inducing ferroptosis, which has been advocated as an iron-dependent cell death, are mentioned. Concept 3) Treatment with drugs that inhibit the synthesis of lipid mediators. In addition to the reduction of inflammatory lipid mediators by inhibiting cyclooxygenase and leukotriene synthesis, we will introduce the possibility of disease treatment with lipoxygenase inhibitors. Concept 4) Disease treatment by inducing the production of useful lipid mediators for disease control. We describe the treatment of inflammatory diseases utilizing pro-resolving mediators and propose potential compounds that activate lipoxygenase to produce these beneficial mediators.

***Key Words*:** **antioxidant, fetal treatment, ferroptosis, specialized pro-resolving mediator, stress**

Reactive oxygen species (ROS) damage cells by oxidative modifying biomolecules (lipids, proteins, nucleic acids, etc.), resulting in disease triggers. The diseases listed in Table 1 as diseases in which ROS is involved in those pathologies have been confirmed using animal model experiments and clinical samples. On the other hand, antioxidant enzymes and antioxidants exist as molecules that protect biomolecules from damage caused by ROS. Although the involvement of ROS in the pathophysiology and development of the disease has been investigated and the usefulness of antioxidants has been demonstrated by animal experiments, antioxidants are not used as therapeutic agents in actual clinical practice. In Japan, antioxidants have been used as therapeutic agents in actual clinical practice only for non-alcoholic steatohepatitis (NASH) and cerebral infarction.[1,2] The progression of NASH has been reported to be suppressed by the administration of vitamin E (a fat-soluble vitamin with antioxidant activity), and vitamin E is listed as a therapeutic agent in the therapeutic criteria.[3] Edaravone, an antioxidant, is administered intravenously for the purpose of protecting the brain from neuronal cell death caused by ROS generated by injury due to cerebral infarction and ischemia-reperfusion injury after resumption of blood flow. In Japan, edaravone is administered to about half of the cases with acute cerebral infarction, and the effect of improving neurological symptoms has been confirmed, but the effect is limited.[4] Recently, edaravone has been approved for use in amyotrophic lateral sclerosis,[5] and the therapeutic effect will be reported in the future.

Although it has been demonstrated that ROS is involved in the pathophysiology of many diseases, and various antioxidants that can suppress the toxicity of ROS by efficiently capturing ROS are known, antioxidants are not widely used in actual clinical practice. Niki[6] explained this problem with the following six possible reasons. #1. Oxidative events are the result of diseases, not the cause. #2. Multiple antioxidants with different functions are required because multiple oxidants with different reactivity and selectivity contribute to the etiology. #3. Healthy subjects who already have sufficient antioxidants may have limited beneficial effects by supplemented antioxidants. #4. Appropriate antioxidant selection, dosage, and duration of supplementation are necessary. #5. Choice of clinical trials and endpoints to be included in the meta-analysis. #6. Oxidative stress may be pivotal for the onset of the disease, but it may become less important at the later stages of the disease.

This manuscript describes four concepts regarding application to disease treatment by controlling ROS, free radicals, and lipid oxidation. The first section of this article refers to the use of antioxidants as disease prophylaxis, based on the findings we have previously reported in experiments with Down syndrome model mice. In addition, the second section explains the concept of utilizing ROS itself for treatment. Lipid oxidation products produced via lipid oxidation enzymes have a pharmacological action as a lipid mediator. We describe how to apply suppression or activation of lipid mediator production to the treatment of disease.

Disease Treatment by Suppressing Oxidative Stress by Antioxidants

Antioxidants used in clinical practice. Antioxidants and antioxidant enzymes are classified into the following three types according to their action:[6,41,42] a) preventive antioxidants;

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Table 1. Diseases involving reactive oxygen species and free radicals

Brain/neurological disorders	cerebral infarction,[4] diabetic neuropathy,[7] Alzheimer's disease/dementia,[8–11] Parkinson's disease[12,13]
Respiratory diseases	pneumonia/infections,[14] smoking,[15] bronchial asthma,[16] chronic obstructive pulmonary disease[17]
Cardiovascular diseases	myocardial infarction,[18] arteriosclerosis,[19] ischemia-reperfusion injury,[18] heart failure,[20] cardiomyopathy[21]
Gastrointestinal disorders	gastric ulcer,[22] non-alcoholic steatohepatitis,[3] inflammatory bowel disease/ulcerative colitis[23]
Endocrine disorders	diabetes,[24] hyperlipidemia[25]
Urologic diseases	chronic nephritis, diabetic nephropathy,[26] nephrotic syndrome[27]
Ophthalmic disorders	cataract,[28] glaucoma,[29] age-related macular degeneration,[30] diabetic retinopathy,[24] retinopathy of prematurity,[31] dry eye[32]
Other diseases	cancer,[33] atopic dermatitis,[34] sunlight dermatitis,[35] chronic granulomatosis,[36] rheumatoid arthritis,[37] systemic lupus erythematosus,[37] chronic fatigue syndrome,[38] aging,[39] stains/wrinkles[40]

compounds that exert antioxidant action by suppressing radical generation such as catalase, peroxidase, and superoxide dismutase (SOD), b) radical-scavenging antioxidants; antioxidants that act by capturing generated radicals and suppress chain initiation such as vitamin E, vitamin C, polyphenols, carotenoids, uric acid, and ubiquinol, c) repair and *de novo* enzymes; compounds that repairs and regenerates damaged molecules such as phospholipase, protease, and DNA repair enzymes. Among these antioxidants, this section specifically discusses the clinical application of radical scavengers.

Among the clinical drugs used in Japan, compounds having an antioxidant activity include vitamin E, vitamin C (hemostatic drug), glutathione (GSH) (liver protection agent), probucol (hyperlipidemic drug), edaravone (treatment for cerebral infarction), rebamipide (protective drug for gastric mucosa), polaprezinc (medicine for gastric ulcer), and pravastatin (medicine for hyperlipidemia). Among these drugs, vitamin E and edaravone are used for their antioxidant properties.

Non-alcoholic fatty liver disease (NAFLD). NAFLD is a pathological condition that can be said to be the expression form of metabolic syndrome in the liver. The prevalence rate of NAFLD is 20–40% in Europe and the United States, 12–30% in Asian countries, and 9–30% in Japan, and the number of patients is extremely large worldwide. In a narrow sense, NAFLD is classified into NASH, which is a progressive liver disease, and non-alcoholic fatty liver (NAFL), which has a good prognosis. The "two-hit hypothesis" has long been proposed as the pathological mechanism of NASH/NAFLD.[43] According to this theory, fatty acids and triglycerides accumulate in the liver (1st hit), making the liver susceptible to external stimuli. In this state, factors that damage hepatocytes such as oxidative stress, lipid peroxidation, and insulin resistance are added as the 2nd hit, and as a result, hepatocyte necrosis and apoptosis progress with inflammation of the liver parenchyma. On the other hand, in recent years, "multiple parallel hits" hypothesis has also been proposed.[44] It is hypothesized that inflammation induced in the liver by many factors such as cytokines derived from adipose tissue and intestinal tract, and alterations in gut microbial functions occurs at the same time as or prior to fatty degeneration and promote NAFLD. In both theories, oxidative stress is involved as a trigger for inflammation and fibrosis in the liver. Therefore, vitamin E is administered as an antioxidant to patients with NAFLD/NASH. In a pilot study, vitamin E was reported to improve e blood biochemical examinations in pediatric NASH patients in the United States.[1] Subsequently, the effect of vitamin E on NASH was confirmed from various institution.[45] The current guideline by the American Association for the Study of Liver Disease (AASLD) has recommended the use of vitamin E in patients with biopsy-proven NASH and without diabetes.[46]

Cerebral infarction. Many patients with cerebral infarction are left with impaired intelligence or physical function as a prognostic symptom. Neurological symptoms such as motor paralysis due to cerebral infarction cannot be expected to improve in the chronic phase, so treatment in the acute phase is important. If blood flow is reperfused at an ischemic region in the brain at an early stage after the onset, recovery of brain function is expected, but if ischemia continues for several hours or more, irreversible neuronal damage occurs. Around the central core of ischemia that has fallen into irreversible changes, there is a region called "penumbra" that survives after ischemia. In this region, cell death is known to occur even if blood flow resumes, which is called "delayed neuronal cell death". Various factors are thought to contribute to this neuronal damage, including the release of excitatory amino acids, the influx of Ca^{2+} into nerve cells, and the production of free radicals. In the ischemic condition, the production of free radicals increases due to the enhancement of the arachidonic acid metabolic system, and the peroxidation of unsaturated fatty acids in the cell membrane causes membrane damage, resulting in worsening of cerebral ischemic injury.[47] The treatment options for the acute phase of cerebral infarction are 1) reperfusion therapy before irreversible brain damage occurs, and 2) brain protection therapy to minimize nerve cell death. Edaravone, which has radical scavenging and lipid peroxidation inhibitory properties, has been developed and used clinically as a drug exhibiting this brain protective effect.[48] Animal studies have also demonstrated that edaravone rapidly crosses the blood-brain barrier.[49] However, according to the results of the YAMATO study, a multicenter prospective randomized controlled trial conducted by Aoki *et al.*[50], edaravone administered before or simultaneously with tissue plasminogen activator (tPA) in the acute phase of cerebral infarction did not significantly improve the early resumption rate or functional prognosis compared with edaravone administration after tPA. These results clarified that the effect of edaravone has a limited on the sequelae of cerebral infarction. On the other hand, the PROTECT 4.5 study conducted in Japan reported an improved incidence of sequelae and symptomatic intracranial hemorrhage when edaravone and tPA were administered within 4.5 h after the onset of cerebral infarction.[2] It is suggested that edaravone cannot restore nerve cells that have already been damaged to irreversible levels by free radicals generated by ischemia. In order to protect neurons from damage caused by cerebral infarction by using antioxidants, it is considered necessary to start administration of antioxidants very early in the onset of ischemia.

Down syndrome. There is no consensus on the effects of antioxidants on Down syndrome. Chromosome 21 is trisomy in Down syndrome. The SOD gene is encoded in chromosome 21, and SOD is expressed 1.5-fold in Down syndrome.[51,52] On the other hand, the expression of catalase and glutathione peroxidase is not increased, indicating that the degradation of hydrogen peroxide produced via SOD is insufficient. The results suggested that tissue injury caused by oxidative stress is related to the pathogenesis of Down syndrome. Increased lipid oxidation product (8-iso-prostagrandin F2α and TBARS) have been reported in the urine of patients with Down syndrome.[53] Following these reports, several clinical studies on the adminis-

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tration of antioxidants to patients with Down syndrome were conducted.[54] Ellis *et al.*[54] administered antioxidants (selenium, vitamin E, vitamin C, vitamin A, and folic acid) to infants with Down syndrome around 4 months of age, but developmental index after 18 months was not improved. Since chromosomal trisomy is associated with the pathology of Down syndrome, it is presumed that this pathology affects cells after fertilization. In fact, lipid oxidation product (8-isoprostane) in amniotic fluid at 16 weeks gestation were 9 times higher in pregnant women who were pregnant with Down syndrome fetuses than in pregnant women who are pregnant with normal fetuses.[55] Analysis using the fetal brains at 18–20 weeks gestation also showed that lipid oxidation products were significantly higher in the brains of Down syndrome fetuses than in normal fetal brains.[56] These results suggest that excessive ROS are generated from the fetal period in Down syndrome.

Therefore, we investigated whether administration of α-tocopherol, the most active form of vitamin E, from the fetal period of Down syndrome affects brain development in Ts65Dn mice, a mouse model of Down syndrome.[57] Female Ts65Dn mice were fed an α-tocopherol-supplemented diet from premating until the newborns were weaned (Fig. 1). As a result, α-tocopherol is administered transplacentally to fetal mice and via breast milk to infant mice. After weaning, infant mice were orally fed an α-tocopherol-supplemented diet, and behavioral experiments and brain tissue collection were conducted at 10 weeks of age. In the Morris water maze test, Ts65Dn mice exhibited marked learning disabilities, but administration of α-tocopherol from the fetal period was able to significantly improve learning disabilities. In addition, lipid oxidation products were increased in the hippocampus of Ts65Dn mice, and the number of neurons in the hippocampal dentate gyrus was decreased in the tissue section. α-Tocopherol reduced lipid peroxidation products in the hippocampus and recovered the decreased number of neurons in the hippocampal dentate gyrus. These results suggest that the administration of α-tocopherol from the fetal period may have a therapeutic effect on the intellectual disability of Down syndrome. Since the results of our study were obtained using model mice, careful clinical researches are required to determine the effect of α-tocopherol administration from the fetal period on human Down syndrome.

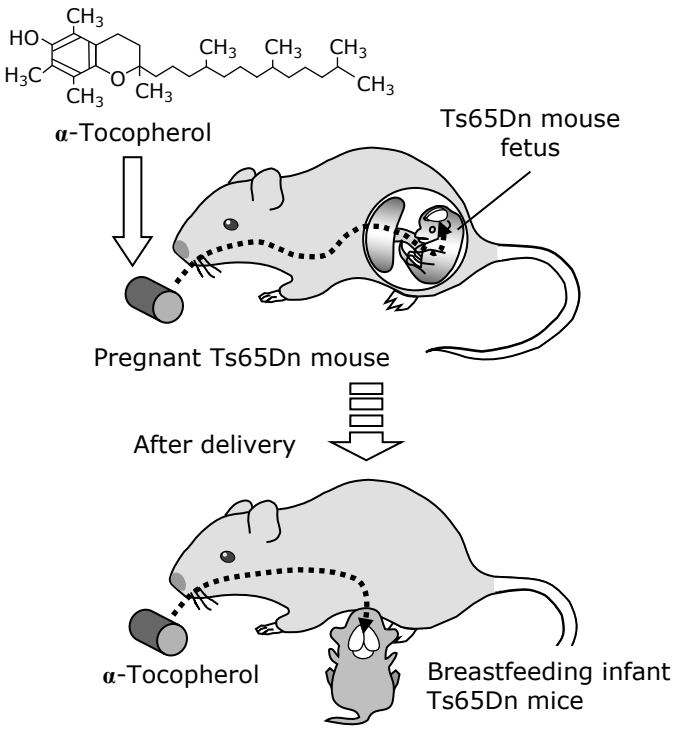

Fig. 1. Fetal treatment with antioxidants in Down syndrome model mice.

In recent years, there is a concept of fetal treatment in which drugs are administered to pregnant women to exert an effect on the fetus. For example, corticosteroids are administered for congenital cystic adenomatous malformation and antiarrhythmic agents are administered for fetal tachyarrhythmia. There is a possibility of fetal treatment by administration of antioxidants for Down syndrome. The effects of fetal treatment with epigallocatechine gallate,[58] the vasoactive intestinal peptides NAPVSIPQ/SALLRSIPA,[59] the selective serotonin re-uptake inhibitor fluoxetine,[60] and apigenin have been investigated in experiments using Down syndrome model mice.[61] Among these compounds, epigallocatechine gallate is a compound having an antioxidant effect. Careful consideration is required for the clinical application of fetal treatment for Down syndrome using vitamin E and these antioxidants.

The report that edaravone is effective for cerebral infarction at an extremely early stage and our results using Down syndrome model mice suggest that starting antioxidants at a very early stage before the progression of ROS-related pathologies may increase the effectiveness of antioxidants. In general, antioxidants are known to be effective when administered prophylactically. Antioxidants have the function of capturing ROS and radicals, but they are not effective in recovering damaged cells and restoring normal functions. On the other hand, the reason why it is effective to start the administration of vitamin E after the onset of NAFLD is considered to be due to the high regenerative capacity of hepatocytes. It is speculated that even if damaged hepatocytes die, the regeneration of hepatocytes is promoted by capturing ROS generated in the newly regenerated hepatocytes by antioxidants.

In order to start administration of antioxidants before the onset of the disease, it is necessary to develop methods to predict the risk of disease onset. In the case of Down syndrome, prenatal diagnosis allows diagnosis at the fetal stage. However, in the case of diseases associated with oxidative stress, such as Alzheimer's disease and diabetes, markers that predict disease onset are needed to determine when to start antioxidant administration.

Disease Treatment by Inducing Production of ROS and Radical Oxidation

Excessive production of ROS causes various diseases, while ROS is effectively utilized for immune function and infection protection. Neutrophils that phagocytose pathogens produce superoxide ($O_2^{\bullet-}$) via NADPH oxidase, and hydrogen peroxide (H_2O_2) is generated non-enzymatically from $O_2^{\bullet-}$.[62] Furthermore, hypochlorous acid (HOCl) is produced from H_2O_2 and chloride ion (Cl^-) by a reaction catalyzed by myeloperoxidase.[62] HOCl is not a radical but one of the ROS. Congenital abnormalities of NADPH oxidase lead to dysregulation of the immune response, resulting in chronic granulomatous disease with a prolonged excessive inflammatory response.[36]

Molecular mechanism of ferroptosis and ferroptosis-inducing compounds. Recently, the idea of treating cancer by inducing the production of ROS and radicals has begun to emerge. Various regulatory mechanisms other than apoptosis have been shown to exist in necrosis-like cell death, which has been considered to be unregulated cell death.[63] Ferroptosis was found by Dixon *et al.*[64] as a novel regulated cell death through the study of Erastin, which was identified in a therapeutic drug screening of for Ras mutation-positive cancer. In ferroptosis, a chain reaction of intracellular iron-mediated phospholipid peroxidation induces cytotoxicity and cell death. Various intracellular networks that regulate ferroptosis have been reported, indicating

that cell death has a complicated and sophisticated mechanism.[65] The main pathways regulating ferroptosis are as follows: 1) the reduction of phospholipid peroxidation product by glutathione peroxidase 4 (GPX4) and GSH, which is GPX4 substrate,[66] 2) the amount of free iron unbound to intracellular proteins,[65] 3) the amount of polyunsaturated fatty acid (PUFA)-containing phospholipids,[67] and 4) the reduced state of CoQ10, which functions as a coenzyme in the mitochondrial electron transfer system.[68] System x_C^- exchanges intracellular GSH for extracellular cystine at a 1:1 molar ratio.[69] After being transferred to cells, cystine is quickly reduced to cysteine and then used for GSH synthesis. GSH is an important antioxidant and free radical scavenger. GPX4 is a peroxide-degrading enzyme and GSH is an essential cofactor for its activation. Ferroptosis is induced by inhibition of GPX4 and depletion of glutathione; but antioxidants such as vitamin E, CoQ10, and selenium act suppressively on ferroptosis. It has also been reported that GPX4 and vitamin E cooperate to prevent the degeneration of hepatocytes and hematopoietic stem cells due to ferroptosis.[70,71] Ferroptosis is attracting attention as a new therapeutic strategy for malignant tumors. The cancer cell types for which the effectiveness of induction of ferroptosis has been verified in cell experiments or animal experiments are as follows: breast cancer, head and neck cancer, acute myeloid leukemia, pancreatic cancer, ovarian cancer, hepatocellular carcinoma, osteosarcoma, prostate adenocarcinoma, B cell lymphoma, renal cell carcinomas, non-small cell lung cancer, and glioblastoma.[72] In order to induce ferroptosis, it was investigated that induction of GSH depletion via inhibition of cystine uptake by Erastin and its derivatives,[64,73,74] sulfasalazine,[75] glutamate,[76] and sorafenib.[73] Ferroptosis is also induced through GPX4 inhibition by (1S,3R)-RSL3,[66] ML162.[77,78] Table 2 lists compounds having a ferroptosis-inducing effect reported previously.

The effect of suppressing the reproduction of Plasmodium malaria by inducing vitamin E depletion. Induction of ferroptosis by suppression of GPX4 and GSH has been investigated,[66,77,78,81–83] but depletion of vitamin E (tocopherols and tocotorienols) may also be effective in inducing ferroptosis. Because vitamin E is a nutrient abundant in foods, it is generally considered difficult to induce vitamin E depletion, and it is difficult to clinically utilize vitamin E depletion to induce ferroptosis.

On the other hand, we had reported that probucol, a therapeutic agent for hyperlipidemia, can suppress circulating blood vitamin E levels in mice, thereby preventing death of mice after malaria infection.[91] Probucol can inhibit vitamin E secretion from the liver to the circulation by inhibiting ATP binding cassette transporter A1 (ABCA1).[92] It has been reported that mice genetically depleted of vitamin E acquire resistance to malaria infection.[93] Plasmodium malaria is known to lack some of the important antioxidant enzymes such as GPX and catalase.[94] In addition, Plasmodium malaria should be exposed to ROS because this parasite parasitizes in iron-rich red blood cells. Reducing circulating vitamin E in the host mouse may have increased the oxidative stress generated in the malaria parasite and inhibited the reproduction of plasmodium malaria. In other words, the reduction of vitamin E due to probucol induced ferroptosis to malaria parasites in erythrocytes.

Furthermore, artemisinin, used as a first-line antimalarial drug, has an endoperoxide in the molecule and is known as a compound that induces ferroptosis.[85] In our experiments, the combined administration of probucol and artemisinin (dehydroartemisinin) showed a remarkable synergistic effect.[91,95]

Probucol is also known as an antioxidant compound,[96] but its antioxidant activity is weaker than that of vitamin E.[97] It was speculated that probucol could not suppress the lipid oxidation caused by decreased vitamin E in plasma. The effect of probucol was also confirmed in monkeys,[98] especially a reduction of vitamin E content and an increase in lipid peroxidation products were shown in erythrocytes. Furthermore, it has been shown that the reduction of vitamin E and the increase in lipid oxidation in plasma and erythrocytes due to probucol administration were restored to the initial levels by withdrawal of probucol.[91] Probucol can be used to temporarily make the condition more susceptible to ferroptosis.

Induction of vitamin E depletion by probucol administration may provide a new tool for anticancer therapeutic strategies utilizing induction of ferroptosis. Combination therapy of the ferroptosis-inducing compound shown in Table 2 and the probucol that can induce vitamin E depletion may synergistically induce ferroptosis.

Table 2. Ferroptosis inducers

Targets	Compound	Inducing mechanisms of ferroptosis	References
System x_c^-	Erastin, Erastin2	Inhibits system x_c^-, Erastin 2 is a potent Erastin analog	(64,73,74)
	Sulfasalazine	Inhibits system x_c^-, prodrug of 5-acetylsalicylic acid	(75)
	Lanperisone	Inhibits the absorption of cyctine and depletes GSH	(79)
	5-Octyl D-Glutamate	Increases intracellular glutamate	(76)
	Sorafenib	Inhibits the absorption of cyctine and depletes GSH	(73)
	Metformin	Inhibits system x_c^- expression	(80)
GPX4	(1S,3R)-RSL3	Inhibits GPX4 by directly binding to active site	(66)
	ML-162, ML-210	Inhibits GPX4 stronger than (1S,3R)-RSL3	(77,78)
	FIN56	Promotes degradation of GPX4	(81)
GSH	Acetaminophen	Parent compound of NAPQI, which lowers intracellular glutathione levels	(82)
	N-acetyl-4-bezoquinone Imine (NAPQ1)	Conjugates with GSH during metabolic process	(82)
	L-Buthionine-(S,R)-Sulfoximine (BSO)	Depletes GSH by inhibiting the enzyme of GSH synthesis	(83)
	Cisplatin	Reduces GSH	(84)
Others	Artemisinin, Artesunate	Generates ROS upon cleavage of their endoperoxide bridge	(85)
	Siramesine, Lapatinib	Inhibits the iron transport system	(86)
	Ferumoxytol	Produces ROS	(87)
	Salinomycin (ironomycin)	Induces a rapid degradation of the iron storage protein ferritin	(88)
	Fenugreek (trigonelline)	Inhibits NRF2	(89)
	Chlorido[*N*,*N*'-Disalicylidene-1,2-Phenylenediamine]iron(III)	Complex containing Fe^{3+}	(90)

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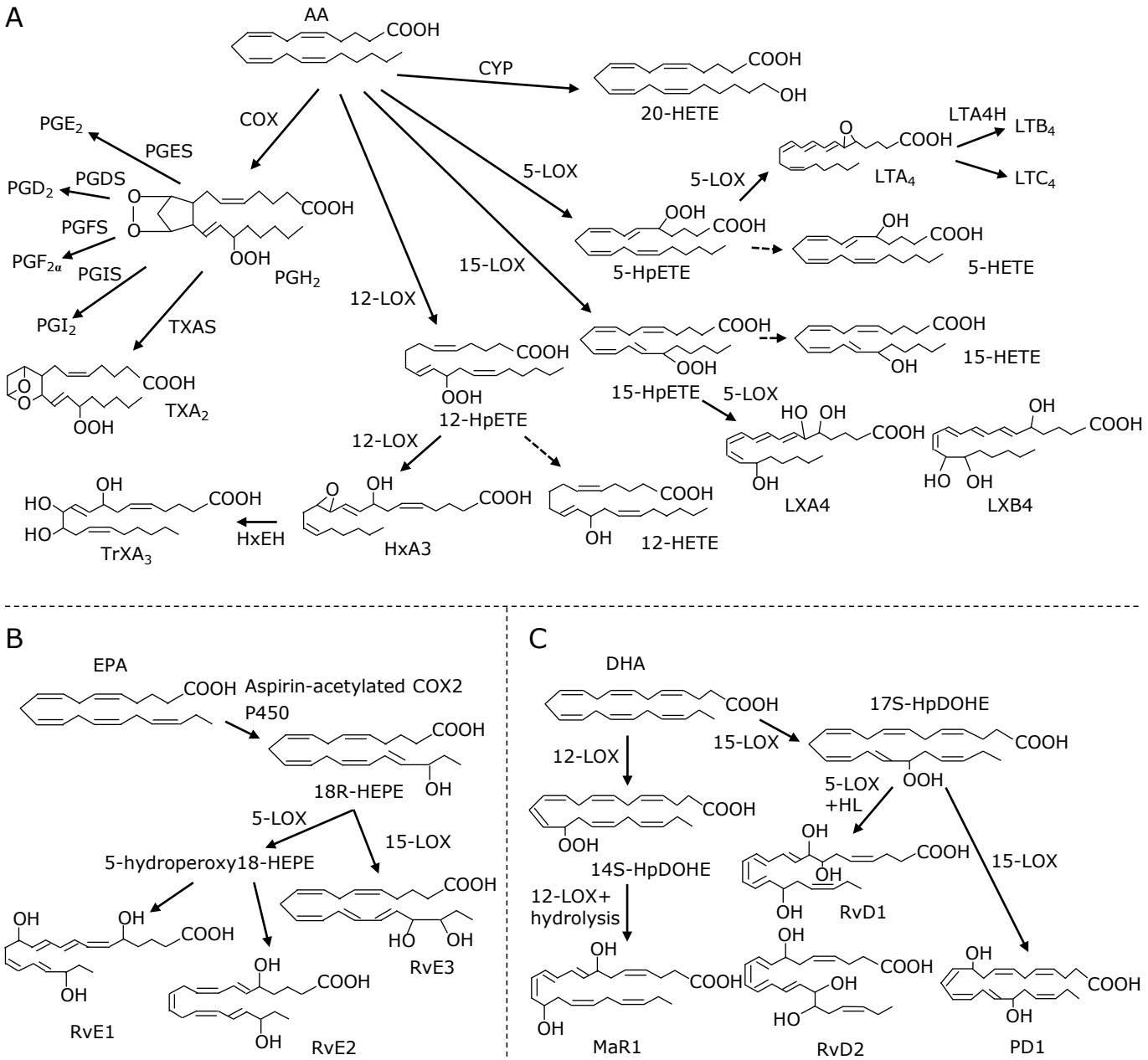

Fig. 2. The synthetic pathways of lipid mediators. Biosynthetic pathways of lipid mediators derived from AA (A), EPA (B), and DHA (C). COX, cyclooxygenase; CYP, cytochrome P450; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HL, hydrolase; HpDOHE, hydroperoxydocosahexaenoic acid; HpETE, hydroperoxyeicosatetraenoic acid; Hx, hepoxlin; HxEH, hepoxlin epoxide hydrolase; LOX, lipoxygenase; LTA4H, leukotriene A4 hydrolase; LX, lipoxin; MaR, maresin; PD1, protectin D1; PGD_2, prostaglandin D_2; PGDS, prostaglandin D synthase; PGE_2, prostaglandin E_2; PGES, prostaglandin E synthase; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGFS, prostaglandin F synthase; PGI, prostaglandin I; PGIS, prostaglandin I synthase; Rv, resolvin; TrX, trioxilin; TXA_2, thromboxane A_2; TXAS, thromboxane A synthase.

Disease Treatment by Suppressing Lipid Mediators Pproduced by Enzymatic Oxidative Reactions

Lipid mediators involving lipid oxidases. Lipids are known to be oxidized by ROS as well as oxidatively modified by enzymes, and some of the lipid oxidation products produced by these lipid oxidases have pharmacological functions as lipid mediators. Arachidonic acid (AA), an omega-6 (*n*-6) PUFA, is oxidatively modified by cyclooxygenase (COX) to produce prostaglandins (PGs) and thromboxanes (TXs) (Fig. 2A). 5-Lipoxygenase (5-LOX) produces leukotrienes (LTs) from AA (Fig. 2A). These processes are called the arachidonic acid cascade, and PGs, TXs, and LTs act primarily as lipid mediators that induce inflammatory responses. On the other hand, lipoxins (LXs) are produced from AA by enzymatic oxidation of 15-LOX and 5-LOX (Fig. 2A). LOXs metabolizes eicosapentaenoic acid (EPA), an omega-3 (*n*-3) PUFA, into E-series resolvins (Rvs) (Fig. 2B), and docosahexaenoic acid (DHA) into maresins (MaRs), D-series Rvs, and protectins (PDs) (Fig. 2C). LXs, Rvs, MaRs, and PDs involves in the resolution of inflammation and infection are termed specialized pro-resolving mediators.[99] LXs inhibit allergic responses.[100] Rvs are involved in the elimination of infection and restoration of injured tissue.[101] MaRs are related to wound healing and neuropathic pain reduction.[102,103] PDs

have anti-apoptotic,[104] neuroprotective,[105] and antiviral function.[106] 12-LOX produces hepoxilins (Hxs) and trioxilins (TrXs) from AA (Fig. 2A), and the biological roles of Hxs and TrXs are involved in the regulation of insulin secretion and lipid metabolism.[107]

Inhibitors of lipid mediator synthesis. In 1971, Vane *et al.*[108] discovered that acetylsalicylic acid and indomethacin suppress the PGs synthesis and are known to inhibit the enzymatic activity of COX. Furthermore, the development of PGs synthesis inhibitors with reduced side effects has been promoted. These drugs are used as non-steroidal anti-inflammatory drugs (NSAIDs) to treat rheumatoid arthritis and to suppress inflammatory reactions such as acute pain and fever. Because LTs are associated with bronchial asthma and allergies, inhibitors of LTs have been developed. Inhibitors (pranlukast and montelukast) that antagonize LTs (LTC4, LTD4, and LTE4), which have cysteine residues in their structures, are currently used as therapeutic agents for bronchial asthma and bronchitis caused by respiratory syncytial virus.[109] Inhibitors of 5-LOX (such as zileuton), which is involved in the production of precursors [5-hydroperoxyeicosatetraenoic acid (5-HpETE)] during LT biosynthesis, are also under development. Although 5-LOX inhibitors have been studied, there are no clinically applicable inhibitors for 12-LOX and 15-LOX, whose physiological functions are not well understood. 20-Hydroxyeicosatetraenoic acid (20-HETE) is produced from arachidonic acid by the enzymatic reaction of cytochrome P450 (CYP450) (Fig. 2A). 20-HETE is known to induce cancer cell proliferation and neovascularization through animal experiments, and studies on the anticancer activity of a CYP450 selective inhibitor (HET0016) have been reported.[110]

Stress induces 12-LOX activity, and inhibition of 12-LOX improves behavioral disorders. We reported that 12-HETE was significantly increased in the plasma of mice by exposure to the water immersion restraint (WIR) stress, which is used in the experiments of stress-induced gastric ulcer.[111] In mice, 12/15-LOX, which has both 12-LOX and 15-LOX activity, is expressed in leukocytes.[112] Exposure of WIR stress to mice lacking 12/15-LOX in leukocytes did not increase 12-HETE in plasma. These results suggest that 12/15-LOX is involved in stress-induced production of 12-HETE. To analyze the physiological function of 12-HETE in stress, a tail suspension test was performed immediately after exposure to WIR stress. Wild-type mice exposed to WIR stress showed a marked elongation in struggling time, whereas 12/15-LOX-deficient mice did not. Increased struggling time is similar to panic behavior in stressful situations. In addition, administration of 12-HpETE (a primary metabolite derived from arachidonic acid via enzymatic oxidation of 12/15-LOX) to stress-exposed 12/15-LOX-deficient mice extended the struggling time. Analysis of monoamines in brain tissue showed that 12-HETE is involved in noradrenaline secretion in the hypothalamus and cerebral cortex. These results suggest that 12-HETE produced by stress-induced activation of 12/15-LOX releases noradrenaline in brain tissue, resulting in panic disorder-like behavior.

Furthermore, 2-week oral pre-administration of tocotrienols, which has been reported to have an inhibitory effect on 12/15-LOX, suppressed the increase in 12-HETE production due to WIR stress and improved stress-induced panic disorder-like behavior. There was also a report that tocotrienols showed significant improvement in composite memory and verbal memory in Japanese adult subjects (around 55 years of age) when taken simultaneously with astaxanthin, and this report indicated that tocotrienols are safe for human administration.[113] These results shed light on the elucidation of the physiological function of 12-HETE, whose function has not been elucidated, and suggest that suppressing 12-LOX enzyme activity may be possible to control stress-induced behavioral disorders. Further research is needed on the association of 12-HETE in stress states and stress-related disorders [post-traumatic stress disorder (PTSD) and panic disorder].

Disease Treatment Strategies by Inducing the Production of Lipid Mediators

While the previous chapter mentioned the therapeutic method using inhibitors of enzymes that promotes the production of lipid mediator, this chapter discusses the therapeutic application of anti-inflammatory lipid mediators themselves and promoting the activity of the enzymes that synthesize the mediators. Since LXs, Rvs, MaRs, and PDs have anti-inflammatory functions, their effects on inflammatory bowel disease, respiratory infections, allergic inflammation, etc. have been verified in animal experiments, and many studies have been reported as shown in Table 3. Each of these pro-resolving mediators is known to exert their effects by binding to one or more receptors (G-protein-coupled receptors).[114] Because these mediators are strong endogenous ligands and have shown great potential in preclinical animal studies, there is interest in developing stable analogs that prevent metabolic inactivation.[114]

We have found a compound that activate an enzyme involved in the production of lipid mediators. The soy isoflavone daidzein was found to induce 5-LOX activation to produce 5-HETE.[142] Furthermore, 5-HETE which was increased in MDCK cells by daidzein administration, inhibited influenza virus replication in the cells.[142] Morita *et al.*[106] reported that PD1 generated from DHA by the enzymatic activity of 15-LOX inhibited influenza virus proliferation, and in our results, similar activity was observed with 5-HETE produced from arachidonic acid via 5-LOX. As mentioned previously, several inhibitors against 5-LOX have been developed so far. The enzymatic activity of 5-LOX is known to be induced by inflammation and allergy reaction, however, to the best of our knowledge, no exogenous compounds that can activate 5-LOX activity have been reported. Because 5-HETE and PD1 are unstable and easily metabolized compounds, their use in treatment of influenza infection is considered diffi-

Table 3. Diseases for which the therapeutic efficacy of pro-resolving mediators is being investigated by animal experiments

Mediators	Diseases (reference)
Lipoxins	ischemic stroke,[115] Alzheimer's disease,[116] multiple sclerosis,[117] gram-negative bacterial pneumonia,[118] pneumococcal pneumonia,[119] allergic rhinitis and asthma,[100] hyperalgesia,[120] periodontitis[121]
Resolvin E1	Alzheimer's disease,[116] myocardial infarction,[122] depression,[123] asthma,[124] inflammatory bowel disease,[125] herpes simplex virus-induced ocular inflammation,[126] psoriatic dermatitis,[127] contact hypersensitivity of skin[128]
Resolvin D1	depression,[129] emphysema,[130] *E. coli*-induced pneumonia,[118] pneumococcal pneumonia,[119] non-alcoholic steatohepatitis,[131] kidney stones,[132] diabetic wounds,[133] oral squamous cell carcinoma[134]
Maresin	Alzheimer's disease,[135] cerebral ischemia/reperfusion injury,[136] asthma,[137] inflammatory bowel disease,[130] diabetic nephropathy,[138] skin inflammation by UVB[139]
Protectins	epilepsy,[140] influenza infection,[106] kidney stones,[132] wound healing[141]

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cult. On the other hand, if the mechanism of 5-LOX activation by daidzein is elucidated and compounds that accelerates the production of lipid mediators via induction of LOX activity are developed, they may become new therapeutic agents for influenza. However, activation of 5-LOX also leads to an increase in LTs, which may result in worsening allergic symptoms. When 5-LOX activators are used to treat disease, combination use of LT synthase inhibitors may be required. Further studies are needed on the method of utilizing the lipid oxidase inducer.

Conclusion

The essential points of each concept are summarized below.

1) Utilization of antioxidants for disease treatment; when antioxidants are administered to treating diseases in which ROS or free radicals are involved in the disease pathology, the target organ of the disease must be in a regenerable or repairable state. In order to start the administration of antioxidants prophylactically at the very early stage of disease, markers that predict the disease need to be developed. Alternatively, if the patient is at high risk of disease, such as age-related diseases, prophylactic administration of antioxidants is a good situation.

2) Treatment strategy using ROS and free radicals; methods of treating cancer with drugs that induce ferroptosis are being developed, and the ferroptosis inducers have the ability to inhibit the antioxidant enzyme GPX4 and to induce GSH depletion. Combination use of drugs that can reduce α-tocopherol may enhance the effects of ferroptosis inducers.

3) Treatment with drugs that inhibit the synthesis of lipid mediators; in addition to the conventional treatment of inflammatory diseases with COX inhibitors and asthma treatment with LT synthetase inhibitors, there is potential for the treatment of cancer by CYP inhibition and stress-related diseases by 12-LOX inhibition.

4) Disease treatment by inducing the production of lipid mediators useful in disease control, including pro-resolving mediators; the development of compounds that induce the activation of lipid mediator synthases such as LOXs may lead to new disease treatment strategies.

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Abbreviations

AA	arachidonic acid
ABCA1	ATP binding cassette transporter A1
COX	cyclooxygenase
CYP450	cytochrome P450
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
GPX4	glutathione peroxidase 4
GSH	glutathione
HETE	hydroxyeicosatetraenoic acid
HpETE	hydroperoxyeicosatetraenoic acid
Hx	hepoxilin
LOX	lipoxygenase
LT	leukotriene
LX	lipoxin
MaR	maresin
NAFL	non-alcoholic fatty liver
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NSAID	non-steroidal anti-inflammatory drug
PD	protectin
PG	prostaglandin
PTSD	post-traumatic stress disorder
PUFA	polyunsaturated fatty acid
ROS	reactive oxygen species
Rv	resolvin
SOD	superoxide dismutase
tPA	tissue plasminogen activator
TrX	trioxilin
TX	thromboxane
WIR	water immersion restraint

Conflict of Interest

No potential conflicts of interest were disclosed.

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