Age-related decrease in 15-lipoxygenase contributes to reduced vasorelaxation in rabbit aorta

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Tang X, Aggarwal N, Holmes BB, Kuhn H, Campbell WB. Age-related decrease in 15-lipoxygenase contributes to reduced vasorelaxation in rabbit aorta. Am J Physiol Heart Circ Physiol 294: H679–H687, 2008. First published November 30, 2007; doi:10.1152/ajpheart.01053.2007.—Rabbit 15-lipoxygenase-1 (15-LO-1) oxygenates arachidonic acid (AA) into 15-hydroperoxyeicosatetraenoic acid, which is then converted to the vasodilatory 15-hydroxy-11,12-epoxyeicosatrienoic acid (HEETA) and 11,12,15-trihydroxyeicosatrienoic acid (THETA). We studied the age-dependent expression of the 15-LO-1 in rabbit aorta and its effects on the synthesis of THETA, HEETA, and vasoactivity. Aortas of 1-wk-old rabbits express greater amounts of 15-LO-1 mRNA and protein compared with aortas of 4-, 8-, or 16-wk-old rabbits. The synthesis of THETA and HEETA in the rabbit aorta was also reduced with age. THETA synthesis was maximal in 1-wk-old aortas but decreased in aortas of 4- (42%), 8- (4%), and 16-wk-old (1%) rabbits. Similarly, THETA and HEETA synthesis decreased with age in mesenteric arteries from 1-, 4-, 8-, and 16-wk-old rabbits. The maximum vasorelaxation response to acetylcholine (10⁻⁶ M) in the presence of indomethacin (Indo) and nitro-L-arginine decreased in the order of 1 wk (64.5 ± 6.9%), 4 wk (52.6 ± 8.9%), 8 wk (53.0 ± 9.4%), and 16 wk (33.3 ± 6.6%). Similarly, the maximum relaxation to AA (3 × 10⁻⁴ M) decreased with age in the order of 1 wk (60.4 ± 8.9%), 4 wk (56.3 ± 5.8%), 8 wk (41.8 ± 12.3%), and 16 wk (28.9 ± 1.6%). In contrast, the vasorelaxation to sodium nitroprusside was not significantly altered by age. These data indicate that aortic 15-LO-1 expression and activity are downregulated with aging in rabbits. This decrease is paralleled by the reduced synthesis of vasoactive THETA and HEETA and aortic relaxations to acetylcholine and AA.

endothelium-derived hyperpolarizing factor; arachidonic acid; acetylcholine; vasodilation

ENDOTHELIAL CELLS SYNTHESIZE and release many factors that control vascular tone in response to stimulation with acetylcholine (ACh), bradykinin, and other hormones as well as by shear stress (11). In rabbit aortic endothelial cells, arachidonic acid (AA) is metabolized through cyclooxygenase (COX), lipooxygenase (LO), and cytochrome P-450 pathways into bioactive eicosanoids that may contribute to these vascular responses (6, 28, 30, 39). The rabbit aorta relaxes to ACh and AA in the presence of indomethacin (Indo) and nitro-L-arginine (NLA), which block COX and nitric oxide (NO) synthase (NOS), respectively (6, 7, 36, 39). These relaxation responses are blocked by 15-LO-1 inhibitors, such as nordihydroguaiaretic acid (NDGA), cinnamyl-3,4-dihydroxy-α-cyanocinnamate, and ebselen (7, 27). The 15-LO pathway oxygenates AA to 15(S)-hydroperoxyeicoso-(5Z, 8Z, 11Z, 13E)-tetraenoic acid, which may be further converted to 15-hydroxy-11,12-epoxyeicosatrienoic (EET) acid (HEETA) and 11,12,15-trihydroxyeicosatrienoic acid (THETA). HEETA and THETA have been described as potent vasodilators (31) that implicate aortic 15-LO-1 in the regulation of the vascular tone. We showed that 15-LO-1 expression and activity are necessary and sufficient for the vasorelaxation in rabbit aorta (1, 41). Thus rabbit aortic 15-LO-1, which initiates the synthesis of these vasodilatory eicosanoids, represents an important regulatory site for vascular activity.

15-LO-1 expression is regulated at both the levels of translation and transcription. For example, 15-LO-1 expression is regulated developmentally in rabbit reticulocytes (3). 15-LO-1 expression is also regulated by transcription factors, cytokines (20), and protein kinase G (9). Therefore, developmental changes in the expression kinetics of the active 15-LO-1 might occur. This might have implications on the vasoactivity and thus alter hemodynamics with aging. AA metabolites of 15-LO-1, THETA, and HEETA also contributed to this decreased endothelium-derived hyperpolarizing factor (EDHF) responsiveness, indicating a role of 15-LO-1. In this study, we investigated the age-dependent regulation of 15-LO-1 expression in 1- to 16-wk-old rabbit aortas and correlated the expression with the synthesis of vasoactive THETA and HEETA and the vascular responsiveness toward ACh and AA.

MATERIALS AND METHODS

Tissue preparation. Animal protocols were approved by the Animal Care Committee of the Medical College of Wisconsin, and procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996). Aortic tissues were dissected from 1-, 4-, 8-, and 16-wk-old New Zealand White rabbits (Kuiper Rabbit Ranch, Gary, IN). Fat and connective tissues were carefully removed in ice-cold HEPES buffer concentration of (in mM) 10 HEPES, 150 NaCl, 5 KCl, 1 EDTA, 1 MgCl₂, and 6 glucose (pH 7.4).

RT-PCR. Total RNA was prepared from rabbit aortic tissue by using TRIzol total RNA isolation reagent (Life Technologies), as described previously (41, 42). PCR reactions were performed using a PCR Superscript kit (Life Technologies). The 50-μM PCR mixture consisted of primers high-fidelity buffer (1X) (0.2 μM), 2'-deoxyxynucleoside 5'-triphosphate (1 μM), MgSO₄ (1 mM), and Platinum Taq (GIBCO). The primers, forward 5'-ACAAGGCGTTGCAACAG-3' and reverse 5'-TTATCTGGTCAAAAATGCCGAG-3', recognize that both 15-LO-1 and 12-LO were designed from the cDNA sequence of rabbit 15-LO-1 and were synthesized by Operon (Huntsville, AL). The program for the thermocycler was 94°C for 30 s, 58°C for 1 min, and 72°C for 1.5 min, repeated 30 times

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followed by a final extension at 72°C for 7 min. PCR products were separated by 1% agarose gel electrophoresis and visualized by ethidium bromide staining. The PCR band was isolated from the gel, excised, subcloned into TOPO2.1 TA cloning vector (Invitrogen), and sequenced.

Cloning and sequencing of 3’ rapid amplification of cDNA ends for 15-LO in rabbit aorta. The 3’ end was amplified using the 3’ rapid amplification of cDNA ends (GIBCO). First-strand cDNA was synthesized using an adapter primer as before. The 3’ amplification of cDNA ends (GIBCO). First-strand cDNA was synthesized using m13 reverse and t7 forward primers (Retrogen). Western blot analysis. Protein lysates were loaded in each lane and separated by SDS gel electrophoresis as described previously (41). The extracts of media and cellular lipids from incubations performed in the presence of Indo were analyzed by reverse-phase high-pressure liquid chromatography (HPLC) using solvent system I and a Nucleosil C-18 (5 μm, 4.6 x 250 mm) column (31). The column effluent was monitored at 210 and 235 nm with a Beckman diode array detector to detect internal standards and the radioactivity was determined. The fractions corresponding to the THETAs (fractions 5–7 min), HEETAs (fractions 13–15 min), and HETEs (fractions 19–21 min) were collected, acidified, and extracted with cyclohexane-ethyl acetate (50:50, vol/vol) and dried under nitrogen.

Analysis of the metabolites. The extracts of media and cellular lipids from incubations performed in the presence of Indo were analyzed by reverse-phase high-pressure liquid chromatography (HPLC) using solvent system I and a Nucleosil C-18 (5 μm, 4.6 x 250 mm) column (31). The extracts of media and cellular lipids from incubations performed in the presence of Indo were analyzed by reverse-phase high-pressure liquid chromatography (HPLC) using solvent system I and a Nucleosil C-18 (5 μm, 4.6 x 250 mm) column (31). The column effluent was monitored at 210 and 235 nm with a Beckman diode array detector to detect internal standards and the radioactivity was determined. The fractions corresponding to the THETAs (fractions 5–7 min), HEETAs (fractions 13–15 min), and HETEs (fractions 19–21 min) were collected, acidified, and extracted with cyclohexane-ethyl acetate (50:50). The THETA fraction was rechromatographed in solvent system II using the same Nucleosil C-18 column. In solvent system II, solventa was water containing 0.1% glacial acetic acid, and solventb was acetonitrile with 0.1% glacial acetic acid. The program consisted of a 5-min isocratic phase with 35% solventb in solventa followed by a 35-min linear gradient to 85% solventb with a flow rate of 1 ml/min. The column elute was collected in 0.2-ml aliquots, and the radioactivity was determined as described above. The fractions containing HETEs were rechromatographed on a normal-phase HPLC system using solvent system III on an Ultrasphere-Si silica column (5 μm, 4.6 x 250 mm; Beckman, Fullerton, CA). The solvent system III consisted of a linear gradient starting at 0.5% to 1.5% 2-propanol in hexane-glacial acetic acid (99:1 vol/vol) over 40 min at a flow rate of 3 ml/min. Extracts from incubations performed in the absence of Indo were reconstituted in 50 μl acetonitrile and 150 μl deionized water. Metabolites were analyzed by a Nucleosil C-18 (5 μm, 4.6 x 250 mm) column using solvent system IV. This solvent system consisted of a 40-min isocratic phase (flow rate = 1 ml/min) with 31% solventb in solventa (acetonitrile in solventa were denoted with 0.025 M phosphoric acid), followed by a 20-min linear gradient to 100% solventb and a 20-min isocratic phase with 100% solventb. The column elute was monitored at 210 nm to detect the internal standards and collected in 0.5-ml fractions, and the radioactivity was determined in each fraction.

Isometric tension and vascular reactivity. Vascular reactivity was measured using a wire myograph (model 610M; Danish Myo Technology) in the precontracted rabbit aortic rings (41, 42). Briefly, aortic rings were suspended in a tissue bath in Krebs-Ringer bicarbonate buffer containing (in mM) 119 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.17 MgSO₄, 25 NaHCO₃, 1.18 KH₂PO₄, 0.027 EDTA, and 5.5 glucose; at 37°C bubbled with 95% O₂-5% CO₂. After equilibration, aortic rings

**Extraction of metabolites from media and cellular lipids.** 12-HETE (50 μg; Cayman Chemicals) and 14,15-EET acid (a kind gift from Dr. J. R. Falc of the University of Texas Southwestern Medical Center) in 50 μl ethanol were added as internal standards in the media from incubations performed with Indo. In media from incubations performed without Indo, 6-keto PGF₁α (50 μg; Cayman Chemicals) and PGE₂ (50 μg; Cayman Chemicals) were the internal standards. The media were extracted using Bond Elute octadecylsil columns as described previously (31, 33). The tissue from each age group was washed twice with 1 ml of cold deionized water and then resuspended in HEPES (0.5 ml) in a new glass tube. The resuspended tissue was homogenized (Polytron homogenizer) on ice. The cellular lipids were extracted by the addition of 4 vol of chloroform and methanol (CHCl₃/CH₃OH = 1:2). The tubes were allowed to stand for 30 min at room temperature with occasional stirring. Water (1.2 vol) and CHCl₃ (1.2 vol) were then added. The extraction tubes were mixed again and centrifuged at 900 g for 5 min. The aqueous phase was removed, and the organic phase was dried under a stream of nitrogen. The extracts were hydrolyzed with 1 N potassium hydroxide in ethanol at 40°C for 1 h. The solution was acidified to a pH of 3 by the addition of 1 N hydrochloric acid dropwise. The metabolites were extracted by liquid-liquid extraction with 5 vol of cyclohexane/ethyl acetate (50:50, vol/vol) and dried under nitrogen.

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were gradually stretched to a resting tension (0.5 g for 1-wk-old and 1.5 g for older rabbits) and stimulated four times with 40 mM KCl. Aortas were pretreated for 15 min with NLA (10 μM), an endothelial NOS (eNOS) inhibitor, and Indo (10 μM), a COX inhibitor. Aortic rings were precontracted with submaximal concentrations of phenylephrine (0.1–1 μM), and relaxations were measured with the addition of a cumulative concentration of ACh (10⁻⁹ - 10⁻⁵ M), AA (10⁻⁷ - 10⁻⁴ M), or sodium nitroprusside (SNP; 10⁻⁹ - 10⁻⁵ M). ACh relaxations were also measured in aortas from 1-wk-old rabbits in the presence or absence of Indo and NLA and in the presence of a LO inhibitor NDGA (1 × 10⁻⁵ M) (Sigma). Vasorelaxations were expressed as percentages of maximum precontraction. The vascular activity data are expressed as means ± SE. A statistical comparison of the data obtained from treated and control groups was performed using a one-way ANOVA, with P < 0.05 considered statistically significant.

RESULTS

15-LO-1 mRNA expression in rabbit aortas. We have previously reported the partial sequence of 15-LO-1 from rabbit aorta and used a restriction enzyme digestion method to differentiate between the 15-LO-1 and 12-LO (41). Here we present a complete sequence-and-expression pattern-15-LO-1 mRNA using the same strategy (Fig. 1). When cDNA was made from 1-wk-old rabbit aortas, a 326-bp PCR product was obtained (Fig. 2A). After restriction enzyme digestion, the PCR product from the rabbit aorta was cleaved to produce a band at about 160 bp, indicating that 15-LO-1 but not 12-LO was expressed (data not shown). Therefore, 15-LO-1 was cloned and sequenced from rabbit aortas. The cloned sequence from an aorta is identical to the reported rabbit reticulocyte 15-LO (Fig. 1). These data indicate that the rabbit aorta expresses 15-LO-1.

Aging decreases aortic 15-LO-1 expression in 1- to 16-wk-old rabbits. Figure 2 shows mRNA and protein expression of aortic 15-LO-1 in rabbits of different ages. mRNA was extracted from the aortic tissue of 1-, 4-, and 8-wk-old rabbits. RT-PCR was performed using a pair of intron-spanning primers specifically directed to 15-LO-1. Among the three age groups, the 15-LO-1 mRNA content was the highest in 1-wk-old rabbits. The band intensity of 15-LO-1 in 4-wk-old rabbits was significantly lower than in 1-wk-old rabbits. As shown in Fig. 2A, the band intensity of 15-LO-1 in 4-wk-old rabbits was reduced by approximately 70%, compared with that in 1-wk-old rabbits. The band intensity of 15-LO-1 in 8-wk-old rabbits was further reduced by approximately 80%, compared with that in 4-wk-old rabbits. These results indicate that 15-LO-1 expression decreases with aging in rabbit aortas.

Fig. 1. Amino acid sequence of the lipoxygenase (LO) from aortas of 1-wk-old rabbits. 15-LO-1 was cloned, sequenced, and compared with the published sequence of rabbit reticulocyte 15-LO (15-LO-1). Matching amino acids are denoted by the asterisk.
rabbits was one order of magnitude lower than that in 1-wk-old rabbits and lowest in 8-wk-old rabbits (Fig. 2A). In accordance, immunoblotting also showed a reduction of 15-LO-1 expression in older animals (Fig. 2B). The rabbit aorta contained a 75-kDa immunoreactive band corresponding to 15-LO-1. Band densities of 15-LO-1 were normalized with the band density of $\beta$-actin (Fig. 2C). When compared with that in 1-wk-old rabbits, aortic 15-LO-1 protein was 10% lower in 4-wk-old rabbits and further decreased by 40% and 50% in 8- and 16-wk-old rabbits, respectively. A vascular endothelial cell marker, PECAM-1, was expressed at higher levels in aortas of 1-wk-old rabbits compared with older animals. PECAM-1 levels in 4-, 8-, and 16-wk-old rabbits were similar, about 50% less than those of 1-wk-old rabbits (Fig. 2B).

**Metabolism of AA by 15-LO-1 in rabbit aortas.** Aortic or mesenteric tissue from rabbits of different ages was incubated with $^{[14]}$C$\text{AA}$ in the presence of Indo. The $^{[14]}$C-labeled metabolites were extracted and analyzed by reverse-phase HPLC. Figure 3 shows the representative chromatograms of AA metabolites by aortas of neonate, 1-, 4-, 8-, and 16-wk-old rabbits. AA was converted by the rabbit aortas to 15-HETE, 15-THETA, 11,12,15-trihydroxyeicosatrienoic acid; HEETA, 15-hydroxy-11,12-epoxyeicosatrienoic acid; CPM, counts per minute.
12-HETE, HEETA, and THETA in neonate, 1-, and 4-wk-old rabbit aortas. Scale amplification also showed the presence of THETA, HEETA, and 15-HETE in 8- and 16-wk-old rabbit aortas (data not shown). In the older animals, less 15-LO-1 metabolites were synthesized per unit mass of aortic tissue. Similarly, AA was metabolized to THETA, HEETA, 15-HETE, and 12-HETE in the mesenteric arteries from 1-, 4-, 8-, and 16-wk-old rabbits (Fig. 4). Synthesis of these 15-LO-1 metabolites decreased progressively in mesenteric arteries from 1- to 16-wk-old rabbits. The ratio of THETA, HEETA, 15-HETE, and 12-HETE remained unchanged in all age groups. The recovery of the internal standards, 12-HETE and 14,15-EET, was not different in any arteries from any age group.

The THETA fraction from aortic incubations was collected, reextracted, and analyzed with HPLC system II (Fig. 5). THETA production from neonate aortas was two times the production from 1-wk-old rabbit aortas. THETA productions from aortas of 4-, 8-, and 16-wk-old rabbits were 42%, 4%, and 1% of aortas of the 1-wk-old rabbit, respectively. 15-HETE and 12-HETE fractions were also pooled, extracted, and resolved by HPLC system III (data not shown). When compared with that in the 1-wk-old rabbit, 15-HETE from 4- and 8-wk-rabbits was reduced by 38% and 96%, respectively. The ratio of 15-HETE to 12-HETE (10:1) was not significantly different among the age groups. These data suggest that aortic 15-LO-1 decreases with aging, which correlates with reduced 15-LO-1 enzyme activity and eicosanoid synthesis. However, eicosanoids can be reincorporated into cellular phospholipids (26, 34, 43). Thus we wondered whether the decreased THETA, HEETA, and HETEs synthesis that we observed in Fig. 3 was due to the reincorporation into the cellular phospholipids. Therefore, the metabolites were also extracted and hydrolyzed from the cellular lipids. Figure 6 shows AA and AA metabolites in the cellular lipids of the aortas from 1- to 16-wk-old rabbits. There was no reincorporation of either THETA, HEETA, or HETEs in the cellular lipids of either 1- or 16-wk-old aorta. However, 4- and 8-wk-old rabbit aortas reincorporated small amounts of 15-HETE and possibly 12-HETE in the cellular lipids but not THETA and HEETA. The data presented in Figs. 3–6 were repeated four times with different groups of animals, and representative figures are shown.

To determine the effect of age on the PG synthesis, AA metabolism was performed in aortas in the absence of Indo. The aortas synthesized 6-keto-PGF1α, a stable metabolite of prostacyclin. The synthesis of 6-keto-PGF1α was not altered with age in aortas of 1- to 16-wk-old rabbits (data not shown).

15-LO-1-mediated vasorelaxation to ACh and AA decreases with aging. Rabbit aortas with intact endothelium were isolated from 1-, 4-, 8-, and 16-wk-old animals, and their vascular activity was studied. Figure 7 summarizes the concentration-dependent vasorelaxation responses to ACh in the presence of Indo and NLA (Fig. 7A), AA in the presence of Indo (Fig. 7B), and SNP (Fig. 7C). The basal tone measured as KCl contractions and sensitivity to the phenylephrine was not different in aortas from 1-, 4-, 8-, and 16-wk-old rabbits. At 10⁻⁶ M ACh, the maximal relaxation responses to ACh were 64.5 ± 6.9%, 52.6 ± 8.9%, 53.0 ± 9.4%, and 33.3 ± 6.6% in aortas from 1-, 4-, 8-, and 16-wk-old rabbits, respectively. Aortas of 1-wk-old rabbits showed significantly higher relaxations to 10⁻⁷ to 10⁻⁵ M ACh than aortas of 4- and 8-wk-old rabbits. Aortas of 4- and 8-wk-old rabbits were similar in their responses to ACh. At 16 wk, the sensitivity and maximal responses to ACh stimulation were markedly reduced compared with those of the other age groups. The maximal relaxation response to AA (3 × 10⁻⁴ M) was 60.4 ± 8.9% for the 1-wk-old group and decreased to 56.3 ± 5.8%, 41.8 ± 12.3%, and 28.9 ± 1.6% in the 4-, 8-, and 16-wk-old groups, respectively. The minimal AA con-

Fig. 4. Metabolism of [14C]AA by mesenteric arteries of 1- (A), 4- (B), 8- (C), and 16-wk-old (D) rabbits. Mesenteric arterial rings with intact endothelium were incubated with [14C]AA in the presence of Indo. The media were extracted, and eicosanoids were resolved by HPLC system I. The production of [14C] metabolites is normalized by tissue weight. Migration times of known standards are shown above the chromatogram in A.
The concentration to induce relaxation responses was $10^{-6}$ M in the 1-wk-old rabbits and $10^{-5}$ M in aortas of all the other age groups. There was no significant difference between the vasorelaxation of the 4- and 8-wk-old groups. The endothelium-independent responses to SNP were similar in the vessels from all four age groups. The maximum relaxations ranged from 126.7 ± 12.3% in 1-wk-old aorta to 113.7 ± 4.4% in 16-wk-old aorta.

Fig. 5. THETA synthesis by aortas of neonate (A), 1- (B), 4- (C), 8- (D), and 16-wk-old rabbits (E). The column fractions from HPLC system I containing THETA fraction (fractions 27–35) were collected, reextracted, and further analyzed using HPLC solvent system II. Migration times of known standards are shown above the chromatograms. Representative chromatograms of 4 separate sets of experiments are shown.

Fig. 6. Metabolism of [14C]AA by aortas of 1- (A), 4- (B), 8- (C), and 16-wk-old (D) rabbits. Aortic rings with intact endothelium were incubated with [14C]AA in the presence of Indo. The aqueous phase was removed, aortic lipids were extracted and hydrolyzed, and eicosanoids were resolved by HPLC system I. The production of [14C] metabolites is normalized by tissue weight. Migration times of known standards are shown above the chromatograms.
The ACh relaxations were also measured in aortas from 1-wk-old rabbits in the absence or presence of NLA and Indo (Fig. 8). In the absence of Indo and NLA, ACh relaxed the aortic rings at a lower concentration and with a greater maximum effect than in the presence of the inhibitors. NDGA shifted the ACh concentration-response curve to the right without affecting the maximum effect. In the presence of Indo and NLA, ACh relaxed the aortic rings. These relaxations were reduced by a NDGA from 71.6 ± 8% to 25.3 ± 9%.

DISCUSSION

A few studies have shown that LO expression and activity are regulated by development or age. In bovine and rabbit lungs, 5-LO expression is higher in fetal vessels than in newborns and adults (14–16). We previously showed that 15-LO-1 products in rabbit aorta were reduced in 12-mo-old rabbits compared with 1-mo-old rabbits (29). Our results in this study demonstrate the age-related or developmental-related regulation of the rabbit aortic 15-LO-1 pathway in rabbits of 1 to 16 wk old. Our data show that 15-LO-1, but not 12-LO, is expressed in rabbit aortas. Rabbit 15-LO-1 is a dual LO that produces both 15- and 12-HETE in a 9:1 ratio (2, 22). We also found that both aortic 15-LO-1 mRNA and protein decreased dramatically during the first 4 mo of age. Several factors may contribute to the downregulation of aortic 15-LO-1 expression in older rabbits. 15-LO-1 expression is regulated by numerous cellular processes (for review) (21), such as gene silencing by heterogenous nuclear ribonucleoprotein and transcription factors such as Stat family (38), Ku 70/80 (19), and GATA (18).

We previously determined the distribution of 15-LO-1 in the arterial wall of 4-wk-old rabbit aorta and found that 15-LO-1 expression was strongly associated with endothelial cells and smooth muscle cells adjacent to endothelium (42). The decrease of 15-LO-1 during aging is simultaneous with the
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During development of vascular wall, the vasculature undergoes profound morphological and functional changes including intimal thickening (23). We compared the dynamic expression patterns of aortic 15-LO-1 and endothelial cell marker PECAM-1 in various age groups. The results show less 15-LO-1 and PECAM-1 in aortas of 4-wk-old rabbits than in 1-wk-old rabbits, suggesting that 15-LO decreases with the proportion of endothelial cells in the aortic tissue. From 4 to 16 wk of age, aortic 15-LO-1 continues to decrease, but PECAM-1 content stays stable. This suggests that the proportion of endothelium remains unchanged, whereas 15-LO-1 expression decreases from 4 to 16 wk of age in rabbits. The dynamics of aortic 15-LO-1 and PECAM-1 expression suggest that 15-LO-1 content may relate to endothelial cell growth and development at a very young age, but other mechanisms contribute to 15-LO-1 regulation with aging. The neonates and 1-wk-old rabbits were on milk, whereas 4-, 8-, and 16-wk-old rabbits were on normal chow. These dietary differences could influence 15-LO-1 or PECAM expression in aortas of rabbits between 1 and 4 wk of age.

As 15-LO-1 protein content changes, the 15-LO-1 metabolism of AA in rabbit aorta or mesenteric arteries is also altered. Our previous study of AA metabolism by aortas of 1- and 12-mo-old rabbits indicated that 1-mo-old rabbit aortas produced 10 times more 15-HETE than 12-mo-old rabbit aortas (29). Consistent with these results, we demonstrated an age-related decrease in 15-LO-1 from newborn to adult rabbits. We also found that the aortic or mesenteric 15-LO-1 pathway activity starts to decrease at an early age, with the highest activity in neonatal rabbits. Aortic and mesenteric arterial tissue from a 1-wk-old rabbit produces 20 times more 15-HETE than an equal amount of vascular tissue from a 16-wk-old rabbit. The ratio of 15-HETE to 12-HETE is 10:1 in both types of arteries in all age groups, similar to the reported ratio of 15-HETE and 12-HETE produced by purified 15-LO-1 (2, 22). The relative ratios among the major 15-LO-1 pathway metabolites, 15-HETE, THETA, and HEETA, remain the same in all age groups. Similarly, in our previous studies of the aortic 15-LO-1 pathway, the proportions of 15-LO-1 metabolites were also not affected by the induction of 15-LO-1 by IL-13 (42). However, THETA, HEETA, and HETEs could also be reincorporated into the membranes of the cells (26, 34, 43). This could mean that the metabolites were synthesized but not present in the media to be analyzed. Therefore, we also extracted the metabolites from the cellular lipids to confirm whether the decrease in THETA, HEETA, and 15-HETE we observed was actually due to less synthesis. Indeed, there was very little incorporation of the metabolites in the cellular lipids. The metabolism of PGs remained unaffected with age in aortas from 1- to 16-wk-old rabbits. This implies that the expression/activity of COX remains unaltered in this life span of rabbits.

Studies in rats showed a selective loss of EDHF activity with aging and high blood pressure (5, 10, 40). ACh-induced vasorelaxations are mainly mediated by EDHF in young rats, whereas the NO pathway is the major endothelial mediator in old and hypertensive rats (24). Blood pressure of newborn and young rabbits is lower than that in older rabbits (8, 37). In the rabbit aorta, AA is converted by the 15-LO-1 pathway to THETA and HEETA, which relax the preconstricted vessel and act as EDHFs (7). ACh-induced vasorelaxation is partially blocked by NOS and COX inhibitors. The non-NO, non-PG relaxations can be blocked by 15-LO-1 inhibitors (7, 13, 17). The NO- and PG-independent vasorelaxations in 1-wk-old rabbit aortas are significantly higher than those in other age groups. Relaxation responses of 4- and 8-wk-old rabbit aortas to ACh and AA are similar. The maximum vasorelaxation induced by ACh and AA is 52–55% and 35–41%, respectively, in 1 to 2-mo-old rabbits (7, 13, 30). The non-NO, non-PG-mediated vasorelaxations in 16-wk-old rabbits are markedly lower than those in 1- and 4-wk-old rabbits. We have previously reported that the treatment of aortas from 1-wk-old rabbits with a 15-LO-1 antisense oligonucleotide reduced aortic 15-LO and inhibited the relaxations to ACh when compared with the normal aortas (41). Indeed, in the presence of Indomethacin (INDO) and NLA, ACh relaxations of aortas from 1-wk-old rabbits were inhibited by NDGA. These data suggest that 15-LO-1 metabolites of AA are vasodilators and mediate a portion of the relaxations to ACh. Endothelium-independent relaxations induced by SNAP were also tested. The smooth muscle relaxations to SNAP are similar in all age groups. This suggests that the age-related change of vascular activity is mediated by the regulation of endothelium-dependent vasodilatory factors and not due to a change in smooth muscle responsiveness to dilation.

In summary, aortic 15-LO-1 is regulated by the age of the animal. 15-LO-1 expression and activity decrease during aging and development in rabbits. The decrease in 15-LO-1 downregulates vasodilatory eicosanoid synthesis from the 15-LO-1 pathway and reduces vasorelaxation responses induced by ACh and AA. These results support the importance of aortic 15-LO-1 in the regulation of vascular tone. These results also suggest that 15-LO-1 metabolites contribute to the age-dependent reduction EDHF responses and, therefore, may contribute to the increase in blood pressure that occurs in older animals.

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