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Impaired vasomotor function induced by the combination of hypertension and hypercholesterolemia

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Abstract

Background—While it is well known that endothelial function is compromised in the presence of either hypertension (HTN) or hypercholesterolemia (HCh), less is known about whether and how the combination of these risk factors (HTN+HCh) results in impaired endothelium-dependent dilation (EDD). The aims of this study were to evaluate the influence of HTN+HCh on vasomotor function and to identify the mechanisms that underlie the altered vascular reactivity elicited by HTN+HCh.

Methods—Endothelium-dependent and -independent vasomotor responses of aortic vessels were studied in mice with diet-induced HCh and/or HTN induced by chronic administration of either angiotensin II (AngII) or deoxycorticosterone acetate-salt.

Results—HTN+HCh elicited an impairment of EDD that lied between each risk factor alone. Incubation with catalase resulted in more severe EDD impairment. Each risk factor enhanced vascular H_2O_2 production, but a larger response was noted with HTN+HCh. An attenuated EDD was not observed in AngII-type-1a receptor deficient ($AT1r^{-/-}$) mice, but $AT1r^{-/-}$ bone marrow chimeras exhibited more profound impairment compared to WT.

Conclusions—HTN+HCh does not exert an additive effect of vasomotor dysfunction compared to either risk factor alone, and both H_2O_2 and blood cell-associated AT1r contribute to the impaired EDD responses in mice with HTN+HCh.

Keywords

endothelium-dependent vasodilation; angiotensin II type-1 receptors; hydrogen peroxide; risk factors

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Introduction

Hypertension and hypercholesterolemia are two major factors that increase the risk of cardiovascular disease. Long-term exposure to either of these risk factors leads to changes in the structure and function of large and microscopic blood vessels[1]. While the vascular responses vary somewhat between the two risk factors, a characteristic feature of both responses is endothelial dysfunction. Increased leukocyte adhesion, accelerated thrombus formation, diminished endothelial barrier function, and impaired vasomotor responses are common manifestations of the risk factor-induced endothelial cell dysfunction[1-3]. Although the mechanism(s) underlying these endothelium-dependent vascular alterations remain poorly understood, evidence points to reduced nitric oxide bioavailability, secondary to enhanced generation of superoxide, as a critical component of the endothelial cell dysfunction that accompanies HTN and HCh[4]. The capacity of macroscopic and microscopic arteries to dilate in response to acetylcholine, bradykinin, and other endothelium-dependent vasodilators is significantly impaired in humans and experimental animals with either HTN or HCh[5]. This impairment has been linked to different factors, including oxidative stress, circulating immune cells, and angiotensin II type-1 receptors (AT1r)[6]. Among the various sources of reactive oxygen species (ROS) induced by the risk factors, NADPH oxidase has received the most attention. The engagement of angiotensin II with AT1r appears to result in NADPH oxidase-dependent ROS production that has been linked to both the vasomotor dysfunction and inflammatory component (leukocyte adhesion, endothelial barrier failure) of the vascular response to HTN and HCh[7, 8]. Similarly, the involvement of T-lymphocytes in HCh and HTN induced vasomotor dysfunction has also been linked to lymphocyte-associated AT1r and ROS production[1, 9].

While much has been learned about the responses of the vasculature to individual risk factors, less is known about whether, and how, combinations of risk factors (e.g., HTN +HCh) for cardiovascular disease result in endothelium-dependent vascular dysfunction. Epidemiological evidence on the incidence of ischemic diseases of the heart and brain in humans suggest that combinations of risk factors exert a synergistic effect relative to each risk factor alone[10-12]. Since HTN and HCh are known to individually induce similar phenotypic changes (e.g., endothelial cell dysfunction, oxidative stress) in the vasculature and appear to use some common signaling pathways (e.g., AT1r signaling), it may be expected that the combination of these risk factors would yield neither additive nor synergistic vascular responses. However, the limited available evidence in the literature is inconsistent in this regard, with some studies demonstrating synergism between HTN and HCh on vascular reactivity[12, 13] while other reports[14, 15] describe an absence of synergism. For example, in pigs with renovascular HTN and diet-induced HCh, the two risk factors exert a synergistic deleterious effect on endothelium-dependent dilation and oxidative stress in coronary arteries, compared to either risk factor alone[12]. A comparison of the vasomotor responses to both a vasodilator (acetylcholine) and a vasoconstrictor (norepinephrine) between hypercholesterolemia apoE$^{-/-}$ mice, without or without angiotensin II-dependent renovascular hypertension, did not reveal additive effects on vessel function[14]. It remains unclear whether these inconsistent responses to risk factor combinations result from the vascular bed examined and/or models used to induce HTN or HCh. Furthermore, it is uncertain whether the mechanisms that underlie the responses to multiple risk factors differ from the mechanisms that mediate the vascular dysfunction elicited by individual risk factors.

The overall objective of this study was to test the hypothesis that the combination of HTN +HCh alters vascular reactivity to a greater degree than either risk factor alone via a mechanism that involves the activation of AT1 receptors and increased ROS production. Three specific issues were addressed: 1) whether HTN+HCh alters vasomotor function in a

manner that is different from each risk factor alone, 2) the role of ROS in the risk factor-induced alterations in vascular reactivity, and 3) whether AT1a receptors expressed on circulating blood cells vs the vessel wall contributes to the vascular responses elicited by HTN+HCh.

Methods

Animals

All experimental procedures were performed on male wild-type C57Bl/6 (WT) and AT1a-R$^{-/-}$ mice (Jackson Laboratory, Bar Harbor, ME, USA). The animal experiments were performed according to the criteria outlined by the National Institutes of Health and approved by the LSU Health Sciences Center Institutional Animal Care and Use Committee.

Hypercholesterolemia—Mice (6 to 8 weeks old) were placed either on a normal (ND) or high-cholesterol (HC) diet with (Teklad 90221, containing 1.25% cholesterol, 0.5 % sodium choline, 15.8% fat, Harlan Teklad) or without cholate (Teklad 94059, containing 1.25% cholesterol, 15.8% fat, Harlan Teklad) for 2 (AngII groups) or 3 (DOCA-salt groups) weeks, respectively.

Angiotensin II-induced hypertension—In some mice, an osmotic minipump (Alzet) was implanted subcutaneously between the scapulas. The pump contained either saline (sham) or angiotensin II (Sigma Chemical Co) dissolved in saline. The AngII infusion rate was 1.0 ug/kg/min for 2 weeks. Separate groups of mice were treated with the AT1r antagonist losartan (25 mg/kg/per day) in drinking water for 7 days, beginning at day 8 on HCD or Ang II.

Hypertension induced by DOCA-salt—In C57BL6 mice, deoxycorticosterone acetate-salt (DOCA-salt) hypertension were induced by subcutaneous implantation of a 50-mg slow release DOCA pellet. A right flank incision was made and the right kidney removed. Drinking water was replaced by 1.0 % saline/0.2% potassium chloride. Control animals underwent nephrectomy, sham implantation of a pellet, and were given water ad libitum.

Blood Pressure Measurement—On day-14 of Alzet pump (AngII groups) implantation or day-21 of pellet implantation (DOCA-salt groups), blood pressures were measured by tail-cuff plethysmography (model SC-1000, Hatteras Instruments, Inc., North Carolina, USA) in non-anesthetized animals. The average of eight successive measurements was taken as the mean systolic blood pressure in each animal.

Wire Myography—The reactivity of mouse aortic segments to different dilators and constrictors was quantified using wire myography. Briefly, mice were anesthetized with xylazine (7.5 mg/kg body wt ip) and ketamine chloride (150 mg/kg body wt ip) and the thoracic aorta was quickly removed by central sternotomy. The aortic segment was gently washed through the lumen with ice-cold saline and placed in ice-cold physiological salt solution (PSS). 2-mm long segments of aorta were mounted on an eight-channel wire myograph (Randoti Glass' Monrovia CA). Vessel rings were maintained in 15-ml organ baths with oxygenated PSS (95% O2 and 5% CO2) at 37.1°C. The resting tension was increased stepwise to 1.4 g, followed by a 40 min equilibration period. An eight-channel octal bridge and data-acquisition software were used to record all force measurements. After equilibration, aortic rings were rinsed with 120 mM KCl for vascular smooth muscle activation and to determine the maximal contractile response. The aortic rings were then precontracted with 10^{-6} M phenyleprine (PE) to obtain submaximal contraction (60-80% of KCl-induced maximum response), and after obtaining a stable plateau phase of contraction,

the integrity of the endothelium was assessed with acetylcholine (Ach; 3×10^{-9}-10^{-6}). Endothelium-dependent dilation was expressed as the percent dilation from the precontraction response to 10^{-6} M PE. Endothelium independent vasorelaxation was evoked by sodium nitroprusside (SNP; 10^{-9}-10^{-4} M) or papaverine (10^{-9}-10^{-4} M), while endothelium-independent contraction was induced with PE (10^{-9}-10^{-4} M). In some experiments, vascular reactivity to ACh was assessed in the presence (5 min preincubation) of catalase (1200 U/ml; Sigma C-100, Sigma Aldrich) to determine the contribution of H_2O_2 to the vasorelaxation response.

Production of Bone Marrow Chimeras—Chimeras were produced by transplanting bone marrow derived from AT1r$^{-/-}$ mice into WT recipients, as previously described[8]. This procedure normally yields >90% penetrance of the transferred marrow at 6 weeks after transplant. This bone marrow transfer protocol allowed for the creation of mice wherein the genetic deficiency of AT1a receptors is confined to the circulating blood cells[8].

Measurement of H_2O_2—Extracellular H_2O_2 was determined with a fluorometric horseradish peroxidase-linked assay kit (Amplex red assay, Molecular Probes, Eugene, Oregon, USA). Aortic segments were incubated for 60 min at 37 °C in PSS containing 50 μM Amplex red and 0.1 U/ml horseradish peroxidase protected from light. The tissue was removed from the buffer and buffer fluorescence was detected at 590 nm, using an excitation of 530 nm. Background fluorescence, determined using a reaction without tissue, was subtracted from each value. H_2O_2 release, calculated using H_2O_2 standards was expressed as mM per millimeter of vessel. The Amplex red assay is highly specific and sensitive, with detection limit of approximately 5 pmol of H_2O_2[16]. It detects H_2O_2 released by cells and tissues and can be applied to cell-free systems. Resorufin, the end product which, when excited at 530 nm, strongly emits light at 590 nm[17] is a very stable product that allows detection of H_2O_2 both in oxidative and reductive conditions. The specificity of the assay is evidenced by the fact that catalase abolishes the assay signal [16].

Serum Cholesterol Levels—Serum samples were frozen for subsequent measurement of cholesterol levels using a spectrophotometric assay (Sigma Chemicals Co).

Statistical Analysis—Each experimental group included 7-9 mice. All values are reported as means ± SE. Comparisons between groups of animals or treatments were made by one-way ANOVA. When significance was indicated, a Student-Newman-Keuls post hoc analysis was used with statistical significance set at $P<0.05$.

Results

All mice placed on a cholesterol-enriched diet exhibited an increased serum cholesterol concentration (Table 1). Similarly, all mice with an implanted AngII loaded pump or a DOCA pellet exhibited a significantly elevated systolic blood pressure compared to WT mice. In both hypertension models, the elevated serum cholesterol did not alter the blood pressure response. The AngII-induced hypertension response was significantly attenuated in HCh mice treated with losartan (Los) and in AT1r$^{-/-}$ mice, while AT1r$^{-/-}$ chimeras showed no attenuation of blood pressure compared to either WT or WT-HCh mice.

Figure 1 demonstrates that acetylcholine-induced vasodilation was significantly blunted in mice placed on a cholesterol-enriched diet. The HCh-induced impairment of acetylcholine mediated vascular relaxation was largely prevented by treatment with the AT1r antagonist losartan.

Figure 2 summarizes the dose-dependent vasodilation responses to acetylcholine (panels A & B), sodium nitroprusside (panel C) and papaverine (panel D). The data shown in panel A illustrate that aortic segments from mice with AngII-induced hypertension exhibit a blunted relaxation response to acetylcholine and that HTN+HCh exhibited an attenuated response that lies between that produced by either risk factor alone. Losartan completely prevented the blunted relaxation response in AngII hypertensive mice. Panel B illustrates that the blunted acetylcholine-induced relaxation noted with HTN+HCh is more profoundly impaired in the presence of catalase. Panel C demonstrates that while hypercholesterolemia is not associated with an altered relaxation response to different concentrations of sodium nitroprusside, mice with AngII-induced hypertension exhibit a blunted relaxation (at the lower SNP doses). The AngII effect on SNP mediated relaxation is not evidenced when the mice are placed on a cholesterol-enriched diet. The blunted response seen with AngII alone is not evident in losartan-treated mice. Panel D shows that papaverine-mediated relaxation was slightly, but significantly, blunted (at 10^{-5} M) in AngII-infused mice.

Figure 3A provides a comparison of the aortic contraction responses to phenylephrine and the changes in H_2O_2 production by aortic segments derived from mice with AngII-induced hypertension, hypercholesterolemia, or both. The results indicate that PE-induced contraction is blunted in WT-AngII mice at the highest PE concentrations studied. This attenuation of PE-induced contraction was not observed in the WT-HCh or WT-AngII-Los treated groups (panel A). Panel B shows that each risk factor alone enhances vascular H_2O_2 production and that HTN+HCh yields a larger response than seen with either risk factor alone. Losartan treatment significantly attenuated H_2O_2 production in AngII mice.

Figure 4 shows the differences noted between the relaxation (acetylcholine, sodium nitroprusside) or contraction of aortic segments derived from wild type, $AT1r^{-/-}$, and $AT1r^{-/-}$ bone marrow chimeric mice with a combination of AngII-induced HTN+HCh. Panel A shows that the blunted acetylcholine-induced relaxation elicited by HTN+HCh in wild type mice is not observed in $AT1r^{-/-}$ mice. However, $AT1r^{-/-}$ bone marrow chimeras with both risk factors exhibited a more profound impairment of acetylcholine-induced relaxation than WT mice with HTN+HCh. Similar findings were noted with nitroprusside-induced relaxation (panel B). However, in this instance, while the $AT1r^{-/-}$ mice were protected against the impaired relaxation to SNP caused by HCh+HCh, the $AT1r^{-/-}$ chimeras were not. Phenylephrine-induced contraction also showed improvement in $AT1r^{-/-}$ (but not $AT1r^{-/-}$ chimeras) mice with HTN+HCh, compared to WT-HTN-HCh mice (panel C). The large increase in aortic tissue H_2O_2 production elicited by HTN+HCh in WT mice was not observed in aortic segments from $AT1r^{-/-}$ mice (panel D). A significant, but partial, attenuation of H_2O_2 production was detected in $AT1^{-/-}$ chimeras with HTN+HCh.

Figure 5 compares different vascular reactivity responses and H_2O_2 production of aortic segments derived from mice with hypercholesterolemia ± DOCA salt hypertension. Panel A summarizes the acetylcholine-induced relaxation responses in the different groups and demonstrates a significant but modest attenuation of the relaxation response in DOCA-salt hypertensive mice and in mice with both risk factors. While DOCA-salt hypertension was associated with an attenuated relaxation response to nitroprusside (at the lowest concentrations studied) no other differences were noted between groups (panel B). Phenylephrine-induced contraction did not exhibit large differences between groups (panel C), although small significant differences were noted for the DOCA-salt only (hyper-reactivity) and DOCA + HCh (blunted response) groups at low and high PE concentrations, respectively. H_2O_2 production by aortic segments from the different mice is shown in panel D. While both hypercholesterolemia and DOCA-salt hypertension alone produced significant elevations in H_2O_2 production, HTN+HCh did not yield a larger response.

Discussion

Endothelial dysfunction and impaired vascular reactivity are two well-known responses of macroscopic and microscopic blood vessels to hypertension and hypercholesterolemia[18-21]. While the mechanisms underlying the hypertension-induced alterations in endothelial function may differ between the large conducting vessels and peripheral resistance vessels[22-24], hypertension as well as other cardiovascular risk factors are generally elicit an enhanced production of ROS and a reduction in NO bioavailability in both macroscopic (eg, aorta) and microscopic (eg, arterioles) blood vessels, which has been linked to the impaired vasomotor function[25-27]. Although much has been learned about the influence of each risk factor on vascular function, less attention has been devoted to addressing the combined actions of HTN and HCh. Such an effort is justified based on epidemiological studies that reveal a high prevalence of co-existing HTN and HCh in the human population, with 30-40% of men and women exhibiting both risk factors[28]. Clinical evidence also indicates that the concurrence of HTN and HCh increases the incidence of atherosclerosis and cardiac events relative to the changes noted with either risk factor alone[29]. Animal studies have generally confirmed these clinical observations by demonstrating accelerated atherosclerotic lesion development and more profound vascular dysfunction in response to HTN + HCh, compared to either risk factor alone[12, 30]. However, the few studies that have addressed the influence of concurrent HTN and HCh on vascular reactivity have yielded somewhat inconsistent results, with some describing no effect of diet-induced HCh on vasomotor function in spontaneously hypertensive rats[15] and other studies in pigs show a synergistic effect of diet-induced HCh and renovascular HTN on coronary artery reactivity to acetylcholine [27, 31]. In this study, we re-examined the effects of HTN and HCh, either alone or in combination, on vasomotor function using murine models of both risk factors, and capitalized on the availability of mutant mice to assess the contributions of angiotensin II type-1 receptors and oxidative stress to vasomotor dysfunction elicited by the combination of risk factors.

Our study confirms the results of previous studies that demonstrate the ability of HTN and HCh to independently impair vasomotor function. This effect of the risk factors was more clearly manifested in the aortic vessel responses to acetylcholine. We noted that AngII-induced hypertension tends to diminish the capacity of blood vessels to respond to all dilators and vasoconstrictors studied, although this impairment was rather modest for agents other than acetylcholine. A comparison of the vasomotor responses between mice with angiotensin II vs DOCA-salt induced HTN revealed a more pronounced attenuation of ACh mediated vasodilation in the angiotensin II model, suggesting greater impairment of endothelial function in the presence of elevated angiotensin II levels. We also noted that mice with HTN+HCh exhibited an impairment of endothelium-dependent dilation that lies between that observed with each risk factor alone. In the AngII model, this was evident over the entire range of acetylcholine concentrations studied, but only at the lower Ach concentrations in the DOCA-salt model. The more subdued influence of HCh on vascular reactivity is also reflected in the fact that this condition was not accompanied by a significant elevation in blood pressure. Our findings contrast reports that describe either a mild (or no) impairment of vasomotor function in hypercholesterolemic SHR[15, 32] or a more pronounced impairment of vasoreactivity to both endothelium-dependent and -independent dilators in pigs with combination of renovascular HTN and diet-induced HCh[12]. An explanation for the differences between these published observations and our results is not readily apparent but may result from differences in animal species (rat vs pig), vessels (aorta vs coronary artery), and/or models of hypertension studied. The absence of an additive response to HTN+HCh in the SHR model has been attributed to the resistance of rats in developing HCh-induced inflammation and atherosclerosis[15]. An alternative explanation for the absence of an additive (or synergistic) effect of HTN+HCh on impaired vascular

reactivity in our model and others is that the two risk factors exert their actions on endothelial cells via common signaling pathways, such as AT1r or ROS signaling.

Our findings confirm that both HTN and HCh independently promote the production of H_2O_2 in arterial tissue. In addition, we provide the first evidence that concurrent HCh and angiotensin II-induced HTN exerts an additive effect on ROS production, compared to each risk factor alone. This effect on ROS production was not observed with DOCA-salt induced HTN, underscoring the importance of AngII as a signal for risk factor mediated oxidant stress[33, 34]. In order to monitor the production of H_2O_2 in response to HTN and/or HCh in this study, the Amplex red assay was used. This assay is specific for H_2O_2 and does not detect superoxide, which is well known to inactivate nitric oxide (NO) and interfere with endothelium-dependent vasodilation. Since NADPH oxidase is known to generate superoxide, which rapidly is dismutated to form H_2O_2, our measurements of enhanced H_2O_2 production are likely to reflect an equally large increase in the production of superoxide[17, 35] Whether the risk factor-induced impairment of Ach mediated vasodilation noted in our study reflects a response to increased superoxide, hydrogen peroxide, or both remains unclear. In order to analyze the contribution of H_2O_2 to the vasorelaxation response in the HTN+HCh group, ACh vasoreactivity was assessed in the presence of catalase. Incubation with catalase resulted in more severe impairment of ACh vasoreactivity, which implicates H_2O_2 in the ACh-induced relaxation mediated by both risk factors. The contribution of enhanced H_2O_2 production to the impaired ACh-induced dilation associated with HTN+HCh warrants more attention given the differential vasoactive properties of superoxide and H_2O_2 and their ability to alter the vascular responses to different agonists. Furthermore, ACh-induced vasodilation in mouse aorta has previously been linked to H_2O_2 production by neuronal NO synthase (nNOS)[35] and Nox4 NADPH oxidase[36]. Similarly, H2O2 has been shown to significantly enhance the production of NO by endothelial nitric oxide synthase[37]. Additional evidence for the involvement of ROS-dependent mechanisms is provided by a report describing more pronounced reductions in arterial wall levels of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and vitamins (E and C) with HTN +HCh, compared to either risk factor alone[12].

A likely link between the enhanced ROS production and the risk factor-mediated impairment of vascular reactivity is the AngII type-1 receptor[38]. The improved vasoreactivity to acetylcholine noted in Losartan-treated WT mice and in $AT1r^{-/-}$ mice with HCh ± angiotensin II-induced HTN underscores the importance of AT1r signaling. It is noteworthy that the large increase in ROS production normally elicited by HTN+HCh was also blunted in mice that are genetically deficient in AT1a receptors, which supports a link between ROS and AT1r in the impaired vasomotor function. A novel and potentially important observation in our study was the response of $AT1r^{-/-}$ bone marrow chimeras with concurrent exposure to angiotensin II-induced HTN and HCh. These chimeras, in which circulating blood cells (but not endothelial cells) were deficient in AT1ar, exhibited a more profound impairment of acetylcholine-induced relaxation than WT mice with the risk factor combination, suggesting a protective role of blood cell-associated AT1r, i.e., by the AT1 receptor expressed on circulating blood cells. The detrimental effect of AT1r deficiency on circulating cells was noted despite a small and insignificant attenuation of ROS production by vascular tissue in these mice. Previous work has implicate d T-lymphocytes as mediators of the impaired endothelium-dependent dilation induced by either AngII-induced HTN[39] or HCh[40] in mice. In both instances, evidence was provided to implicate pro-inflammatory cytokines (TNF-α, IFN-γ) as potential chemical mediators of the T-cell dependent responses. Our findings would suggest that, in the presence of HTN+HCh, an AT1r-dependent protective agent is liberated by circulating blood cells, perhaps T-cells. A candidate molecule is interleukin-10, which has been shown to preserve endothelium-dependent vasodilation in diabetic mice via a superoxide-dependent mechanism[41].

Interleukin-10 appears to counteract impaired endothelium-dependent relaxation and upregulation of NADPH oxidase induced by AngII in murine aortic rings[42]. Regardless of the AT1r-dependent chemical mediator liberated from blood cells in the presence of HTN +HCh, our data lend support to the view that circulating cells play a role in mediating the vasomotor dysfunction associated with risk factors for cardiovascular disease.

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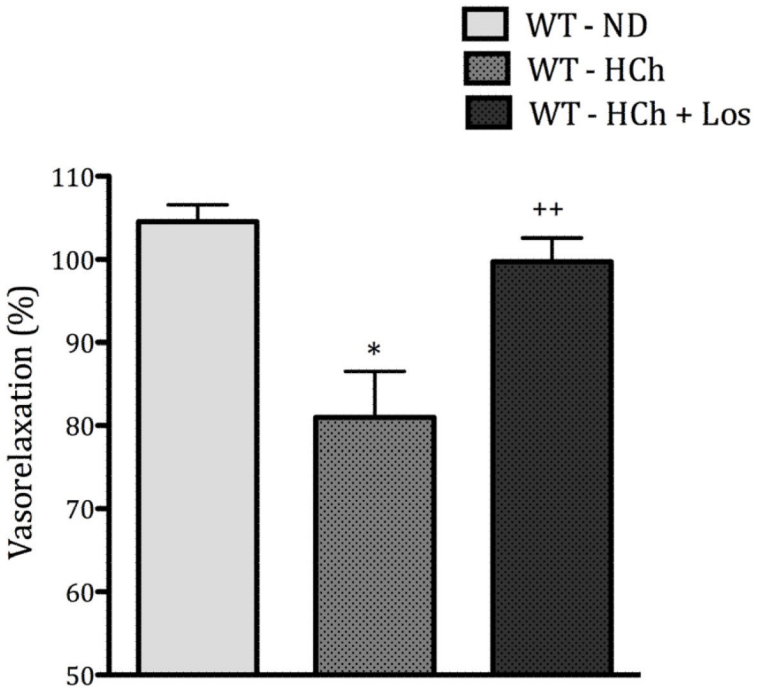

Figure 1.
Effects of AT1-receptor blockage with losartan (Los) on maximal relaxations produced by acethycholine (ACh). HCh; Diet-induced hypercholesterolemia. **, $p<0.01$ vs WT-ND group. ++, $p<0.01$ vs WT-HCh group (Ach induced dilation - 10^{-4}).

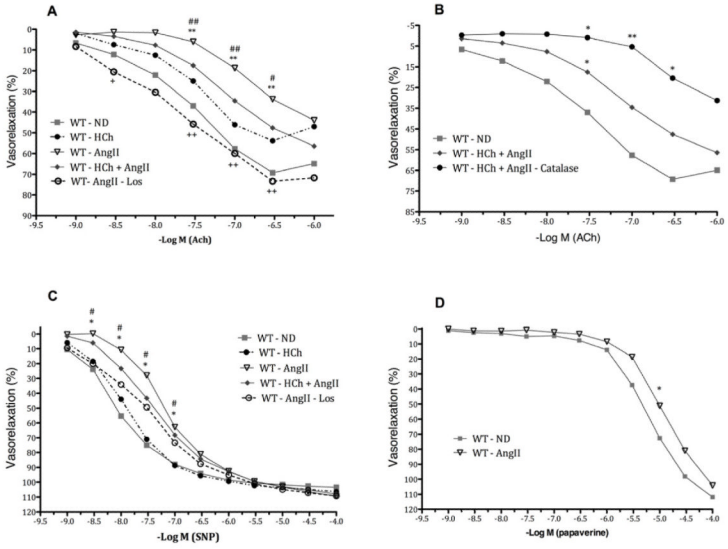

Figure 2.

Endothelium-dependent vasorelaxation to acetylcholine (ACh) (A and B), endothelium-independent vasorelaxation to sodium nitroprusside (SNP) (C) and endothelium/nitric oxide (NO)-independent vasorelaxation to papaverine (D) during diet-induced hypercholesterolemia (HCh) and/or angiotensin II administration, with or without losartan (Los). *, **, $p<0.05$ and 0.01 vs WT-ND group. ++, $p<0.01$ vs WT-AngII group. #, $p<0.05$ vs WT-HCh group.

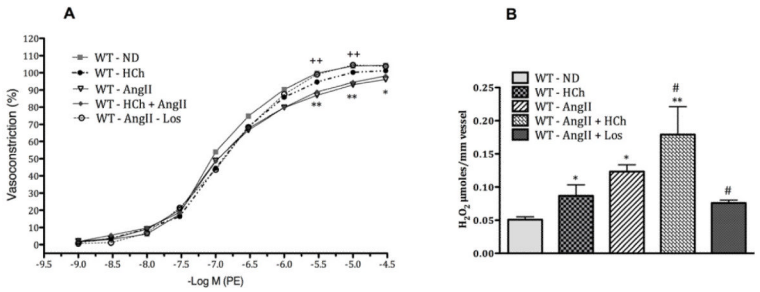

Figure 3.

Panel A: Endothelium-dependent vasoconstriction to phenylephrine (PE) during diet-induced hypercholesterolemia (HCh) and/or angiotensin II (AngII) induced hypertension, with or without losartan (Los) treatment (A). *, **, $p<0.05$ and 0.01 vs WT-ND group. ++, $p<0.01$ vs WT-AngII group. **Panel B**: Changes in H_2O_2 production in response to diet-induced hypercholesterolemia (HCh), angiotensin II (AngII) infusion, AngII infusion + HCh, and AngII infusion + losartan (Los) treatment. *, **, $p<0.05$ and 0.01 vs WT-ND group. #, ##, $p<0.05$ and 0.01 vs WT-AngII group.

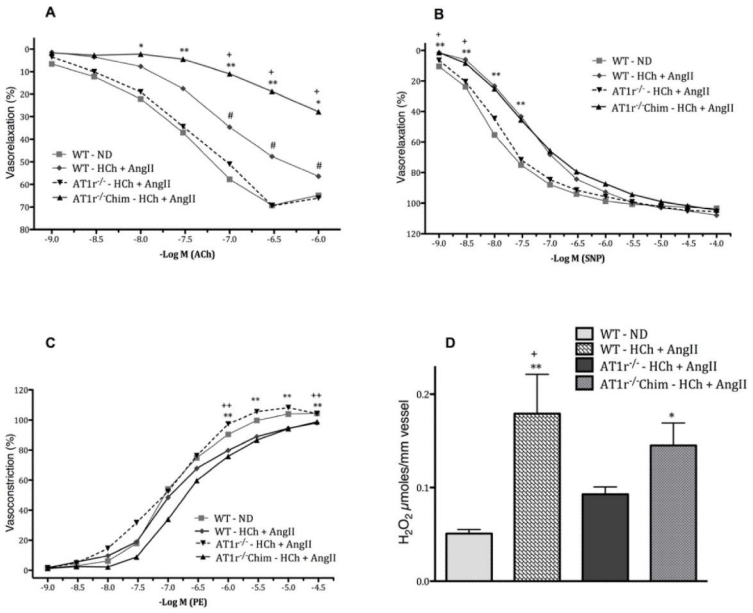

Figure 4.

Endothelium-dependent vasorelaxation to acetylcholine (ACh) (A), endothelium-independent vasorelaxation to sodium nitroprusside (SNP) (B), vasoconstriction to phenylephrine (PE) (D) and changes in H_2O_2 production (D) associated with the combination of diet-induced hypercholesterolemia (HCh) and angiotensin II (AngII)-induced hypertension in WT, AT1r$^{-/-}$ mice or AT1r$^{-/-}$ bone marrow chimeras (AT1r$^{-/-}$Chim). *, **, p<0.05 and 0.01; WT-HCh + AngII and/or AT1r$^{-/-}$Chim-HCh + AngII groups vs WT-ND. +, ++, p<0.05 and p<0.01; vs WT-HCh + AngII or AT1r$^{-/-}$Ch-HCh+AngII groups vs AT1r$^{-/-}$-HCh + AngII. #, p<0.05 vs AT1r$^{-/-}$Chim-HCh+ AngII group.

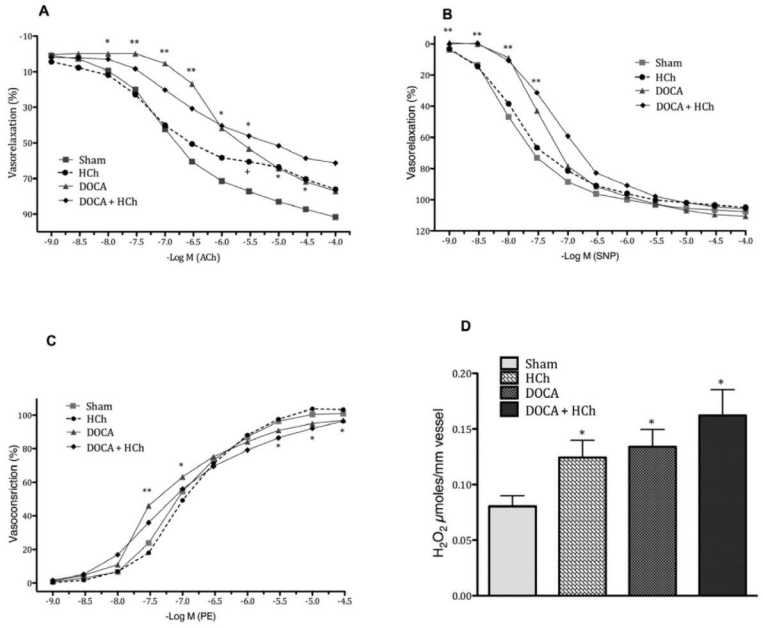

Figure 5.

Endothelium-dependent relaxation to acetylcholine (ACh) (A), endothelium-independent relaxation to sodium nitroprusside (SNP) (B), vasoconstriction to phenylephrine (PE) (D) and changes in H_2O_2 production (D) during diet-induced hypercholesterolemia (HCh) and/or DOCA-salt (DOCA)-induced hypertension. *, **, $p<0.05$ and 0.01; DOCA and/or DOCA +HCD groups vs Sham. +, ++, $p<0.05$ and $p<0.01$; HCh group vs Sham.

Table 1

Serum cholesterol concentration and systolic blood pressure in wild-type (WT), AT1a receptor knockout (AT1a-R−/−), chimeric (AT1r$^{-/-}$ into WT recipient; AT1aCH) and DOCA-salt mice maintained on a normal diet (ND) or high-cholesterol diet (HCD) with or without cholate.

	Cholesterol (mg/dl)	***Systolic Blood Pressure (mmHg)***
Angll model		
WT-ND	57.31 ± 4.18 (*n*=6)	103.75 ± 0.88 (*n*=3)
WT-HCh	112.25 ± 2.89 ** (*n*=10)	101.73 ± 1.80 (*n*=5)
WT-AngII	63.41 ± 5.06 (*n*=7)	141.50 ± 3.00 ** (*n*=5)
)WT-AngII-Los	61.83 ± 1.54 (*n*=5)	116.84 ± 1.68 ++ (*n*=6)
WT-AngII-HCh	128.12 ± 4.28 ** (*n*=8)	143.55 ± 3.53 ** (*n*=4)
AT1r$^{-/-}$AngII-HCh	102.60 ± 2.22 ** (*n*=6)	101.18 ± 0.60 (*n*=4)
AT1r$^{-/-}$Chim-AngII-HCh	112.00 ± 5.27 ** (*n*=6)	152.98 ± 3.74 ** (*n*=4)
DOCA salt model		
WT-ND	61.05 ± 3.24 (*n*=6)	102.78 ± 1.32 (*n*=6)
WT-HCh	115.53 ± 3.93 ** (*n*=6)	104.13 ± 0.84 (*n*=6)
WT-DOCA-salt	77.52 ± 3.58 (*n*=4)	136.52 ± 1.68 ** (*n*=4)
WT-DOCA-salt-HCh	102.33 ± 4.02 ** (*n*=5)	136.58 ± 3.59 ** (*n*=6)

WT indicates wild-type; ND, normal diet; HCh, hypercholesterolemia; Angll, Angiotensin II; Los, Losartan; AT1r$^{-/-}$, Angiotensin II-type-1a receptor deficient; AT1r$^{-/-}$ Chim, Angiotensin II-type-1a receptor deficient chimera; DOCA, deoxycorticosterone acetate.

Values are expressed as mean ± s.e.m

**, p<0.01 vs WT-ND group.

++, p<0.01 vs WT-AngII group.