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Low oxygen microenvironment and cardiovascular remodeling: Role of dual L/N-type Ca²⁺ channel blocker

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Abstract:

OBJECTIVE: Patients exposed to chronic sustained hypoxia frequently develop cardiovascular disease risk factors to ultimately succumb to adverse cardiovascular events. In this context, the present study intends to assess the role of cilnidipine (Cil), a unique calcium channel blocker that blocks both L-type and N-type calcium channels, on cardiovascular pathophysiology in face of chronic sustained hypoxia exposure.

MATERIALS AND METHODS: The study involved Wistar strain albino rats. The group-wise allocation of the experimental animals is as follows - Group 1, control (21% O₂); Group 2, chronic hypoxia (CH) (10% O₂, 90% N); Group 3, Cil + 21% O₂; and Group 4, CH (10% O₂, 90% N) + Cil (CH + Cil). Cardiovascular hemodynamics, heart rate variability, and endothelial functions (serum nitric oxide [NO], serum endothelial nitric oxide synthase [NOS3], and serum vascular endothelial growth factor [VEGF]) were assessed. Cardiovascular remodeling was studied by histopathological examination of the ventricular tissues, coronary artery (intramyocardial), and elastic and muscular arteries. Normalized wall index of the coronary artery was also calculated.

RESULTS AND CONCLUSION: The results demonstrated altered cardiovascular hemodynamics, disturbed cardiovascular autonomic balance, increased levels of VEGF and NOS3, and decreased bioavailability of NO on exposure to chronic sustained hypoxia. The histopathological examination further pointed toward cardiovascular remodeling. Treatment with Cil ameliorated the cardiovascular remodeling and endothelial dysfunction induced by CH exposure, which may be due to its blocking actions on L/N-type of calcium channels, indicating the possible therapeutic role of Cil against CH-induced cardiovascular pathophysiology.

Keywords:

Cardiovascular histopathology, cardiovascular remodeling, chronic hypoxia, cilnidipine, heart rate variability, normalized wall index

Introduction

A among a host of factors, hypoxia has been considered as one of the potential factors inducing cardiovascular remodeling.^[1,2] Patients with cardiovascular remodeling demonstrate a progressive deterioration of cardiovascular functions to ultimately succumb to adverse cardiovascular events such as hypertension, ischemic heart

disease, and cardiac arrhythmias.^[3,4] The trigger for hypoxia-induced cardiovascular remodeling could be an alteration of cardiovascular autonomic balance on exposure to low oxygen microenvironment.^[5] These observations lead us to seek for an adjunct therapeutic approach targeting the altered cardiovascular autonomic balance to prevent or postpone the occurrence of the adverse cardiovascular events in patients exposed to systemic hypoxia.

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Cilnidipine (Cil) is a novel calcium channel blocker (CCB). Like the conventional CCBs, Cil inhibits L-type Ca²⁺ channels on the vascular smooth muscle (VSM). It additionally inhibits N-type Ca²⁺ channels on sympathetic nerve terminals, thereby regulating sympathetic neurotransmission. This pharmacological profile of Cil makes it a suitable option that could be used to target cardiovascular autonomic balance which may be disturbed in chronic hypoxia (CH) exposure. Besides this action, Cil also exhibits potential antioxidant, renoprotective, and direct cardioprotective actions.^[6,7]

In this context, the current study was carried out to assess the cardiovascular autonomic balance and its role in cardiac remodeling including the coronary artery and systemic vascular remodeling (elastic and muscular artery) in experimental animals exposed to chronic sustained hypoxia and the effect of treatment with dual L-/N-type CCB, Cil. We hypothesize that cardiovascular remodeling induced by CH exposure could be ameliorated by the treatment with Cil, an L- and N-type CCB.

Materials and Methods

The institutional animal ethical committee approved the study (Ref: BLDE/BPC/641/2016-2017 dated October 21, 2016). The guidelines by the Committee for the Purpose and Control and Supervision of Experiments on Animals (CPCSEA), Government of India, were adhered to throughout the study.

Study design

Twenty-four laboratory-bred adult male albino rats Wistar strain (*Rattus norvegicus*), aged 8–10 weeks, weighing 180–250 g were obtained from the institution's animal house. The experimental animals were maintained at 22°C–24°C and 12-h light/dark cycle with food and water made available *ad libitum*. Before the initiation of the experimental protocol, all the experimental animals were acclimatized to the conditions in the laboratory for 7 days.

Table 1 presents the group-wise allocation of experimental animals. The bodyweight of the experimental animals was recorded before the commencement of the experimental protocol (day 0, preintervention) and repeated after 21 days of intervention (day 22, postintervention) and the percentage change in body weight was calculated. The bodyweight of the experimental animals of all groups was matched before the commencement of the experimental protocol. All the experimental animals were subjected to baseline/preintervention evaluation that included the recording of electrocardiogram (ECG), blood pressure (BP), and heart rate variability (HRV) analysis. Following this, the animals were subjected to

intervention for 21 days as depicted in Table 1. After completion of the intervention period, the experimental animals were subjected to postintervention evaluation (on day 22) that included the recording of ECG, BP, and HRV analysis. This was followed by the collection of blood in a plain tube with a clot activator. After a waiting period of 45 min, the collected blood was centrifuged at 600 ×g for 15 min to separate serum which was pipetted out and preserved at –20°C for further biochemical analysis, which included estimation of nitric oxide (NO), endothelial nitric oxide synthase (NOS3), and vascular endothelial growth factor (VEGF). The experimental rats were sacrificed between 9.00 AM and 11.00 AM by an overdose of ketamine (150 mg/kg, i.p.) as per the CPCSEA guidelines, Government of India, after the completion of the intervention period (day 22). Heart, thoracic aorta, and femoral artery were carefully dissected out and were immediately washed in ice cold saline to clear off excess blood. 10% neutral-buffered formalin was used to fix the tissues. The fixed tissues were embedded in paraffin, sectioned to 3–5 μm thickness, deparaffinized, rehydrated, and stained with hematoxylin and eosin (H and E) stain.^[8] The H and E-stained tissue sections were subjected to histopathological examination.

Exposure to chronic hypoxia

CH conditions were simulated by keeping the caged rats (4 per cage) in an acrylic chamber (300 l) that could accommodate up to 4 cages (16 rats). Normobaric hypoxia was induced by subjecting the experimental animals to 10% inspired oxygen (O₂) and 90% nitrogen (N₂). An influx of a combination of nitrogen and air was used to create a hypoxic environment. Soda-lime granules were used to remove CO₂, and humidity was maintained by using a desiccator. A temperature of 24°C–26°C was maintained. The cages were cleaned; food and water replaced twice every week. In the current study, the experimental animals were subjected to 21 days hypoxia.^[9]

Administration of drug

Cil was obtained from Laksh Finechem Pvt. Ltd, Gujarat, India. The calculated dose of Cil was 2.0 mg/kg body weight/day. The dose of Cil was estimated by the following formula.^[10]

Table 1: Study groups

Serial number	Groups	Intervention
1	Group 1 (control)	Na CMC (0.5%) by oral gavage for 21 days
2	Group 2 (CH)	CH + Na CMC (0.5%) by oral gavage for 21 days
3	Group 3 (Cil)	Cil (2.0 mg/kg/day) in Na CMC (0.5%) by oral gavage for 21 days
4	Group 4 (CH + Cil)	CH + Cil (2.0 mg/kg/day) in Na CMC (0.5%) by oral gavage for 21 days

n=6 in each group. Cil=Cilnidipine, Na CMC=Sodium carboxymethylcellulose, wt=Weight, CH: Chronic hypoxia

Rat equivalent dose (mg/kg) = Human dose (mg/kg) × km ratio.

Since Cil is poorly soluble in water, it was suspended in sodium carboxymethyl cellulose 0.5%. The suspension was made fresh every day and given to Cil-treated (Group 3) and CH-exposed Cil-treated (Group 4) groups every morning by oral gavage for 21 days.

Hemodynamic evaluation

The hemodynamic evaluation included the recording of ECG (lead II) for the determination of heart rate (HR) and measurement of BP. ECG was recorded using needle subcutaneous electrodes (Biopac Student Lab 4.1). BP was recorded noninvasively using a tail-cuff sensor (BioPac 200A) after the animal was placed in a restrainer. Mean of the three measurements was considered for each rat. The hemodynamic evaluation was performed before starting the intervention (preintervention/baseline, day 0) and repeated after 21 days of intervention (postintervention, day 22). All the recordings were performed after an overnight fast in the morning hours.

Heart rate variability analysis

Short-term HRV analysis using 5-min ECG RR interval data was done to assess the cardiovascular autonomic balance. Frequency-domain method of HRV analysis was done to assess the level of sympathetic activity, parasympathetic activity, and sympathovagal balance. The recorded ECG was inspected for artifacts and ectopic beats were manually deleted from the recording. For HRV analysis, the RR intervals obtained from the recorded ECG were exported to Kubios software version 2.0 (developed by the Department of Applied Physics, University of Eastern Finland, Finland). Low-frequency (LF) (normalized units, nu) component, high-frequency component (HF) (nu), and LF/HF ratio indicated the levels of sympathetic activity, parasympathetic activity, and sympathovagal balance, respectively.^[11]

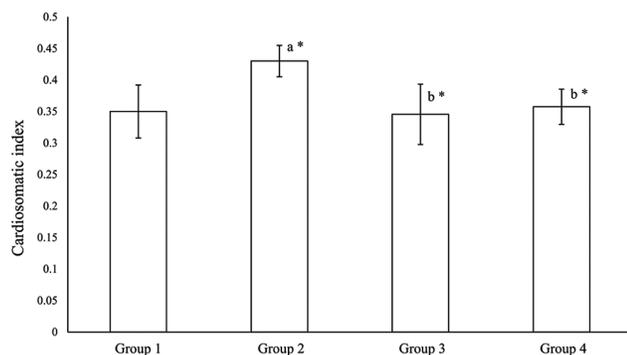


Figure 1: Comparison of cardiosomatic index among groups ($n = 6$ in each group). Superscripts a, b, c represent a significant difference between groups. "a" depicts comparison with Group 1, "b" depicts comparison with Group 2. * $P < 0.05$

Biomarkers of endothelial function

Endothelial function was evaluated by estimating the serum levels of VEGF, NOS3, and NO. NOS3 and VEGF were estimated by enzyme-linked immunosorbent assay (ELISA) (Merilyzer Eiaquant, Meril Diagnostics Pvt. Ltd) using commercially available ELISA kits (Chongqing Biospes Co., Ltd, China). NO was estimated by Griess reaction.^[12]

Histopathological examination for the assessment of cardiovascular remodeling

The stained specimens of the ventricles with coronary artery and the vasculature (elastic and muscular arteries) were assessed for CH-induced cardiovascular remodeling and the effect of treatment with Cil by examining the microscopic changes in the architecture.^[13]

Calculation of normalized wall index

Normalized wall index (NWI) indicates the portion of the total vessel area (TVA) constituted by the wall area (WA).^[14] It is considered a measure of arterial remodeling.^[15] In this study, NWI was calculated for coronary artery by using the histopathological images.^[13] ImageJ software (U. S. National Institutes of Health, Bethesda, Maryland, USA <https://imagej.nih.gov/ij/>) was used to analyze the histological images of the coronary artery. Lumen area (LA) and the TVA of the coronary artery were manually traced, and the values were obtained using the software. WA was calculated using the formula shown below.^[13,14]

$$WA = TVA - LA.$$

NWI for the coronary artery was calculated by using the formula shown below:^[13,14]

$$NWI = WA/TVA.$$

Statistical analysis

Statistical analysis was done using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The values are presented as mean ± standard deviation. For the analysis of the statistical significance of data across groups, one-way analysis of variance was used. Tukey's *post hoc* test was used to ascertain significant intergroup differences. Paired *t*-test was done for both pre- and post-intervention comparison within each group. Pearson's correlation was done to establish a relationship between a pair of variables. A statistical significance was considered at $P < 0.05$.

Results

Heart rate variability analysis

Table 2 depicts a comparison of pre- and post-intervention LF (nu), HF (nu), and LF/HF ratio among groups.

Table 2: Comparison of heart rate variability and cardiovascular hemodynamics among groups of experimental animals (n=6 in each group)

Parameter	Group 1 (control)	Group 2 (CH)	Group 3 (cil)	Group 4 (CH+cil)	ANOVA	
					F	P
LF (nu)						
Pre	47.26±1.73 ^a	49.14±2.16 ^a	47.12±2.08 ^a	48.26±2.08 ^a	0.87	0.483
Post	45.59±1.87 ^a	61.39±2.52 ^b	33.17±2.09 ^c	53.24±2.99 ^d	99.02	0.000*
Paired t-test	0.088	0.0008*	0.0002*	0.0055*		
Percentage change	3.7±2.46 ^a	24.98±4.01 ^b	29.61±2.62 ^b	10.28±2.73 ^c	65.31	0.000*
HF (nu)						
Pre	53.53±2.63 ^a	51.15±2.70 ^a	52.69±2.89 ^a	51.72±3.35 ^a	0.522	0.675
Post	55.18±2.41 ^a	37.79±2.04 ^b	58.03±3.58 ^a	49.85±2.53 ^a	43.73	0.000*
Paired t-test	0.08	0.004*	0.001*	0.38		
Percentage change	3.10±2.34 ^a	25.95±5.78 ^b	10.11±1.71 ^a	6.70±2.64 ^a	33.16	0.000*
LF/HF						
Pre	0.88±0.05 ^a	0.95±0.05 ^a	0.88±0.042 ^a	0.93±0.07 ^a	1.65	0.228
Post	0.84±0.02 ^a	1.62±0.13 ^b	0.55±0.05 ^c	0.96±0.15 ^a	73.89	0.000*
Paired t-test	0.06	0.002*	0.000*	0.59		
Percentage change	5.19±3.46 ^a	70.33±16.88 ^b	40.70±8.15 ^c	10.91±6.74 ^a	35.31	0.000*
Heart rate (beats/min)						
Pre	321.61±42.80 ^a	290.00±22.44 ^a	314.75±28.36 ^a	323.66±15.82 ^a	1.331	0.314
Post	294.29±49.49 ^a	284.52±20.33 ^a	262.95±8.37 ^a	306.00±14.00 ^a	1.489	0.271
Paired t-test	0.004*	0.04*	0.000*	0.01*		
Percentage decrease in heart rate	8.65±3.22 ^a	2.74±1.51 ^b	16.38±1.07 ^c	5.44±1.87 ^a	27.62	0.000*
MAP (mmHg)						
Pre	91.25±2.69 ^a	92.37±4.78 ^a	93.00±3.16 ^a	91.00±2.58 ^a	0.306	0.821
Post	96.75±1.70 ^a	108.50±6.60 ^b	99.75±2.87 ^a	84.00±4.32 ^c	23.39	0.000*
Paired t-test	0.001*	0.01*	0.002*	0.02*		
Percentage change in MAP	6.05±1.22 ^a	17.54±6.63 ^b	7.26±1.76 ^a	-6.61±1.70 ^a	9.309	0.002*

Values are presented as Mean±SD. Intergroup comparison of each parameter is presented horizontally. Horizontally the superscripts a, b, c, d are indicative of values that differ significantly from one another ($P<0.05$). Intragroup comparison (pre vs. post) of each parameter by paired t-test is presented vertically, * $P<0.05$. LF=Low frequency, HF=High frequency; nu=Normalized units, MAP=Mean arterial pressure, SD=Standard deviation

Low frequency (normalized units)

Intergroup comparison

Preintervention (day 0) LF (nu) was comparable between groups. Postintervention (day 22) LF was significantly elevated in Group 2 (CH) and Group 4 (CH + Cil) with a greater increase in Group 2. In contrast, it was significantly reduced in Group 3 (Cil).

Intragroup comparison

Comparison of pre- and post-intervention LF (nu) within each group revealed significantly elevated postintervention LF (nu) in Group 2 (CH) and Group 4 (CH + Cil). On the contrary, postintervention LF (nu) was significantly reduced in Group 3 (Cil) in comparison to its preintervention values. In Group 1 (control), pre- and post-intervention LF (nu) was comparable.

High frequency (normalized units)

Intergroup comparison

Preintervention (day 0) HF (nu) was similar between groups. On comparison of postintervention HF (nu) (day 22), it was significantly reduced in Group 2 (CH), Group 4 (CH + Cil) did not show any significant change in HF, and it was mildly elevated in Group 3 (Cil).

Intragroup comparison

A comparison of pre- and post-intervention HF (nu) within each group was done. There was a significant reduction in the postintervention HF in Group 2 (CH). In Group 4 (CH + Cil), no statistically significant differences were noted. In Group 3 (Cil), postintervention HF (nu) was significantly elevated compared to preintervention values. No significant differences were noted in Group 1 (control).

Low frequency/high frequency ratio

Intergroup comparison

Preintervention (day 0) LF/HF ratio was comparable between groups. Postintervention (day 22) LF/HF ratio was significantly different between groups. It was significantly increased in Group 2 (CH) and decreased in Group 3 (Cil) compared to Group 1 (control). In Group 4 (CH + Cil), the ratio was almost comparable with Group 1 (control).

Intragroup comparison

A comparison of pre- and post-intervention LF/HF ratio within each group was done. No significant differences existed in Group 1 (control). Postintervention LF/HF

HF ratio was significantly increased in Group 2 (CH) and decreased in Group 3 (Cil) compared to their respective preintervention values. In Group 4 (CH + Cil), postintervention LF/HF ratio was comparable to its preintervention value.

Cardiovascular hemodynamics

Table 2 depicts a comparison of hemodynamic parameters among groups.

Heart rate

Intergroup comparison

Preintervention (day 0) HR was comparable among groups. Interestingly, no significant differences were noted in the postintervention (day 22) HR among groups.

Intragroup comparison

Within each group, preintervention (day 0) HR was compared with their respective postintervention (day 22) HR by paired *t*-test. Postintervention HR was significantly decreased when compared to their respective preintervention HR in Group 1 (control). A similar pattern was observed in all other groups. Interestingly, the lowest (2.74 + 1.51%) and the highest (16.38 + 1.07%) decline in HR were observed in Group 2 (CH) and Group 3 (Cil), respectively.

Mean arterial pressure

Intergroup comparison

Preintervention (day 0) mean arterial pressure (MAP) was comparable among groups. Significant differences existed in the postintervention (day 22) MAP with significant elevation noticed in Group 2 (CH) and decline in Group 4 (CH + Cil) in comparison to Group 1 (control).

Intragroup comparison

Within each group, preintervention (day 0) MAP was compared with their respective postintervention (day 22) values by paired *t*-test. Postintervention MAP was significantly elevated when compared to their preintervention values in all groups, except for Group 4 (CH + Cil). Percentage change in MAP revealed an increase of 17.54% ± 6.63% in Group 2 and a decrease of 6.61% ± 1.70% in Group 4.

Assessment of endothelial functions

Table 3 presents a comparison of serum VEGF, serum NOS3, and serum NO among groups.

VEGF was significantly increased in Group 2 (CH) and Group 4 (CH + Cil). However, the magnitude of increase in VEGF in the two groups was dissimilar with higher values in Group 2 (CH) compared to Group 4 (CH + Cil). NOS3 was significantly elevated in Group 2 (CH) and Group 4 (CH + Cil) compared to other groups. Surprisingly, serum NO was significantly reduced in Group 2 (CH) despite elevated NOS3. Interestingly, in Group 4 (CH + Cil), serum NO was almost comparable to Group 1 (control) and significantly higher compared to Group 2 (CH).

Assessment of cardiovascular remodeling

Cardiosomatic index

Figure 1 shows a comparison of cardiosomatic index (heart weight to body weight ratio) among groups. The cardiosomatic index was significantly increased in Group 2 (CH) compared to other groups. No significant differences were observed in the cardiosomatic index in Group 1 (control), Group 3 (Cil), Group 4 (CH + Cil). Correlation between cardiosomatic index and LF/HF ratio, an indicator of sympathovagal balance, showed a statistically significant positive correlation ($R^2 = 0.6213$, $P = 0.000$).

Histopathological study of the ventricle and intramyocardial coronary artery

Figure 2 depicts a comparison of H and E-stained sections of the ventricular tissues with coronary microvasculature among groups. In Group 1 (control) and Group 3 (Cil), the microscopic examination of the ventricles revealed branching and anastomosing cardiac muscle fibres laid parallel and segregated by capillaries with intercalated discs seen connecting the cardiac muscle fibers. The coronary arteries appeared normal on histopathological examination.

In Group 2 (CH), mild hypertrophy of cardiac myocytes with nuclear enlargement and capillary congestion was noted. Coronary artery demonstrated moderate arteriosclerosis and congestion. Group 4 (CH + Cil) showed normal histology of the myocardium with mild arteriosclerosis and dilatation of the coronary artery.

Table 3: Comparison of biomarkers of endothelial function (n=6 in each group)

Parameter	Group 1 (control)	Group 2 (CH)	Group 3 (cil)	Group 4 (CH+cil)	ANOVA	
					F	P
Serum VEGF (pg/ml)	60.00±9.93 ^a	110.82±7.61 ^b	60.50±11.47 ^a	89.33±7.02 ^c	27.32	0.000*
Serum NOS3 (pg/ml)	16.59±2.86 ^a	35.04±6.62 ^b	12.67±2.36 ^a	31.54±2.993 ^b	26.69	0.000*
Serum NO (µmol/L)	11.21±2.63 ^a	6.95±1.76 ^b	10.04±1.25 ^a	9.38±1.81 ^a	3.24	0.056

Values are presented as mean±SD. Intergroup comparison of each parameter is presented horizontally. Horizontally the superscripts a, b, c are indicative of values that differ significantly from one another ($P < 0.05$). * $P < 0.05$. NOS3=Endothelial nitric oxide synthase, NO=Nitric oxide; VEGF=Vascular endothelial growth factor, SD=Standard deviation

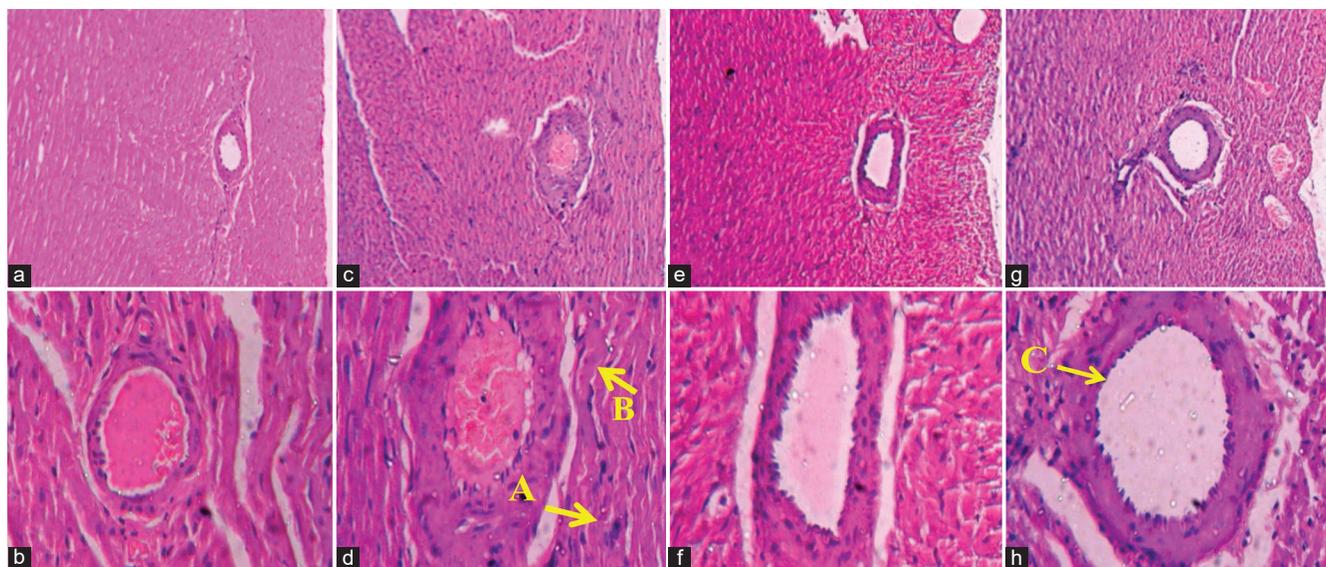


Figure 2: Photomicrograph of the ventricular tissue with coronary artery stained with hematoxylin and eosin stain from (a) Group 1 (control) ($\times 10$), (b) Group 1 (control) ($\times 40$); (c) group 2 (CH) ($\times 10$), (d) Group 2 (CH) ($\times 40$); (e) Group 3 (Cil) ($\times 10$), (f) Group 3 (Cil) ($\times 40$); (g) Group 4 (CH + Cil) ($\times 10$), (h) Group 4 (CH + Cil) ($\times 40$). A: Nuclear enlargement, B: Myocardial hypertrophy, C: dilatation of coronary artery. CH: Chronic hypoxia, Cil: Cilnidipine

Histopathological study of the elastic artery

Figure 3 depicts the H and E-stained sections of the elastic artery. In Group 2 (CH), tunica intima demonstrated a mild thickening, tunica media showed hyperplastic VSM, and an overall augmentation of the wall thickness of the elastic artery was noted. Group 4 (CH + Cil) demonstrated mild thickening of the tunica intima and aortic dilation. Remaining groups showed normal histopathology of the elastic artery.

Histopathological study of the muscular artery

Figure 4 depicts H and E-stained sections of the muscular artery. In Group 2 (CH), tunica intima demonstrated a mild thickening, tunica media showed hyperplastic VSM, and an overall augmentation of the wall thickness of the muscular artery was noted. Group 4 (CH + Cil) demonstrated mild thickening of the tunica intima. Remaining groups showed normal histopathology.

Normalized wall index of the coronary artery

Table 4 depicts a comparison of NWI among groups. Group 2 (CH) demonstrated a significantly higher NWI while Group 4 (CH + Cil) revealed a significantly lower NWI as compared to control (Group 1). Correlation between NWI of coronary artery and LF/HF ratio showed a statistically significant positive correlation ($R^2 = 0.6162$, $P = 0.001$). Correlation between NWI and serum VEGF showed a statistically significant positive correlation ($R^2 = 0.2431$, $P = 0.014$).

Discussion

The current study assessed the role of Cil, a CCB on cardiovascular remodeling in face of altered

cardiovascular autonomic balance induced by chronic sustained hypoxia. Cil inhibits calcium channels of L- and N-type, a unique property that distinguishes it from the conventional CCBs that block only the L-type calcium channels.

HRV analysis was done to assess the sympathetic activity, parasympathetic activity, and overall sympathovagal balance [Table 2]. In the present study, exposure to chronic sustained hypoxia increased sympathetic activity, decreased parasympathetic activity, and disturbed the overall sympathovagal balance. These alterations in the cardiovascular autonomic balance are a result of the activation of chemoreceptor reflex triggered by the stimulation of the carotid bodies by hypoxia. This reflex in addition to inducing respiratory changes elicits a powerful activation of the sympathetic nervous system (SNS). The elevated sympathetic drive, in turn, increases HR and BP.^[16] Paradoxically, the HR decreased [Table 2] in chronic sustained hypoxia (Group 2) despite the enhanced sympathetic drive in the present study. This could be due to direct depressive action of hypoxia on the heart that surpassed the stimulatory effects of enhanced sympathetic drive or maybe the β -adrenergic receptors in the heart were downregulated by hypoxia. The increased sympathetic drive tries to maintain the tissue perfusion by adjusting the cardiac output and altering the vascular conductance as evidenced by increased MAP in chronic hypoxia-exposed group (Group 2) in the current study [Table 2].^[17,18] In addition, the role of other factors such as increased viscosity secondary to raised hematocrit and decreased bioavailability of NO causing endothelial dysfunction in elevating the MAP has to be considered.^[19]

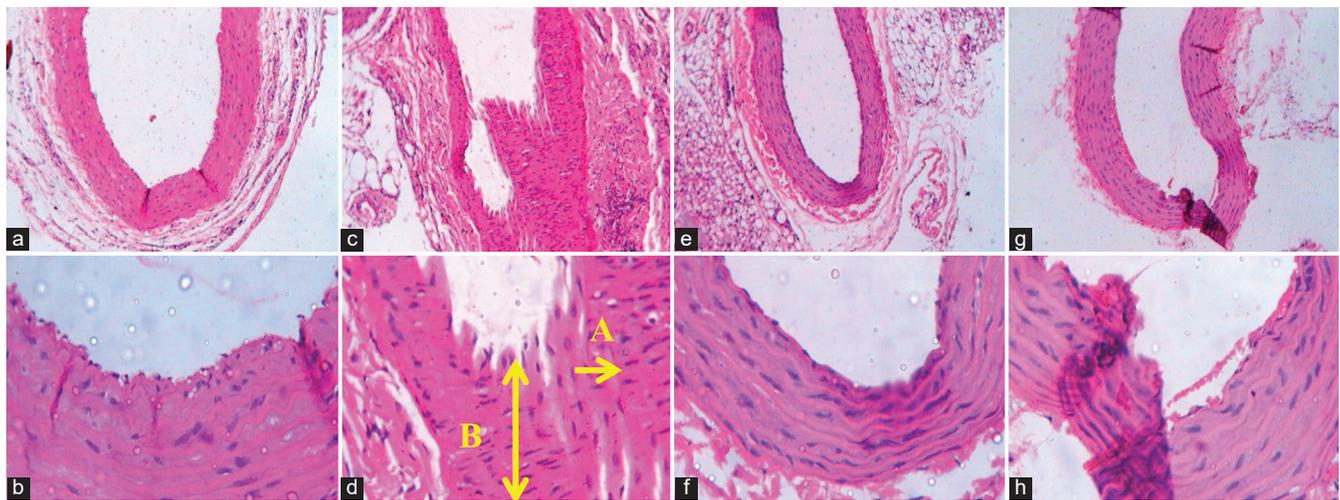


Figure 3: Photomicrograph of elastic artery stained with hematoxylin and eosin stain from (a) Group 1 (control) (×10), (b) Group 1 (control) (×40); (c) Group 2 (CH) (×10); (d) Group 2 (CH) (×40); (e) Group 3 (Cil) (×10); (f) Group 3 (Cil) (×40); (g) Group 4 (CH + Cil) (×10); (h) Group 4 (CH + Cil) (×40). A: Hyperplastic smooth muscle cells, B: Increase in wall thickness. CH: Chronic hypoxia, Cil: Cilnidipine

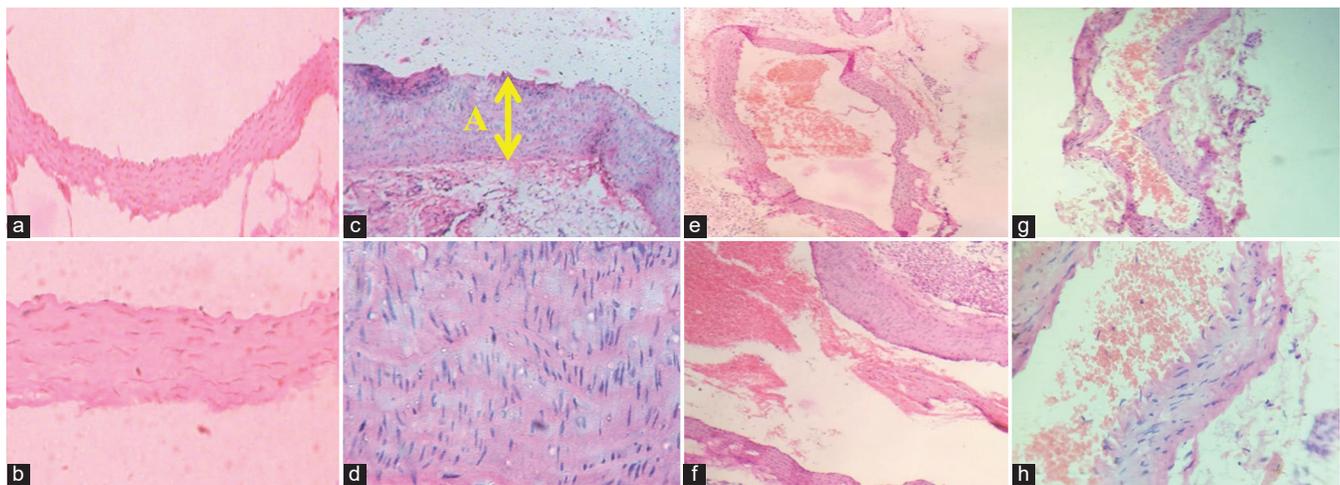


Figure 4: Photomicrograph of muscular artery stained with hematoxylin and eosin stain from (a) Group 1 (control) (×10); (b) Group 1 (control) (×40); (c) Group 2 (CH) (×10); (d) Group 2 (CH) (×40); (e) Group 3 (Cil) (×10); (f) Group 3 (Cil) (×40); (g) Group 4 (CH + Cil) (×10); (h) Group 4 (CH + Cil) (×40). A: Increased wall thickness. CH: Chronic hypoxia, Cil: Cilnidipine

Table 4: Comparison of normalized wall index of coronary artery

Parameter	Group 1 (control)	Group 2 (CH)	Group 3 (Cil)	Group 4 (CH+Cil)	ANOVA	
					F	P
NWI	0.620±0.02 ^a	0.71±0.01 ^b	0.55±0.02 ^c	0.54±0.02 ^c	83.935	0.000*

Values are presented as mean±SD (n=6 in each group). Intergroup comparison of each parameter is presented horizontally. Horizontally the superscripts a, b, c are indicative of values that differ significantly from one another (P<0.05). *P<0.05. NWI=Normalized wall index

Histopathological examination of the ventricles revealed hypertrophy of the ventricular myocytes [Figure 2]. Besides, increased cardiosomatic index in the CH-exposed group [Figure 1] is in support of ventricular hypertrophy. The observed ventricular hypertrophy can be explained by the elevated sympathetic drive that induces cardiac muscle hypertrophy either by direct action or indirectly by inducing a sustained increase in the MAP (pressure overload).^[20-22] Sympathetic activity and vascular function are interlinked, and any perturbation

orchestrates the initiation and the progression of cardiovascular diseases.^[23] Increased sympathetic drive induces proliferation and hypertrophy of the VSM cells that increase the overall vessel wall thickness.^[24] In the present study, the histopathological examination of the coronary artery, elastic artery (thoracic aorta), and muscular artery (femoral artery) [Figures 2-4] revealed hyperplastic VSM in the tunica media and overall increased wall thickness in the CH-exposed group. Further, the increased NWI of the coronary artery in

CH-exposed rats pointed toward thickened vessel wall and arterial remodeling. In addition, NWI of the coronary artery and LH/HF ratio were positively correlated substantiating the role of sympathetic activity in the genesis of vascular remodeling.

In the present study, CH exposure increased serum VEGF and serum NOS3. VEGF and NOS3 are among several target genes induced by hypoxia-inducible factor (HIF)-1, a transcription factor that governs the cell response to hypoxia. VEGF is a key molecule governing angiogenic response to hypoxia.^[25] It also stimulates the production of NO by NOS3.^[26] NO is a potent vasodilator that brings about dilation of the systemic arteries increasing blood flow to the peripheral tissues.^[27] Surprisingly, in our study, serum NO was significantly reduced on CH exposure despite the elevated levels of VEGF and NOS3 [Table 3]. The endothelial dysfunction resulting from the reduced NO levels is observed in several cardiovascular disease risk factors. There is sufficient evidence showing a reciprocal relationship between SNS activity and markers of endothelial function.^[23] Increased sympathetic activity compromises endothelial function as evidenced by decreased NO in the current study. Thus, elevated sympathetic activity induces cardiovascular remodeling and compromises endothelial function culminating into the development of adverse cardiovascular events and diseases. Hence, it can be concluded that the enhanced sympathetic drive, which was originally initiated as an adaptive signal in response to CH exposure, may herald the onset of maladaptive signaling when chronically elevated.

Cil treatment in CH-subjected rats (Group 4) lowered the CH-induced elevation in the sympathetic activity [Table 2]. These actions of Cil could be explained by its ability to block Ca²⁺ channels of the N-type on the sympathetic nerve endings, thereby reducing sympathetic neurotransmission. Thus, we propose that Cil has a potential role to control the chronic sustained hypoxia-induced enhanced sympathetic drive, owing to its pharmacological profile.

Cil treatment in Group 4 lowered the HR and MAP that were elevated by CH exposure [Table 2]. The observed decrease in HR and MAP in Group 4 (CH + Cil) was due to the action of drug Cil, L- and N-type Ca²⁺ channel blocker. It brings about vasorelaxation by blocking the Ca²⁺ channels of the L-type on the VSM and reduces the sympathetic neurotransmission by blocking the Ca²⁺ channels of the N-type on the sympathetic nerve endings.^[28] Thus, overall action of cilnidipine was to reduce the HR and BP.

Hence, it may be stated that chronic sustained hypoxia may be vital in the pathophysiology of hypertension and Cil may have a potentially beneficial action in its management.

Cil-administered CH-subjected rats (Group 4) demonstrated serum NO comparable to that of control in contrast to CH-exposed group (Group 2) [Table 3]. Cil possibly increased the production of NO by enhancing the NOS3 in the elastic arterial tissues. Further, the Cil was also found to increase the release of NO from vascular endothelium.^[29] Hence, Cil probably has a role in improving endothelial functions.

CH-subjected Cil-administered rats (Group 4) demonstrated cardiosomatic index comparable to that of the control [Figure 1]. Examination of H and E-stained sections of the ventricles was comparable to control. Besides, the coronary artery demonstrated mild arteriosclerosis and dilatation [Figure 2]. Thus, Cil ameliorated CH-induced cardiac remodeling. This action of Cil may be ascribed to its ability to suppress sympathetic hyperactivity, improve NO, and control the increase of arterial blood pressure induced by CH.^[30,31] Cil has also been demonstrated to improve coronary microvascular remodeling.^[31] This unique profile of Cil makes it a potential drug against CH-induced cardiovascular pathophysiology.

Conclusion

The present study demonstrates CH-induced alterations of cardiovascular autonomic functions. These alterations culminated into hemodynamic changes, early changes related to cardiovascular remodeling, and endothelial dysfunction. The CCB Cil probably has a beneficial role in improving CH-induced cardiovascular remodeling on account of L- and N-type calcium channel inhibitory actions.

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Conflicts of interest

There are no conflicts of interest.

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