Low oxygen microenvironment and cardiovascular remodeling: Role of dual L/N-type Ca\(^{2+}\) 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% O\(_2\)); Group 2, chronic hypoxia (CH) (10% O\(_2\), 90% N); Group 3, CH + Cil; and Group 4, CH (10% O\(_2\), 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
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 Ca\(^{2+}\) channels on the vascular smooth muscle (VSM). It additionally inhibits N-type Ca\(^{2+}\) 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.\(^{[9]}\) 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 (O\(_2\)) and 90% nitrogen (N\(_2\)). An influx of a combination of nitrogen and air was used to create a hypoxic environment. Soda-lime granules were used to remove CO\(_2\) 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.\(^{[10]}\)

| Serial number | Groups       | Intervention                                      |
|---------------|--------------|---------------------------------------------------|
| 1             | Group 1      | 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.

**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). It is considered a measure of arterial remodeling.[14] 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]

\[
NWI = \frac{WA}{TVA}.
\]

**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 |
|----------------------------------|-------------------|--------------|--------------|------------------|-------|
| LF (nu)                          |                   |              |              |                  |       |
| Pre                              | 47.26±1.73        | 49.14±2.16   | 47.12±2.08   | 48.26±2.08       | 0.87  |
| Post                             | 45.59±1.87        | 61.39±2.52   | 33.17±2.09   | 53.24±2.99       | 99.02 |
| Paired t-test                    | 0.08              | 0.0008       | 0.0002       | 0.0055           |       |
| Percentage change                | 3.7±2.46          | 24.98±4.01   | 29.61±2.62   | 10.28±2.73       | 65.31 |
| HF (nu)                          |                   |              |              |                  |       |
| Pre                              | 53.53±2.63        | 51.15±2.70   | 52.69±2.89   | 51.72±3.35       | 0.522 |
| Post                             | 55.18±2.41        | 37.79±2.04   | 58.03±3.58   | 49.85±2.53       | 43.73 |
| Paired t-test                    | 0.08              | 0.004        | 0.001        | 0.38             |       |
| Percentage change                | 3.10±2.34         | 25.95±5.78   | 10.11±1.71   | 6.70±2.64        | 33.16 |
| LF/HF                            |                   |              |              |                  |       |
| Pre                              | 0.88±0.05         | 0.95±0.05    | 0.88±0.04    | 0.93±0.07        | 1.65  |
| Post                             | 0.84±0.02         | 1.62±0.13    | 0.55±0.05    | 0.96±0.15        | 73.89 |
| Paired t-test                    | 0.06              | 0.002        | 0.000        | 0.59             |       |
| Percentage change                | 5.19±3.46         | 70.33±16.88  | 40.70±8.15   | 10.91±6.74       | 35.31 |
| Heart rate (beats/min)           | 321.6±42.80       | 290.00±22.44 | 314.75±28.36 | 323.66±15.82     | 1.331 |
| Post                             | 294.29±49.49      | 284.52±20.33 | 262.95±8.37  | 306.00±14.00     | 1.489 |
| Paired t-test                    | 0.04              | 0.04         | 0.000        | 0.01             |       |
| Percentage decrease in heart rate| 8.65±3.22         | 2.74±1.51    | 16.38±1.07   | 5.44±1.87        | 27.62 |
| MAP (mmHg)                       |                   |              |              |                  |       |
| Pre                              | 91.25±2.69        | 92.37±4.78   | 93.00±3.16   | 91.00±2.58       | 0.306 |
| Post                             | 96.75±1.70        | 108.50±6.60  | 99.75±2.87   | 84.00±4.32       | 23.39 |
| Paired t-test                    | 0.001             | 0.01         | 0.002        | 0.02             |       |
| Percentage change in MAP         | 6.05±1.22         | 17.54±6.63   | 7.26±1.76    | −6.81±1.70       | 9.309 |

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 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 (CH) compared to other groups. In Group 2 (CH), moderate hypertrophy of cardiac myocytes with nuclear enlargement and capillary congestion was noted. Coronary artery demonstrated moderate arteriosclerosis and congestion. In Group 4 (CH + Cil), postintervention LF/HF ratio was comparable to Group 2 (CH). 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 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) compared to Group 4 (CH + Cil). NOS3 was significantly elevated in Group 2 (CH) compared to other groups. No significant differences were observed in the cardiomatic index in Group 1 (control), Group 3 (Cil), Group 4 (CH + Cil). Correlation between cardiomatic 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±9.93a       | 110.82±7.61a | 60.50±11.47a  | 89.33±7.02c       | 27.32   | 0.000* |
| Serum NOS3 (pg/ml) | 16.59±2.86a       | 35.04±6.62a  | 12.67±2.36a   | 31.54±2.993a      | 26.69   | 0.000* |
| Serum NO (µmol/L) | 11.21±2.63a       | 6.95±1.76a   | 10.04±1.25a   | 9.38±1.81a        | 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 different 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.
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]
Histopathological examination of the ventricles revealed hypertrophy of the ventricular myocytes [Figure 2]. Besides, increased cardio-somatic 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). Sympathetic activity and vascular function are interlinked, and any perturbation orchestrates the initiation and the progression of cardiovascular diseases. Increased sympathetic drive induces proliferation and hypertrophy of the VSM cells that increase the overall vessel wall thickness. 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

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 |
|-----------------|---------------------|---------------|----------------|------------------|-------|
| NWI             | 0.620±0.02a         | 0.71±0.01b    | 0.55±0.02c     | 0.54±0.02c       | 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
Bagali, et al.: Hypoxia, cardiovascular remodeling, and Ca\(^{2+}\) channel blocker

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 Ca\(^{2+}\) 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 Ca\(^{2+}\) channel blocker. It brings about vasorelaxation by blocking the Ca\(^{2+}\) channels of the L-type on the VSM and reduces the sympathetic neurotransmission by blocking the Ca\(^{2+}\) 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 cardio somatic 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.

**References**

1. Essop MF. Cardiac metabolic adaptations in response to chronic hypoxia. J Physiol 2007;584:715-26.
2. Adeoye OO, Bouthors V, Hubbell MC, Williams JM, Pearce WJ. VEGF receptors mediate hypoxic remodeling of adult ovine carotid arteries. J Appl Physiol (1985) 2014;117:777-87.
3. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling—Concepts and clinical implications: A consensus paper from an International Forum on Cardiac Remodeling. Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol 2000;35:569-82.

4. Turnbull CD. Intermittent hypoxia, cardiovascular disease and obstructive sleep apnoea. J Thorac Dis 2018;10:S33-S39.

5. Falcone C, Colonna A, Bozzini S, Matrone B, Guasti L, Paganini EM, et al. Cardiovascular risk factors and sympato-vagal balance: Importance of time-domain heart rate variability. J Clin Exp Cardiolog 2014;5:289.

6. Aritomi S, Harada E, Sugino K, Nishimura M, Nakamura T, Takahara A. Comparison of the cardioprotective and renoprotective effects of the L/N-type calcium channel blocker, cilnidipine, in adriamycin-treated spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 2015;42:94-112.

7. Hishikawa K, Takase O, Idei M, Fujita T. Comparison of antioxidant activity of cilnidipine and amlodipine. Kidney Int 2009;76:230-1.

8. Reddy RC, Devaranavadagi B, Yendigeri SM, Bagali S, Kulkarni RV, Das KK. Effect of L-ascorbic acid on nickel-induced alteration of cardiovascular pathophysiology in wistar rats. Biol Trace Elem Res 2020;195:178-86.

9. Das KK, Jargar JG, Saha S, Yendigeri SM, Singh SB. α-Tocopherol supplementation prevents lead acetate and hypoxia-induced hepatic dysfunction. Indian J Pharmacol 2015;47:285-98.

10. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 2016;7:27-31.

11. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. Front Public Health 2017;5:258.

12. Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: A critical evaluation. Clin Chem 1995;41:892-6.

13. Patil BS, Kanthi PS, Reddy CR, Das KK. Emblica officinalis (Amla) ameliorates high-fat diet induced alteration of cardiovascular pathophysiology. Cardiovasc Hematol Agents Med Chem 2019;17:52-63.

14. Hartevedt AA, Denswil NP, Van Hecke W, Kujif HJ, Vink A, Spriet WG, et al. Data on vessel wall thickness measurements of intracranial arteries derived from human circle of Willis specimens. Data Brief 2018;19:6-12.

15. Zhu C, Teng Z, Sadat U, Young VE, Graves MJ, Li ZY, et al. Normalized wall thickness specific and MRI-based stress analysis of atherosclerotic carotid plaques: A study comparing acutely symptomatic and asymptomatic patients. Circ J 2010;74:2360-4.

16. López-Barneo J, González-Rodríguez P, Gao L, Fernández-Agüera MC, Pardal R, Ortega-Sáenz P. Oxygen sensing by the carotid body: Mechanisms and role in adaptation to hypoxia. Am J Physiol Cell Physiol 2016;310:C629-42.

17. Walsh MP, Marshall JM. The early effects of chronic hypoxia on the cardiovascular system in the rat: Role of nitric oxide. J Physiol 2006;575:263-75.

18. Kacimi R, Richealet JP, Corsin A, Abousahl I, Crozatier B. Hypoxia-induced downregulation of beta-adrenergic receptors in rat heart. J Appl Physiol (1985) 1995;72:1377-82.

19. Calbet JA. Chronic hypoxia increases blood pressure and noradrenaline spillover in healthy humans. J Physiol 2003;551:379-86.

20. Hunter JJ, Chien KR. Signalling pathways for cardiac hypertrophy and failure. N Engl J Med 1999;341:1276-83.

21. Julian RJ. The response of the heart and pulmonary arteries to hypoxia, pressure, and volume. A short review. Poult Sci 2007;86:1006-11.

22. Ito H, Adachi S, Tamamori M, Fujisaki H, Tanaka M, Lin M, et al. Mild hypoxia induces hypertrophy of cultured neonatal rat cardiomyocytes: A possible endogenous endothelin-1-mediated mechanism. J Mol Cell Cardiol 1996;28:1271-7.

23. Bruno RM, Ghidoni L, Seravalle G, Dell’Oro K, Taddei S, Grassi G. Sympathetic regulation of vascular function in health and disease. Front Physiol 2012;3:284.

24. Fisher JP, Young CN, Fadel PJ. Central sympathetic overactivity: Maladies and mechanisms. Auton Neurosci 2009;148:5-15.

25. Rodríguez-Miguelez P, Lima-Cabello E, Martínez-Flórez S, Almar M, Cuebas MJ, González-Gallego J. Hypoxia-inducible factor-1 modulates the expression of vascular endothelial growth factor and endothelial nitric oxide synthase induced by eccentric exercise. J Appl Physiol (1985) 2015;118:1075-83.

26. Le Moine CM, Morash AJ, McClelland GB. Changes in HIF-1α protein, pyruvate dehydrogenase phosphorylation, and activity with exercise in acute and chronic hypoxia. Am J Physiol Regul Integr Comp Physiol 2011;301:R1098-104.

27. Yoon G, Oh CS, Kim HS. Distinctive expression patterns of hypoxia-inducible factor-1α and endothelial nitric oxide synthase following hypergravity exposure. Oncotarget 2016;7:33675-88.

28. Takahara A. Cilnidipine: A new generation Ca channel blocker with inhibitory action on sympathetic neurotransmitter release. Cardiovasc Ther 2009;27:124-39.

29. Fan L, Yang Q, Xiao XQ, Grove KL, Huang Y, Chen ZW, et al. Dual actions of cilnidipine in human internal thoracic artery: Inhibition of calcium channels and enhancement of endothelial nitric oxide synthase. J Thorac Cardiovasc Surg 2011;141:1063-9.

30. Takahara A, Koganei H, Takeda T, Iwata S. Antisympathetic mechanism. J Mol Cell Cardiol 1996;28:1271-7.