Original Article

Study on the therapeutic effect of Imdu on chronic mountain sickness in a rat model

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Abstract: Aims: Isosorbide mononitrate has been used as a long-term treatment for coronary heart disease and the prevention of vasospasm and mixed-type angina pectoris. However, its use in the treatment of chronic mountain sickness (CMS) in a rat model has not been reported. This study aimed to use isosorbide mononitrate for CMS therapy in a rat model to guide and expand the scope of its clinical application. Methods: In this study, we simulated a high altitude environment using hypoxic pulmonary hypertension (HPH) as a guide for the CMS rat model. Echocardiography, cardiac pathological sections, and serum indices were employed to evaluate the model. We studied the effect of isosorbide mononitrate on levels of endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), and nitric oxide (NO) in CMS rat serum and their correlations with the pathology and cardiac ultrasound findings. Result: Results showed that a moderate dose of isosorbide mononitrate given to CMS rats improved all indicators in the positive control group, except for pulmonary arterial pressure, blood oxygen saturation, and partial pressure of oxygen. When subjected to a high dose of isosorbide mononitrate, most of the indicators in the CMS rats improved in the positive control group. Conclusion: Isosorbide mononitrate can be used in the treatment of CMS in rats to protect the heart and improve systemic hypoxemia.

Keywords: Isosorbide, hypoxic pulmonary hypertension, chronic mountain sickness

Introduction

Imdur®, also known as isosorbide 5-mononitrate sustained-release tablets, is a commonly used nitrate medication used in clinics [1]. It releases nitric oxide (NO) through multi-step enzymatic reactions in the body that relaxes vascular smooth muscle [2, 3]. It can reduce heart burden and myocardial oxygen consumption by expanding the vascular system, lowering peripheral resistance, and reducing myocardial oxygen consumption [4]. It can also improve the blood supply to the heart by dilating the coronary arteries, opening or increasing the collateral blood flow, and increasing the coronary blood flow, while also promoting the re-distribution of the myocardial blood flow [5]. The specific mechanism by which Imdur® acts has not been reported.

Pulmonary arterial hypertension (PAH) is a common clinical disorder. It serves as an important pathophysiological basis for chronic pulmonary heart disease. There are many causes for this disease even though the mechanism is unclear. Hypoxia-induced pulmonary hypertension is more common than PAH. Hypoxic pulmonary hypertension is one of the important physiological causes of chronic mountain sickness (CMS) [6]. Mitochondrial swelling is an early morphological change of myocardial cells during hypoxia. Low oxygen can damage the mitochondria [7] through swelling of the mitochondrial cristae, which expands the separation between sparse and crest [8]. Acute hypoxia can cause mitochondrial degeneration and necrosis [9]. Mitochondria change as metabolism changes. These changes include variation in quantity, size, arrangement, and altered activity of the oxidative phosphorylation-related enzymes in mitochondria [10]. We have found substantial damage in epicardial arteries and in the myocardium in CMS rat models. In this study, we simulated hypoxic conditions. Hypoxic pulmo-
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Coronary hypertension (HPH) was used as an indicator of the CMS rat model, supplemented by echocardiograms and heart biopsy results, to evaluate the model and study the effects of isosorbide mononitrate on changes in endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), and nitric oxide (NO), in CMS rat sera and its correlations with the pathology and cardiac ultrasound findings.

Material and methods

Laboratory equipment


Low-pressure oxygen chamber: The artificial testing chamber for peacetime and wartime at the Northwest Territories (located in the Lanzhou Military Region, Urumqi Hospital, Xinjiang). Conditions: simulated altitude: 5000 m; temperature range: 18°C-26°C; humidity: 40%-60%; pressure: 54.1 KPa (391.4 mmHg); partial oxygen pressure: 10.84 KPa (80.8 mmHg).


Investigated drugs

Imdur® was purchased from the Astrazeneca Company (product lot number: 1206085). The main ingredient was isosorbide mononitrate. Nifedipine tablets (Shanxi Yunpeng Pharmaceutical Co., Ltd., product lot number C1-20304).

Reagents


Animal preparation and animal model establishment

A total of 120 healthy SD rats (body weight 160-200 g) were provided by the Animal Center of Xinjiang Medical University, license number: SCXK (xin) 2011-0004.

Control group (CG): This group comprised 20 healthy SD rats, half male and half female, weighing 160-200 g. The control experimental conditions were as follows: simulated altitude: 720 m, temperature range: 18°C-26°C, humidity range: 40%-60%, pressure: 93.2 KPa, and oxygen partial pressure: 19.54 KPa. The rats were kept in these laboratory conditions for 45 days. Food was readily available with no medical intervention. The behavior of the SD rats was observed daily to monitor their movement, food and water intake, hair, feces, urine, and secretions from their eyes, ears, nose, and mouth. The body weights of the rats were measured daily.

CMS model group (MG): This group comprised 100 healthy SD rats (half male and half female), with body weight ranging from 160 g to 200 g. The rats were kept in a low-oxygen pressure cabin for 30 days. The experimental conditions were as follows: simulated elevation: 5000 m, temperature: 18°C-26°C, humidity: 40%-60%, pressure: 54.1 KPa, Oxygen partial pressure: 10.84 KPa. The behavior of these SD rats was observed daily to monitor their movement, food and water intake, hair, feces, urine, and secretions from their eyes, ears, nose, and mouth. The body weights of the rats were measured daily.

The experimental design and implementation were approved by the Animal Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (approval number: IACUC-20130217063).

Laboratory animals and grouping

The 120 SD rats were randomly divided into six groups by stratified randomization. In each model, 60 female and 60 male rats were divided randomly by gender stratification. Using a
random number table, the rats were divided into six groups with an equal number of male and female rats in each. There were 20 rats per group. The conditions in each group were as follows: Plain control group (CG): No medication intervention, feed 45 days in plain environment; Mountain model group (MG): No medication intervention, feed 45 days in plateau environment; Positive control group (NE): Feed 30 days in plateau environment, after successfully reaching plateau condition. Nifedipine tablets (2.7 mg) were given once daily by gavage for 15 days. Imdur® low-dose group (LDIM): Feed 30 days in plateau environment, after successfully reaching plateau condition. Imdur® (0.8099 mg) was given once daily by gavage for 15 days. Imdur® dose group (MDIM): Feed 30 days in plateau environment, after successfully reaching plateau condition. Imdur® (1.6199 mg) was given once daily by gavage for 15 days. Imdur® high-dose group (HDIM): Feed 30 days in plateau environment, after successfully reaching plateau condition. Imdur® (3.2398 mg) was given once daily by gavage for 15 days.

Method for determination of pulmonary arterial pressure (PAP)

After anesthesia, rats were held in supine position along the neck midline to perform an incision and blunt dissection. The trachea was then exposed along with the parts of the subline inverted-T incision. The endotracheal intubation was fixed and directly connected to the ventilator. The ventilator frequency was adjusted to 60 times/min, with a tidal volume of 6 mL/kg and breathing ratio of 3:2. Rats were fixed in supine position; chest was cut open along the midline of the sternum, and lungs and heart were fully exposed. Ventricular pressure and pulmonary artery pressure were measured using a heparinized saline needle at the upper right corner of the right ventricle, and the position of the needle was observed by eye. The further end of the needle and catheter obtained using a pressure transducer were connected to the pressure variation of the biological signal recorder to record the experimental data.

Pathological examination method

Preparation of wax heart specimens: Heart tissue samples fixed with 10% formaldehyde solution were placed in 70% alcohol solution for 3 h, 80% alcohol solution for 2 h, 90% alcohol solution for 1.5 h, 95% alcohol solution for 2 h, and 100% alcohol solution for 1 h. They were rendered transparent by transferring to p-xylene for 30 min and infiltrated with molten paraffin wax for 3 h at a low melting point of 54°C. The wax-impregnated specimens were solidified naturally as wax blocks at room temperature.

Heart tissue slice preparation: Wax-embedded tissues were used to obtain paraffin sections of 4 µm each. They were subsequently expanded in 30%-40% alcohol solutions and 38°C water to obtain slices for microscopic observation. The microscopic slides were transferred to an oven at 37°C overnight and left at room temperature.

Routine H&E stain: The slices were transferred to p-xylene solution three times in a row; dewaxed for 15 min; transferred to 100% alcohol solution for 2 min, 95% alcohol for 2 min, and 80% alcohol for 2 min, twice in a row; and washed with tap water. They were then subjected to hematoxylin staining for 6 min and washed with tap water. The colors were separated using 0.1% hydrochloride, alcohol, and tap water. They were treated with 0.5% ammonia water and washed with tap water three times. The specimens were transferred to eosin dye for several seconds and washed with tap water. Finally, they were transferred to 80% alcohol, 95% alcohol, and 100% alcohol for a few seconds and then dehydrated with 100% alcohol for 2 min. After drying the slices for 10 min in a thermostat at 64°C, we prepared dry mounts for observation under an optical microscope.

Echocardiographic examination

One bottle of ketamine (100 mg/2 mL), one bottle of diazepam (10 mg/2 mL), and one bottle of atropine (0.5 mg/mL) were mixed and diluted to 10 mL. The diluted liquid was injected into the abdominal cavity of anesthetized SD rats (0.75 mL/100 g). The SD rats were fixed on a plate, and 8% barium sulfide solution was used prior to shaving the fur from the chest of the rats. The examination was conducted using a color Doppler ultrasonic diagnostic instrument (Philips HDII XE 453561263181), with a probe frequency of 7.5 MHz (speed 12-4). The SD rats were probed by the sternum, and apical four-chamber view showed the structure of the rats' four cavities of the heart, including SD rats left ventricular systolic and diastolic diameter, right ventricular diameter,
right ventricular outflow tract and right ventricular anterior wall thickness and interventricular septum thickness, and left ventricular ejection fraction. In the SD rats, a left ventricular long axis plane was made near the sternum by using M-type ultrasonic measurement ventricular movements and cardiac function parameters.

Serological marker testing method

The SD rats were anesthetized by intraperitoneal injection with 0.75 mL/100 g of the 1:1 diluted solution from a mix containing 2 mL of ketamine (100 mg/2 mL), 2 mL of diazepam (10 mg/2 mL), and 1 mL of atropine (0.5 mg/mL). Blood was drawn from the abdominal aorta. The blood was set at room temperature for 30 min and centrifuged at 3000 rpm for 20 min at a low temperature. The supernatant was collected and tested for the presence of inflammatory mediators in the serum including ET-1, NO, and VEGF by using the appropriate kits purchased.

Hematocrit and hemoglobin measurement

Hematocrit and hemoglobin levels were measured by using the BC-5300Vet Automatic Hemacytometer and its ancillary reagents from WanRui Co., Shenzhen, China. The tests were completed within 0.5-1 h after blood sampling.

Blood oxygen saturation and blood oxygen partial pressure measurements

The blood oxygen saturation and blood oxygen partial pressure of the plain control group were tested in the plain environment, and all other groups were tested in the hypobaric chamber that simulated a high-altitude environment (artificial environment laboratory testing chamber in Northwest Territories, Urumqi Hospital, Xinjiang).

Statistical analysis

All measurement data were tested for normality and homogeneity. Data that followed a normal distribution were expressed in mean and standard deviation. Single-factor ANOVA was used to compare means among different groups. The mean differences between groups were compared using LSD and SNK 22 methods. The inspection level α was 0.05.

Results

Pathological examinations from each group of CMS rats

Pathological findings are shown in Figure 1. In the control group (CG), the heart tissue structure was normal under a low- and high-magnification. No obvious lesions were observed. The myocardial fibers aligned well, and the cellular stain was clear with dense nuclei.

In the mountain model group (MG), severe hyperemia of the sub-epicardial blood vessels was visible under a low-magnification microscope. The epicardial vessels were severely congested, and the stripes were unclear. Under a high-magnification microscope, some heart muscles were swollen, and the cytoplasm showed granular degeneration. Some of the myocardial fiber eosinophilia degenerated significantly and were infiltrated by inflammatory cells.

In the positive control group (NE), the sub-epicardial blood vessels were slightly dilated and congested under a low-magnification microscope. The myocardial interstitial blood vessels were occasionally dilated and congested. The stripes were not clearly observed. Under a high-magnification microscope, mild edema could be seen in the myocardial cells. A small amount of eosinophilic degeneration in cardiac myocytes could be detected, and inflammatory cell infiltration was visible.

In the Imdur® low-dose group (LDIM), the epicardial blood vessels were dilated and congested significantly under a low-magnification microscope. Diffuse hemorrhage from myocardial vasculature was obvious. Under a high-magnification microscope, there were visible scattered bleeding points in the myocardial interstitia, few inflammatory cells, some eosinophilic lesions, and occasional granular degeneration.

In the Imdur® medium-dose group (MDIM), the sub-epicardial blood vessels showed mild hyperemia under a low-magnification microscope. There was no bleeding from the myocardial vessel. Under a high-magnification microscope, the myocardial fibers showed normal arrangement. Very few eosinophilic lesions, granular degenerations, and few inflammatory cell infiltrations were observed.
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In the Imdur® high-dose group (HDIM), under a low-magnification microscope, the myocardial arrangement was normal, and there were random bleeding points in the myocardial interstitia. A high-magnification microscope revealed no eosinophilic lesion cells and no significant infiltration of inflammatory cells.

Results of the serological marker testing from each group of CMS rats

The serological testing results of the ET-1 marker are shown in Figure 2. Compared with CG, the ET-1 content from MG, NE and LDIM increased, whereas that from MDIM and HDIM decreased significantly ($P < 0.05$). Compared with MG, the ET-1 content from CG, NE, LDIM, MDIM, and HDIM all decreased significantly ($P < 0.05$).

The serological testing results of the NO marker are shown in Figure 3. Compared with the CG, the NO content from MG and NE decreased, whereas that from MDIM and HDIM increased significantly ($P < 0.05$). Compared with MG, the NO content from CG, NE, HDIM, MDIM, and LDIM all increased significantly ($P < 0.05$).

The serological testing results of the VEGF marker are shown in Figure 4. Compared with the CG, the VEGF contents from MG, NE, HDIM, MDIM, and LDIM all increased significantly ($P < 0.05$). By contrast, compared with the MG, the VEGF contents from CG, NE, HDIM, MDIM, and LDIM all decreased significantly ($P < 0.05$).

Results of the PAP, hemoglobin, hematocrit, blood oxygen saturation, and oxygen partial pressure tests from each group of CMS rats

As shown in Table 1, PAP in MG, NE, LDIM, and MDIM was significantly higher than that in the CG ($P < 0.05$), but no significant difference was found between the HDIM group and the CG ($P > 0.05$). Compared with the MG group, PAP for NE, LDIM, MDIM, and HDIM was significantly reduced ($P < 0.05$).

Compared with CG, Hb in MG, NE, LDIM, MDIM, and HDIM significantly increased ($P < 0.05$). Compared with MG, the Hb level in LDIM, MDIM, and HDIM significantly decreased ($P < 0.05$).

Compared with CG, the Hct level in MG, NE, LDIM, MDIM, and HDIM significantly increased...
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As for blood oxygen saturation (SaO₂), compared with CG, the level of SaO₂ in MG, NE, LDIM, MDIM, and HDIM significantly decreased (P < 0.05). The SaO₂ levels in NE and HDIM were significantly higher than that in MG (P < 0.05), whereas those in LDIM and MDIM were not significantly different from that in MG (P > 0.05).

Echocardiography results

As shown in Figure 5, compared with MG, the left ventricular diastolic vertical diameter in each DIM group was decreased, and the difference of each dose group was minimal. In the high-dose group the left ventricular systolic pressure reduced, the effects of the low dose-group and middle-dose group were not obvious. The right ventricular pressure of every dose group decreased, but the effect was not obvious. The increase in the heart ejection fraction was minimal. The vertical diameter of the right atrium decreased in the same give medicine group, in the high-dose group the right atrium vertical diameter decreased significantly, and in the middle-dose group and low-dose group the relative reduction was less.
**Table 1.** Results of the pulmonary arterial pressure, hemoglobin, hematocrit, blood oxygen saturation and oxygen partial pressure tests from each group of CMS rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pulmonary artery pressure (mmHg)</th>
<th>Hb (g/L)</th>
<th>Hct (%)</th>
<th>SaO$_2$ (%)</th>
<th>PaO$_2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>19.51 ± 2.3</td>
<td>151.9 ± 15.7</td>
<td>36.3 ± 4.2</td>
<td>99.3 ± 1.6</td>
<td>99.9 ± 4.6</td>
</tr>
<tr>
<td>MG</td>
<td>41.1 ± 4.3*</td>
<td>243.1 ± 21.8'</td>
<td>69.6 ± 3.5'</td>
<td>60.3 ± 2.5'</td>
<td>31.2 ± 9.1'</td>
</tr>
<tr>
<td>NE</td>
<td>29.2 ± 2.5*</td>
<td>217.9 ± 18.2'</td>
<td>60.1 ± 6.8'</td>
<td>68.1 ± 3.6'</td>
<td>39.2 ± 7.2'</td>
</tr>
<tr>
<td>HDIM</td>
<td>22.3 ± 3.2*</td>
<td>169.3 ± 12.9*</td>
<td>48.6 ± 5.3*</td>
<td>70.3 ± 2.5*</td>
<td>37 ± 3.1*</td>
</tr>
<tr>
<td>MDIM</td>
<td>30.3 ± 4.1*</td>
<td>182.3 ± 15.3*</td>
<td>52.4 ± 4.3*</td>
<td>65.5 ± 3.2*</td>
<td>33.2 ± 2.3*</td>
</tr>
<tr>
<td>LDIM</td>
<td>39.1 ± 3.5*</td>
<td>199.5 ± 13.8*</td>
<td>56.5 ± 5.9*</td>
<td>62.3 ± 1.1*</td>
<td>32.4 ± 4.1*</td>
</tr>
</tbody>
</table>

Note: VS control group, *P < 0.05; VS model group, ▲P < 0.05.

This demonstrates that in DIM the high-dose is effective in the treatment of chronic plateau disease.

**Discussion**

The central mechanism in the progression of HPH is the increase in pulmonary vascular resistance. The main pathological changes are pulmonary vascular structure remodeling, characterized by enhanced pulmonary vasoconstriction and proliferation of smooth muscle cells [11]. Early studies suggested that high PAP results from the enhanced pulmonary vasoconstrictive response [12]. When the pulmonary vascular smooth muscle cells are hypoxic, excess oxygen free radicals will be produced. The consumption of large amounts of superoxide dismutase (SOD) leads to SOD and catalase (CAT) reduction. When the accumulated oxygen free radicals in the body cannot be eliminated, they provoke a cascade of lipid peroxidation reactions that disrupt cell membrane integrity and lead to intracellular calcium overload, causing lethal damage to cells and resulting in weakened vascular endothelium-dependent vasodilation and increased vasoconstriction [13]. An increasing number of experimental results showed that pulmonary vascular reconstruction is the pathological basis that leads to different diseases with elevated pulmonary pressure. The specific mechanism underlying the cause of pulmonary vascular reconstruction remains unknown. Current studies have shown that several factors interrupt the proliferation of pulmonary vascular smooth muscle cells, thereby causing extracellular matrix components, including collagen fibers and stretch fibers, to accumulate. The non-muscle-type arteries form new muscle layers, and endothelial cell swelling eventually leads to the thickening of the pulmonary vascular wall, and narrowing the vascular cavity [14].

**Figure 5.** The results of echocardiograph.
Our study showed that the PAP increased significantly in MG compared with that in the normal CG, indicating that the CMS rat model reliably revealed the disease phenotypes. Hypoxia can cause myocardial morphological changes. The hypoxic damage of the cardiomyocytes is characterized by damage to mitochondria, capillaries, and intercalated discs. In this study, the rats in MG showed epicardial, arterial, and myocardial interstitial vein swelling; fuzzy stripes; swelling in a portion of the heart muscle; granular degeneration of the cytoplasm; significant degeneration of eosinophilic cells in myocardial fibers; and infiltration of inflammatory cells. Ultrasonic examination also proved that hypoxia could damage the heart. All these findings further demonstrated the successful modeling of the CMS condition in rats and the improvement after the drug intervention.

Modern research has shown that endothelin (ET) and NO are important cytokines secreted by vascular endothelial cells. They help regulate vascular relaxation and contraction [14]. ET can be divided into three subtypes: ET-1, ET-2, and ET-3 [15], where ET-1 is particularly important. ET-1 is an acidic polypeptide that consists of 21 amino acids. It is a highly potent vascular contracting [16] and mitosis-inhibiting substance [17]. Aortic, pulmonary, and limb arteries and other vessels are very sensitive to ET-1. The vasoconstrictive function of ET-1 is achieved through the endothelin subtype 1A receptor. Once activated, the receptor can block the voltage-gated potassium channels, causing vasoconstriction [18]. NO is a vascular relaxation factor produced by endothelial cells in blood vessels. It is synthesized from L-arginine by NO synthase. Under normal conditions, due to its high hydrophobicity, it can quickly diffuse (or is mediated by certain carriers) into smooth muscle cells to activate soluble guanylate cyclase and produce a large amount of guanosine 3', 5'-cyclic phosphate (cGMP) [13]. The increased intracellular level of cGMP consequently activates cGMP-dependent protein kinase, an enzyme that phosphorylates proteins and decreases the intracellular Ca

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to vascular smooth muscle relaxation causes an increase in pulmonary artery pressure.

In this study, the ET-1 level in MG was higher than that in CG, whereas the NO level in MG was lower than that in CG. This result further confirmed the above conclusion. Isosorbide mononitrate-sustained release tablets are NO donors [2]. We found that different dosages of the medication significantly improved the NO content in CMS rats. This finding further indicated that exogenous NO replenished the reduced endogenous NO reservoir. Medium and high doses of Imdur® significantly reduced the ET-1 level in CMS rats. Imdur® relaxes vascular smooth muscle [3] and enhances endothelium-mediated vasoactive effects. It can also inhibit the proliferation of vascular smooth muscle [23, 24]. Subsequently, Imdur® can balance ET-1 and NO to alleviate pulmonary hypertension induced by hypoxia.

Hypoxia can cause polycythemia [25]. Erythrocytes are important components of the blood, and their primary function is to transport oxygen and carbon dioxide. Hemoglobin is the main component in erythrocytes [26]. The volume ratio of erythrocyte per liter of blood is known as the hematocrit count (Hct). Its value reflects the increase or decrease of red blood cells in the body. In a certain range, Hct is proportional to the oxygen-carrying capacity of the blood. The higher the Hct value, the higher the blood oxygen-carrying capacity will be [27]. In this study, the rats in the plateau model had significantly higher hemoglobin and Hct values than the rats in the plain CG. After Imdur® treatment, their hemoglobin and Hct values were lower than the plateau model group. The percentage of blood oxygenation content in the total oxygen-carrying capacity of the blood is known as blood oxygen saturation. It is an important index reflecting the body's oxygen levels, which is commonly used to evaluate the blood oxygen saturation degree of the body in low oxygen environments. It is widely appreciated as an indicator that monitors body condition in hypoxic environments [28]. Studies have shown that ordinary people in hypoxic conditions exhibit decreased oxygen saturation [29]. This study found that the SaO

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level in the
Imdur® high-dose group was significantly increased in comparison with that in the MG group. Thus, Imdur® could enhance SaO₂ in vivo under hypoxic conditions.

VEGF is a new type of mitogen that can specifically bind to the receptors on the surface of endothelial cells [30]. It is a hypoxia-inducible protein, and hypoxia is a strong factor that induces VEGF expression in vitro and in vivo. In normal conditions, VEGF sustains normal organ development and function. Under hypoxic conditions, VEGF gene expression increases [14]. This increase may be due to induction by oxygen-sensitive transcription factors [31] such as HIF-1 [32]. Low oxygen can lead to the generation of new blood vessels [33]. Under this condition, the organism can establish collateral circulation through enhanced VEGF expression to compensate for its needs [34]. In this study, we also found that the level of VEGF significantly increased in the MG model group. Acarregui et al. reported that hypoxia mediates the up-regulation of VEGF mRNA expression [35]. We also found significantly reduced VEGF levels in the CMS rat model after Imdur® was given, compared with the groups without treatment; this result suggested that Imdur® could prevent increasing VEGF levels.

**Conclusion**

Imdur® can adjust the balance of ET-1 and NO; affect serum levels of VEGF; and influence changes in PAP, Hb, Hct, PaO₂, and SaO₂ indices in CMS rats. We found that the CMS model group, compared with CG, had significant increases in ET-1 and VEGF and a decrease in NO. The positive control drug, nifedipine, as well as Imdur® at high, medium, and low doses could reduce the levels of ET-1 and VEGF and increase the level of NO. The Imdur® high-dose group showed optimal results on normalization of serum levels of VEGF. A medium dose of Imdur® reduced ET-1 levels to that of the plain CG level. The NO level in the Imdur® low-dose group reached the same level as that in the plain CG. We also found that the CMS model group, compared with CG, had significant increases in PAP, Hb, and Hct and decreases in SaO₂ and PaO₂. Positive control drug (i.e., nifedipine) and Imdur® at high, medium, and low doses could reduce PAP, Hb, and Hct and increase SaO₂. The Imdur® high-dose group showed the best improvement among all the groups in this study. The positive control drug, nifedipine, could effectively increase PaO₂, whereas the high-, medium-, and low-dose groups had no obvious effects on this index. We concluded that isosorbide mononitrate had successful effects on rats with CMS, and it could protect cardiac function and improve systemic hypoxemia.

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**Disclosure of conflict of interest**

None.

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