Available online at www.medicinescience.org

ORIGINAL RESEARCH

Medicine Science International Medical Journal

Medicine Science 2018;7(4):748-53

The use of serum hypoxia-inducible factor two alpha levels and diagnostic values in adult carbon monoxide poisoning

Ismail Altintop[1], Mehmet Tatli[1], Cigdem Karakukcu[2], Ahmet Ozturk[3]

[1]Kayseri Training and Research Hospital, Department of Emergency Medicine, Kayseri, Turkey
[2]Kayseri Training and Research Hospital, Department of Biochemistry, Kayseri, Turkey
[3]Erciyes University Medical Faculty, Department of Biostatistics, Kayseri, Turkey

Received 22 March 2018; Accepted 09 April 2018
Available online 19.04.2018 with doi: 10.5455/medscience.2018.07.8780

Abstract

Carbon monoxide poisoning (COP) is a life-threatening intoxication which should be interfered immediately to be prevented from serious damages. The effect of carbon monoxide (CO) in the body at the cell level is mediated by a CO-releasing molecule. This molecule results in heme degradation via heme oxygenase. Because of the high affinity of CO on heme, many enzymes with biological signal transduction cause heme protein increase. Universally, this signal activation with similar enzymes and mechanisms are used in COP. The most widely used test is Carboxyhaemoglobin (COHb) in serum. The COHb is used as a standard parameter in the diagnosis of COP. COHb levels, are high after CO poisoning and decrease rapidly with oxygen therapy in the first four hours. So COHb is insufficient to demonstrate chronic ischemic damage of hypoxia. Hypoxia-inducible factors (HIFs) are the master regulators of oxygen homeostasis. The best known HIF isoforms in the literature are HIF-1α and HIF-2α. Recently, more data have found about selective HIF-2α responsive genes and indicate the importance of this isoform in hypoxic gene regulation. The present study purposed to determine the change in HIF-2α values in adult patients in diagnosing COP. A significant statistical correlation was found between (p=0.05) serum HIF-2α levels and the first COHb level. HIF-2α levels at 4th hour in COP patients were significantly higher like first COHb. HIF-2α levels can be used as a new biomarker in long-term for monitoring COP patients.

Keywords: Carbon monoxide, carboxyhemoglobin, hypoxia-inducible factor, poisoning

Introduction

Carbon monoxide (CO) is a toxic gas in an environment and is one of the most common causes of poisoning-related deaths worldwide. Carbon monoxide poisoning (COP) is increasing due to the widespread use of carbon-based fuels universally. Carbon monoxide has various transient and permanent effects on the human body depending on the duration of CO exposure and effectiveness of therapy [1]. Long and high dose exposure even with immediate treatment may lead to permanent damages especially in organs vulnerable to hypoxia [2].

Carbon monoxide poisoning has an effect on both the neurological and cardiovascular systems, and recent studies have demonstrated prognosis [2]. Hence, the most important problem for emergency that neurological involvement in COP patients shows poor physicians is to cope with the neurological damage. Even if the neurological effect is understood in COP patients, the degree of influence cannot be predicted. Carboxyhaemoglobin levels in blood gases have been used as the diagnostic test but recent studies found out that there was a weak relationship between COHb levels and clinical presentation [3]. In addition, COHb levels decrease rapidly after oxygen therapy so when investigating for long-term or delayed effects of CO, it becomes invisible in blood [3,4]. New, long-acting diagnostic markers seem to be needed not to fail in diagnosing COP.

Carbon monoxide poisoning can be discussed pathophysiologically in two parts. The first is the hypoxic theory and the second is the cellular theory. According to the hypoxic theory, CO molecule binds to haemoglobin 230-270 times more than oxygen [4]. The haemoglobin-bound CO reduces oxygen transport capacity [4,5]. After all, it causes hypoxia in all tissues in the body [4]. According to cellular theory; CO inhibits the mitochondrial electron transport enzyme system in a cell [5,6]. Due to the high affinity of the CO on the heme, many enzymes induce the increase of the heme protein with biological signal activation [4]. Polymorphonuclear leukocytes increase and cause lipid peroxidation in the brain. This lipid peroxidation is responsible for the delayed neurological effects of COP [4,7]. In COP, the oxidative stress plays an important role in hypoxic-ischemic brain injury [5]. Furthermore, COP increases the production of reactive oxygen species [1].

***Coresponding Author:** Ismail Altintop, Kayseri Training and Research Hospital, Department of Emergency Medicine, Kayseri, Turkey
E-mail: draltintop1@hotmail.com

Levels of free oxygen radicals in hypoxic conditions are not high enough to eliminate them [6]. Various markers have been used to determine the degree of damage.

The diagnosis of COP is based on the level of carboxy haemoglobin measured by the co-oximeter [1,2]. Hypoxia has several effects on organs and systems [1]. Troponin is the most commonly used agent for determining heart damage [6]. But no consensus has been reached on determining ischemic brain damage. For this reason, our study aimed to use HIF-2α levels to diagnose and evaluate the severity of patients' ischemic brain during COP. According to the literature review we conducted, there was no study that evaluated HIF levels in patients with acute CO poisoning.

Hypoxia-inducible factor (HIF)

When the tissues exposed to hypoxia the amount of oxygen in tissues is reduced. Reduced oxygen levels stimulate posttranslational modification of proteins and cell metabolism. This event simultaneously initiates gene transcription. HIFs are the main regulator molecules in oxygen hemostasis [8]. HIFs have 3 isoforms (HIF-1α, HIF-2α, and HIF-3α) [9]. The most known and studied forms are HIF-1α and HIF-2α. While HIF-1α is mainly involved in endothelial cells, HIF-2α isoform is involved in hypoxic gene regulation mentioned above. Although HIV-1α and HIV-2α coexist in tissues, their amounts and response to hypoxia are different in different conditions (e.g. ischemic diseases, vascular diseases or tumour presence) [8,9]. HIF-2α play role in certain tissues containing brain and kidney [10,11]. As a result, HIF-2α may be used as a beneficial tool to diagnose and follow-up COP in patients.

Material and Method

Study population

A single-center, diagnostic randomize controlled study included patients older than 17 years of age who presented to the hospital with CO poisoning between December 2016 and June 2017, and a healthy control group. Prior to the study, a protocol was approved by the local hospital ethics committee. An informed consent form was approved by patients and control groups included in the study. This study confirmed to the Helsinki Declaration.

Study protocol

A total of 87random numbers were generated by SPSS software and assigned to a study and a control group. The study group was instructed with 66 participants at first who were exposed to CO and had clinical findings including a headache, dizziness, vomiting, fatigue and some degrees of unconsciousness. Unfortunately, 26 of them had to be excluded due to some chronic illnesses i.e. chronic kidney failure, severe lung disease, pregnancy, low age, the last but not the least low COHb levels. And the other five patients had been excluded due to insufficient samples. Finally, all the study were composed of 35 patients of a study group and 21 patients of control groups. The details of groups are shown in figure 1. Medical history was obtained from conscious patients and from the relatives of unconscious patients, and all home medications were recorded. Demographic data and Glasgow coma scores were also recorded. Blood was drawn at the time of hospital admission to measure arterial blood gas, COHb, electrolytes, and troponin I, along with other tests. The study's inclusion and exclusion criteria are shown in Table 1 below. The diagnosis of COP was made with the patients' medical history and blood carboxyhaemoglobin (COHb) levels above 10%.

Biochemical measurements

Blood gases were analysed immediately in emergency departments (ED) laboratory in a blood gas analyser (ABL700). COHb levels were measured and recorded. Troponin I was measured using an automatic autoanalyzer. Biochemical analyse were done with serum creatinine, AST, ALT, GFR, Lactate, CK-MB, and troponin. To analyse HIF-2α all other blood samples taken from the patients were stored at -80 °C after turning for 15 minutes at 3,000 cycles. Finally, blood samples were analysed in biochemical tubes to measure the value of HIF-2α by ELISA (Cayman Chemical Co, Ann Arbor, Michigan, USA).

Statistical analysis

Patients were divided into two groups (study, control) according to their clinical findings of COP and serum COHb levels. Power analysis was performed with a power-size for 2-sample t-test with the Minitab 17 program (α.0005, stdev 0.65, difference 1.0). The sample size was calculated as 17 by power analyse. Biochemical markers and blood gas analyses data were transferred to the computer environment. Chi-square test was used to examine categorical variables. Categorical variables were analyzed using the chi-squared test. Also, continuous variables with a normal distribution were compared using the independent t-test and the one-way analysis of variance (ANOVA). Pearson's correlation analysis was used to examine the correlation between serum HIF-2α and other tests. A receiver operating characteristics (ROC) curve was calculated for serum HIF-2α levels and other biomarkers. CCHb, cTnI, GFR. SPSS version 22.0 (SPSS Inc, Chicago, Illinois) statistical software and MedCalc® (2011statistical software version 11.5.1) were used for statistical analysis. The measured p-value of less than 0.05 was considered statistically significant.

Results

There were 34 females and 22 male patients, with an age range of 57.2±19.6 in the former study group and 41.6±14.8 in the control group. There was no significant difference between age and genus. There were no significant vital findings on admission to the hospital among groups. A headache, dizziness, vomiting, weakness and fatigue were statistically significant in a study group ($p<0.001$). Loss of consciousness and syncope were only observed in 6 patients in study group and were not statistically significant. The cause of poisoning was stove (coal or wood) in 30 (85.7%) cases. The demographic, clinical and laboratory characteristics of the patients are given in Table 2.

Table 1. Exclusion criteria.

Exclusion criteria
Acute hypoxia caused by different factors and non-current diagnosis criteria of COPa.
Severe lung, heart, liver, kidney and blood system diseases.
Hematologic diseases.
Pregnancy, lactating
Inadequate medical record.
Age <18 years.
COHb level of <10%.

[a]World Health Organization-Examination COP criteria(24).

Glasgow Coma Scale of 32 (91.4%) patients of the study group were GCS 14-15. GCS was found 9-13 in two patients and 9 in one patient. In the study group, 29 (82.9%) of the patients were discharged urgently and 6 patients (17.1%) were hospitalized. Mortality was not detected.

Initial COHb (%) was 22.14±10.74 in the study group and 2.42±2.47 in the control group. And it was statistically significant (p<0,001). Control COHb (%) was found similar in two groups as expected (p=0,452). The CK-MB level was 1.53±0.63 U/L, the WBC level was 9.55±2.16 /mm3, the AST level was 25.37±11.4 U/L, the ALT level was 23.51±11.05 U/L, the GFR level was 101.49±15.36 U/L, and the Lactate level was 2.2±0.9 U/L in a study group. Creatinine Kinase-MB, WBCs, ALT, GFR and Lactate levels were all higher in the study group than the control group and statistically significant; only AST levels between groups were not statistically significant.

Table 2. Laboratory, clinical and findings, group I and group II of serum HIF-2α levels

Variables	Groups		pvalue
	Study Group (n=35)	Control Group(n=21)	
Age (y)	57.2±19.6	41.6±14.8	<0.001
Sex (Female/Male)	21/14	13/8	1.000
Cause of exposureto COP	n(%)		
1 Stove (coalorwood)	30 (85.7%)	-	-
2 Gasboiler	5 (14.3%)	-	-
Exposure time of COP gas (h)	2.89±0.4	-	-
Vitalsigns	mean±SD	mean±SD	
SBP (mm Hg)	110.8±13.5	113.3±18.5	0.108
DBP (mm Hg)	68.8±9.0	70.0±12.2	0.178
Heart rate (beats/min)	87.5±14.8	79.4±16.2	0.062
Respiratory rate (breaths/min)	23.1±6.1	21.4±3.5	0.647
Temperature (°C)	36.5±0.5	36.4±0.4	0.711
Symptoms	n(%)	n(%)	
Headache	26 (46.4%)	0 (0.0%)	<0.001
Dizziness and Vomiting	24 (42.9%)	1 (1.8%)	<0.001
Weakness and fatigue	23 (41.1%)	0 (0.0%)	<0.001
Lose of consciousorsyncope	6 (10.7%)	1 (1.8%)	0.237
Glasgow Coma Score (GCS)	n(%)		
GCS 14-15	32 (91.4%)	-	-
GCS 9-13	2 (5.7%)	-	-
Final outcome	n(%)		
Discharged	29 (82.9%)	-	-
Hospitalisation	6 (17.1%)	-	-
Exitus	0 (0.0%)	-	-
Blood gas analysis	mean±SD	mean±SD	
pH	7.38±0.4	7.41±0.5	0.830
PO2 (mm Hg)	79.6±53.2	57.2±35.2	0.162
PCO2 (mm Hg)	32.8±8.1	38.5±4.1	0.216
SaO2 (%)	81.2±18.1	75.7±21.3	0.688
Other laboratory findings	mean±SD	mean±SD	
HIF 2 α (pg/mL)	1.72±0.65	1.16±0.48	<0.001
First COHb (%)	22.14±10.74	2.42±2.47	0.001
Control COHb (%)	7.12±2.43	2.42±2.47	0.452
cTnI (ng/mL)	0.0078±0.25	0.0037±0.011	0.218
CK-MB (U/L)	40.0±59.5	25.06±16.10	0.007
WBCs (/mm3)	9.55±2.16	8.57±3.58	0.011
AST (U/L)	25.37±11.4	21.57±8.7	0.196
ALT (U/L)	23.51±11.05	16.76±9.13	0.022
GFR	101.49±15.36	81.76±23.32	0.040
Lactate	2.2±0.9	1.84±0.2	0.017

The data are expressed as arithmetic mean and standard deviation (mean±SD).
Abbreviations: Systolic blood pressure (SBP); diastolic blood pressure (DBP); aspartate aminotransferase (AST); alanine aminotransferase (ALT), glomerular filtration rate (GFR), white blood cells (WBCs), creatine kinase-MB fraction (CK-MB), carboxyhemoglobin (COHb).

Table 3. Sensitivity and specificity values of the HIF-2α, GFR, CTnI and lactate counts tests at different cut-off levels.

	Cut-offvalue	Sensitivity	Specificity	+LR	-LR	PP	NP
HIF-2α	>0.575	100.0	9.52	1.11	0.00	64.8	100.0
HIF-2α	>0.786	100.0	19.05	1.24	0.00	67.3	100.0
HIF-2α	>1.101	82.86	66.67	2.49	0.26	80.6	70.0
HIF-2α	>1.309	71.43	71.43	2.50	0.40	80.6	60.0
HIF-2α	>1.817	31.43	95.24	6.60	0.72	91.7	45.5
GFR	>76	97.14	47.62	1.85	0.06	75.6	90.9
cTnI	>0.015	5.71	95.24	1.20	0.99	66.7	37.7
Lactate	>2.4	40	90.48	4.20	0.66	87.5	47.5

PP, positivepredictivevalue; NP, negativepredictivevalue, LR, positivelikelihoodratio; –LR, negativelikelihoodratio

And finally, the average serum HIF-2α level was 1.72±0.65pg/mL in the study group and 1.16±0.48 pg/mL in control group. HIF-2α levels were significantly higher in the study group (p=0.001).

HIF-2α levels, COHb, cTnI, and GFR were analyzed by receiver operating character analysis (ROC) and were shown in Figure 2. Areas under the receiver operating characteristic curves (AUC) were calculated. AUC for HIF-2α levels was found significantly different between areas (p<0.001). AUC was found 0.765 (95% confidence interval, 0,632 - 0,868, p<0.001) for HIF-2α levels. A cut-off value of 1,101 ng/ml was found with 83.9% sensitivity and 66.7% specificity for higher levels of HIF-2α. A significant statistical correlation was found between serum HIF-2α and first COHb (p<0,005) levels and shown in Table 3.

According to bivariate Pearson correlation test HIF-2α and COHb had a significant correlation (p=0,005, 0.464). No significant correlation was found between other parameters and shown in Table 4.

Table 4. Correlation test (Bivariate pearson correlation coefficients (r value)

Variable	r	pvalue
Age (years)	0.069	0.696
First COHb (%)	0.464	0.005
Control COHb (%)	0.105	0.549
cTnI (ng/mL)	0.222	0.201
CK-MB (U/L)	0.195	0.262
WBCs (/mm3)	0.082	0.641
ALT (U/L)	0.114	0.543
AST (U/L)	0.095	0.587
GFR	0.042	0.812
Lactate	0.321	0.060

r, correlation coefficient.

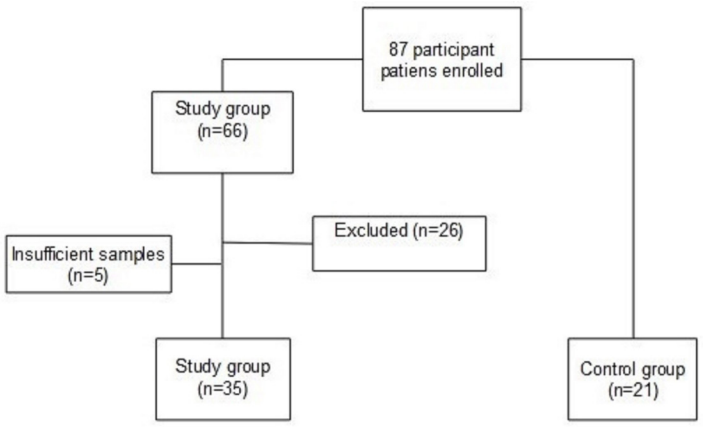

Figure 1. Consolidated Standards of Randomised controlled flow diagram for groups

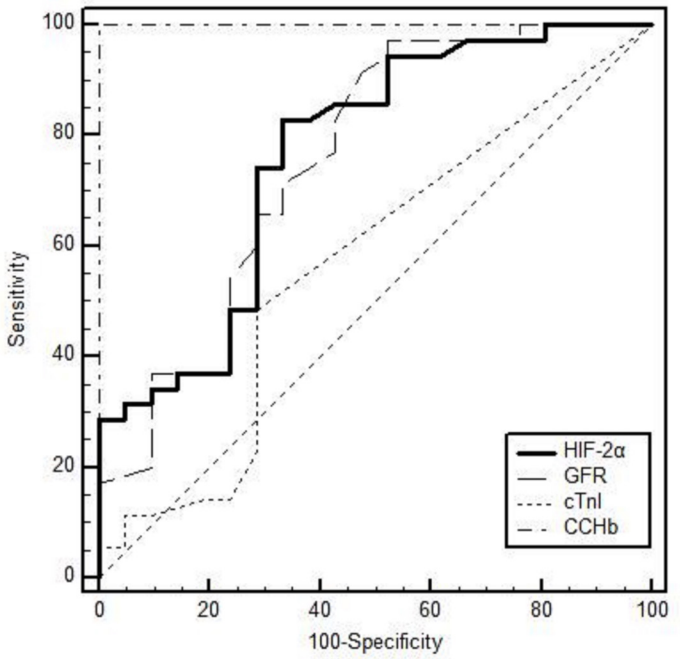

Figure 2. ROC curve analysis showed the prognostic value of HIF-2α levels,CCHb, CTnI, GFR and COHb in COP. Diagnostic values of HIF-2α , CCHb, cTnI, and GFR tests to make an assumption in acute CO poisoning. AUC for HIF-2α, CCHb, cTnI and GFR levels. HIF-2α (AUC; 0.765 (95 % [CI], 0.632 to 0.868, pv0.01), CCHb (AUC; 1,00 (95 % CI, 0.936 to 1,00, pv0.001),cTnI (AUC; 0.586 (95 % CI, 0.446 to 0.716)

Discussion

All body systems, especially the central nervous system, are affected by COP [3]. Symptoms in COP patients are not fully evident and varies from an ordinary dizziness to coma. Even in such studies, 20-30% of patients are generally asymptomatic though high COHb levels [2]. Common symptoms in patients are a headache, dizziness, vomiting, gastroenteritis, lethargy, convulsions, arrhythmias, pulmonary edema and coma [12]. Patients involved in this study had approximately 50% of headache, dizziness, vomiting and fatigue similarly but only six of them lost they're conscious. Glasgow Coma Scales of 32 (91%) patients at ED admission were 14 and 15 so this study group seemed to moderately be poisoned by CO. However, Türedi S et al reported that there was a limited correlation of symptoms with COHb levels in their study [5].

Most effected systems in COP are a Central nervous system (CNS) and cardiovascular system in particular [13]. Measurement of COHb is the key factor to determine COP but its level reduces

soon after oxygen therapy [5,13]. The fact that the COHb level is normal on the follow-up, the clinicians have trouble to decide how often the patients will be followed up or how long oxygen therapy will be given to minimize the acute and chronic effects of COP. So, monitoring the patients is important to determine the cellular damage.

In COP patients, many researchers have searched for a new biomarker Giuseppe L et al. advocated a new biomarker, fatty acid-binding protein (FABP) and troponin could be used as an alternative for monitoring and determining of cardiac damage of COP [6]. Wunderlich et al. used heart-type fatty acid-binding protein (H-FABP) to demonstrate myocardial damage in COP patients [14,15] It was found that H-FABP levels reached its peak level in 3-4 hours of exposure to CO and showed myocardial damage in COP patients. Türedi S et al. found at 1-6 hours of exposure to CO that albumin concentration increases statistically significant in rat COP model [5]. Xiaofang Yu et al defined HIFs, heterodimeric nuclear transcription factor, as crucial in the defence mechanisms against hypoxia [16]. Stavik et al demonstrated stimulated angiogenesis through the upregulation of HIF sand VEGF expression after hypoxia [17]. There is a strong correlation between vascula endothelial growth factor (VEGF) and hypoxia-inducible factors (HIFs). In particular, HIF-2α plays an important role in ischemic damage to proliferation and regeneration, unlike other HIFs [16]. Some proangiogenic and proinflammatory factors are regulated by HIF-2α [18,19]. While IL-8 is suppressed by HIF-1α in humans, NF-E2-related factor 2 (Nrf2) is induced by HIF-2α [20]. HIF-2α have been implicated in physiological and pathological processes such as inflammation, cell survival in ischemic tissue [21]. Kapitsinou PP et al, found increased HIF-2α of post-ischemic microcirculatory blood flow in the renal ischemia / reperfusion model [10]. HIF-2α seems to be a hypoxia-specific marker as it is detectable in hypoxia in various organs and tissues. Lui Jing et al had found HIF-2α in patients with ischemic kidney damage and showed the critical value of HIF-2α as a marker of hypoxia [22]. Similarly, HIF-2α could be detected through 1-4 hours in serum after inhalation of CO. It reaches a peak level at the end of four hours and the increase lasts for 12-24 hours [7,16,17].

The present study investigated the diagnostic value of HIF-2 α by finding out the correlation with the index test COHb During study the HIF-2α were measured in the first 4 hours of COP. Due to the nature of the study held on in ED, researchers could draw blood only once- few hours after CO exposure in ED – to examine HIF-2 α levels because patients were discharged rapidly. HIF-2α levels were correlated with first COHb measurements (p=0.005) so HIF-2α may be a marker for following patients even at the 12th and 24th hours of CO exposure and be used to diagnose and follow COP as other markers in ED such as troponin. Lactate is another molecule related to ischemic intolerance and increased statistically significant at the 4th hour of CO exposure like HIF-2α during this study (P= 0.017). But extra work should be executed to reveal its specificity to hypoxia.

Conclusion

The crucial point in the follow-up and treatment of COP patients is to understand the presence and degree of ischemic damage happened. The grade of devastation and duration of treatment is critical for prognosis. HIF-2α looked promising as a long-lasting new marker to follow up patients and evaluate hypoxic damage in COP. More detailed studies and large numbers of a patient population are needed to reveal the diagnostic and prognostic value of HIF-2α.

Competing interests
The authors declare that they have no competing interest

Financial Disclosure
The financial support for this study was provided by the investigators themselves.

Ethical approval
This work has been approved by the Institutional Review Board.

References

1. Shen M, Fan D, Zang Y, et al. Neuroprotective effects of methane-rich saline on experimental acute carbon monoxide toxicity. J Neurol Sci. 2016;369:361-7.
2. Bleecker ML. Carbon monoxide intoxication. Handb Clin Neurol. 2015;131:191-203.
3. Hampson NB, Piantadosi CA, Thom SR, et al. Practice recommendations in the diagnosis, management, and prevention of carbon monoxide poisoning. Am J Respir Crit Care Med. 2012;186:1095-101.
4. Zazzeron L, Liu C, Franco W, et al. Pulmonary phototherapy for treating carbon monoxide poisoning. Am J Respir Crit Care Med. 2015;192:1191-9.
5. Turedi S, Yilmaz SE, Mentese A, et al. The diagnostic value of serum ischemia-modified albumin levels in experimentally induced carbon monoxide poisoning and their correlation with poisoning severity. Acad Emerg Med. 2013;20:652-8.
6. Lippi G, Rastelli G, Meschi T, et al. Pathophysiology, clinics, diagnosis and treatment of heart involvement in carbon monoxide poisoning. Clin Biochem. 2012;45:1278–85.
7. Li Q, Cheng Y, Bi MJ, et al. Effects of N-Butylphthalide on the expressions of Nogo/NgR in rat brain tissue after carbon monoxide poisoning. Environ Toxicol Pharmacol. 2015;39:953-61.
8. Szade A, Grochot-Przeczek A, Florczyk U, Cellular and molecular mechanisms of inflammation-induced angiogenesis. IUBMB Life. 2015;67:145-59.
9. Loboda A, Jozkowicz A, Dulak J. HIF-1 and HIF-2 transcription factors--similar but not identical. Mol Cells. 2010;29:435-42.
10. Kapitsinou PP, Sano H, Michael M, et al. Endothelial HIF-2 mediates protection and recovery from ischemic kidney injury. J Clin Invest. 2014;124:2396-409.
11. Nordquist L, Friederich-Persson M, Fasching A, et al. Activation of Hypoxia-Inducible Factors Prevents Diabetic Nephropathy. J Am Soc Nephrol. 2015;26:328-38.
12. Chiew AL, Buckley NA. Carbon monoxide poisoning in the 21st century. Crit Care. 2014;18:221.
13. Lippi G, Rastelli G, Meschi T, Pathophysiology, clinics, diagnosis and treatment of heart involvement in carbon monoxide poisoning. Clin Biochem. 2012;45:1278–85.
14. Yardan T, Meric M, Bozkurt A, Bilge S, Bas DB, Bedir A, Ozdemir T, Baydin A. The role of heart-type fatty acid-binding protein in the evaluation of carbon monoxide poisoning in rats. Hum Exp Toxicol. 2011;30(2):124–8.
15. Wunderlich MT, Hanhoff T, Goertler M, et al. Release of brain–type and heart–type fatty acid–binding proteins in serum after acute ischaemic stroke. J Neurol. 2005;252:718–24.
16. Yu X, Fang Y, Liu H, et al. The balance of beneficial and deleterious effects of hypoxia-inducible factor activation by prolyl hydroxylase inhibitor in rat remnant kidney depends on the timing of administration. Nephrol Dial Transplant. 2012;27:3110–9.

17. Sluimer JC, Gasc J-M, van Wanroij JL, et al. Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. J Am Coll Cardiol. 2008;51:1258–65.

18. Covello KL, Kehler J, Yu H, et al. HIF-2α regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev. 2006;20:557–70.

19. Wiesener MS, Jürgensen JS, Rosenberger C, et al. Widespread hypoxia-inducible expression of HIF-2α in distinct cell populations of different organs. FASEB J. 2003;17:271–3.

20. Loboda A, Stachurska A, Florczyk U, et al. HIF-1 induction attenuates Nrf2-dependent IL-8 expression in human endothelial cells. Antioxid Redox Signal. 2009;11:1501–17.

21. Cramer T, Yamanishi Y, Clausen BE, et al. HIF-1α is essential for myeloid cell-mediated inflammation. Cell. 2003;112:645–57.

22. Liu J, Wei Q, Guo C, et al. Hypoxia, HIF, and Associated Signaling Networks in Chronic Kidney Disease. Int J Mol Sci. 2017;18:950.

23. Rosenberger C, Heyman SN, Rosen S, et al. Up-regulation of HIF in experimental acute renal failure: Evidence for a protective transcriptional response to hypoxia. Kidney Int. 2005;67:531–42.

24. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia. 2008;22:14–22.