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Angiotensin-converting Enzyme Inhibition Improves the Effectiveness of Transcutaneous Carbon Dioxide Treatment

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Abstract. *Aim: To study the effect of carbon dioxide (CO_2) therapy on the nitric oxide (NO) pathway by monitoring plasma asymmetric dimethylarginine (ADMA) concentrations. Patients and Methods: Forty-seven hypertensive patients who underwent transcutaneous CO_2 therapy were enrolled. Thirty healthy individuals were recruited for the control group. Blood samples were taken one hour before, as well as one hour, 24 hours and 3 weeks after the first CO_2 treatment. Controls did not undergo CO_2 treatment. Plasma ADMA levels were measured by ELISA. Results: ADMA levels decreased significantly one hour after the first CO2 treatment compared to the baseline concentrations (p=0.003). Significantly greater reduction was found among patients in whom angiotensin converting enzyme inhibitors (ACEIs) were administered (p=0.019). Conclusion: The short- and long-term decrease of ADMA levels suggests that CO_2 is not only a vasodilator, but also has a beneficial effect on the NO pathway. ACE inhibition seems to enhance the effect of CO_2 treatment.*

Transcutaneous administration of carbon dioxide (CO_2), further referred to "CO_2 therapy" has been used for curative purposes since 1932 (1). CO_2 passes freely through membranes and has a well-known vasodilation effect. Both *in vitro* and *in vivo* studies have demonstrated a rightward shift of the oxygen-hemoglobin (O_2-Hb) dissociation curve after administration of CO_2. This "artificial Bohr-effect" seems to be responsible for decreased pH and increased partial pressure of oxygen (PO2) demonstrated in tissues after CO_2 therapy (2). These effects have been confirmed by Minamiyama and Yamamoto by using intra-vital microscopy video images to demonstrate subcutaneus vasodilation after CO_2 administration (3). In addition, CO_2 has been shown to increase blood flow rate in the observed subcutaneus vessels (3). Taking advantage of its easy use, low cost and high efficiency, CO_2 treatment is used to cure several diseases notably, peripheral arterial and venous disorders (*e.g.*, claudication, lower limb ulcer, *etc.*), heart diseases (*e.g.*, hypertension, heart failure, *etc.*) and immunological disorders (*e.g.*, Raynaud's syndrome, Sudeck's disease, *etc.*) (4, 5).

The pathophysiological link between these conditions is the presence of excessive oxidative stress. Investigating the relation between oxidative stress and CO_2, Veselá and Wilhelm found CO_2 to play a protective role in scavenging free radicals and suppressing oxidative metabolism (6).

Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide synthase (NOS) inhibitor. Increased plasma ADMA concentrations were shown in patients with classic cardiovascular risk factors (*e.g.*, hypertension, diabetes mellitus, smoking, obesity, *etc.*) (7, 8). Its outstanding predictive value in cardiovascular risk assessment, already demonstrated by several authors, has been confirmed by a recent systematic review involving nearly 20,000 individuals. Willeit *et al.* showed a 1.42 risk ratio of adverse cardiovascular disease (CVD) outcomes comparing ADMA values of the top tertile with the bottom tertile (9, 10). Furthermore, strong correlations were found between ADMA levels and intima-media thickness and ADMA levels and aortic augmentation index, which are early markers of endothelial dysfunction (11, 12). Not only is ADMA capable

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of indicating endothelial dysfunction, but also uncoupling electron transport between NOS and L-arginine. It also inhibits NO formation and induces vascular free radical production leading to increased oxidative stress (13). Accordingly, ADMA is a useful tool for studying the NO system especially in conditions with inflammation and oxidative stress (14).

ADMA is produced intracellularly, as a result of methylated protein degradation, and metabolized mainly by dimethylarginine dimethylaminohydrolase enzyme (DDAH). Expression and function of DDAH is impaired by several conditions, *e.g.* increased glucose levels, hypercholesterinemia, hyperhomocysteinemia and inflammatory milieu (15). Furthermore, loss of efficiency of DDAH is directly proportional to the severity of oxidative stress (16). In short, ADMA is considered as a marker and mediator of oxidative stress and an indicator of vascular well-being.

Patients and Methods

Study design. The present study has been performed at our ISO 9001 accredited Cardiology Rehabilitation Inpatient Unit from April 2016 to November 2016. Non-smoker, abstinent, hypertensive patients with an ejection fraction over 55% were enrolled. Patients who had previously received CO_2 therapy were excluded. Moreover, patients who had suffered from myocardial infarction, stroke or undergone open surgery less than a year before the study were also excluded. Additionally, individuals diagnosed with cancer or kidney injury were also excluded. To monitor the changes of plasma ADMA concentrations, blood samples were obtained one hour before and 1 hour, 24 hours and 3 weeks after the first CO_2 treatment, respectively. The patients received three transcutaneous CO_2 treatments per week for 3 weeks. CO_2 gas was administered for 35 minutes in a plastic bag sealed at mid-thoracic level, as previously described by Fabry *et al.* (4). Clinical data (medical history, age, weight, height, drugs, ejection fraction, laboratory data, *etc.*), were registered by the same investigator, respectively. Healthy individuals were recruited for the control group. Controls did not undergo CO_2 treatment. Only one sample was obtained from controls.

Informed written consent was obtained from every patient. The study protocol was approved by the Regional Ethics Committee of University of Pécs, Pécs, Hungary (Permission No.: 5919.), in accordance with the 2008 Helsinki declaration.

Sampling and measurement of ADMA. Venous blood samples were taken into K-EDTA-containing tubes using a closed blood sampling system (Becton Dickinson Vacutainer; Becton Dickinson, Franklin Lakes, NJ, USA). After centrifugation (1,500 × *g*, 10 min) plasma was collected and stored at –70˚C until analysis.

Plasma ADMA concentrations were determined by using enzyme-linked immunosorbent assay (ELISA) method (product No: MBS264847; Mybiosorce, San Diego, CA, USA). The measuring range of the assay was 0.078-5.0 μmol/l. Measurements were performed according to manufacturer's protocol.

Statistical analysis. Statistical analysis was performed by IBM SPSS Statistics for Windows Version 22 (IBM Corp., New York, NY, USA). According to Shapiro-Wilk test, ADMA samples showed normal distribution and, thus, error bar was used to demonstrate results (95% confidence interval for mean). Independent sample *t*-test was used to compare the ADMA levels of patients with those of the ADMA levels of the controls. Differences during the follow-up were investigated by paired-sample *t*-test. To reveal correlations, the Pearson's correlation test was used. All *p*-values less than 0.05 were considered statistically significant.

Results

We enrolled 47 patients and 30 controls. Clinical characteristics of the subjects are shown in Table I. Baseline plasma ADMA concentrations were significantly higher in the patients compared to the controls (0.41 μmol/l *vs.* 0.35 μmol/l; $p=0.018$). Patients suffering from diabetes mellitus had significantly higher baseline plasma ADMA concentrations compared to non-diabetic patients (0.47 μmol/l *vs.* 0.37 μmol/l; $p=0.038$). Relatively weak but significant positive correlation was found between baseline ADMA levels and age ($p=0.011$, r=0.392).

ADMA levels decreased significantly one hour after the first CO_2 treatment compared to the baseline concentrations ($p=0.003$; Figure 1). Twenty-four hours after the treatment, ADMA levels increased approximately to the baseline. Comparing ADMA concentrations measured 1 and 24 hours after the first CO_2 treatment, a modest but statistically not significant increase was observed. After receiving 9 CO_2 treatments in an interval of 3 weeks, ADMA levels were found to be modestly lower than the baseline ($p=0.210$).

We investigated the effects of baseline medication (angiotensin converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers, beta-receptor blockers, diuretics, antidiabetics, antiplatelet therapy, proton pump inhibitors, H2-receptor blockers) on the lowering of ADMA levels that was demonstrated one hour after the first CO_2 treatment. Significantly greater reduction was only found among patients in whom ACEIs were administered ($p=0.019$). Other medications showed no effects on ADMA levels.

Discussion

In the present study, we investigated the effect of CO_2 therapy by monitoring ADMA levels in patients with cardiovascular diseases. The observed baseline elevation in ADMA concentration is probably due to the ongoing disease. Young individuals were enrolled as controls to rule out possible undiscovered diseases that could alter ADMA concentrations. In line with literature data, elevated ADMA levels were found in patients suffering from diabetes mellitus and positive correlation was found between ADMA and age (7, 12).

To the best of our knowledge, this is the first study investigating the effect of CO_2 treatment on ADMA levels. The prompt decrease of ADMA levels demonstrated 1 hour after CO_2 treatment indicates that the treatment had

Table I. *Clinical characteristics of the participants.*

	Control group (n=30)	Patient group (n=47)	*p*-Value
Mean age, years	28±8.4	67±12.7	<0.001
Male, n (%)	13 (43)	20 (43)	0.808
Diabetes type 2, n (%)	-	18 (38)	-
CABG, n (%)	-	10 (21)	-
MI, n (%)	-	8 (17)	-
BMI, kg/m^2	25.2±3.8	29.1±4.7	0.706
WBC count, G/l	6.53±1.81	6.12±1.36	0.238
ADMA 0, µmol/l	0.35±0.07	0.43±0.03	0.018
ADMA 1h, µmol/l	-	0.38±0.02	-
ADMA 24h, µmol/l	-	0.41±0.03	-
ADMA 3w, µmol/l	-	0.40±0.08	-

CABG, Coronary artery bypass surgery; MI, myocardial infarction; BMI, body mass index; WBC, white blood cells; EF, ejection fraction; ADMA, asymmetric dimethylarginine. Mean±SD values are presented.

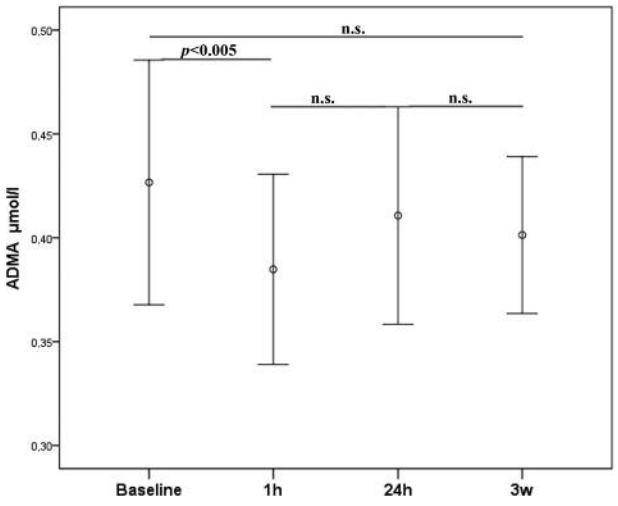

Figure 1. *Plasma ADMA levels (mean and SD) of the involved controls and follow-up of the patients. ADMA, Asymmetric dimethylarginine; n.s., not significant; baseline, before the first CO_2 treatment; 1h, one hour after the first CO_2 treatment; 24h, 24 hours after the first CO_2 treatment; 3w, 3weeks after the first CO_2 treatment.*

beneficial effects on the NO pathway, possibly due to vasodilation. Thus, ADMA levels can represent the current vascular state. Such rapid changes have already been reported previously while monitoring ADMA levels during on-pump and off-pump cardiac surgery. The authors related the changes of ADMA concentrations to the intensity of inflammation and oxidative stress (17). After the initial decrease of ADMA concentrations, we observed an increase within 24 hours after the CO_2 treatment, which may be due to a rebound effect explained by the subsiding of the vasodilator effect. In a recent study, Bolevich *et al*. have proved that CO_2 is "a universal inhibitor of oxidative stress" (18). Reduced oxidative stress results in an increased efficiency of DDAH leading to decreased ADMA levels (16). In line with these findings, long-term CO_2 treatment can decrease the production of reactive oxygen species, which is indicated by the decreasing tendency of ADMA observed after receiving 9 CO_2 treatments within 3 weeks.

Besides presenting the changes of ADMA levels after CO_2 treatment, significantly greater short-term ADMA reduction was found among 25 patients in whom ACEIs were administered. Only a few studies have investigated the relation between ADMA and ACEIs. Veresh *et al*. showed that ADMA activates the renin-angiotensin-aldosterone system (RAAS), which leads to vasoconstriction and increased oxidative stress (19). Moreover, Ito *et al*. found reduced ADMA levels among hypertensive patients after being treated with ACEIs (20). Napoli *et al*. investigated the influence of zofenopril on oxidative stress. ADMA and malondialdehyde (MDA) were used to monitor the oxidative changes. After administering zofenopril, both ADMA and MDA levels decreased. The authors suggest that zofenopril alters the NO pathway and reduces oxidative stress (21). Moreover, ACEIs have been shown to stimulate NO production through their "bradykinin-sparing" property (22, 23). Interestingly, in the present study, baseline ADMA levels were not shown to be significantly lower in patients receiving ACEIs compared to those without ACEIs. However, according to our results and previous findings, ACE inhibition is not only protective against oxidative stress, but it can also improve vascular reactivity demonstrated by the short-term changes of ADMA after CO_2 treatment.

It must be mentioned that this study has certain limitations, such as a relatively small sample size and investigating only ADMA levels. Further research involving more individuals and markers could be useful to examine the relation between CO_2 treatment and oxidative stress in more detail.

Conclusion

The short- and long-term decrease of ADMA levels suggests that CO_2 is not only a vasodilator, but also has a beneficial effect on the NO pathway, possibly by reducing oxidative stress. ACE inhibition seems to enhance the beneficial effect of CO_2 treatment, most likely due to decreased activation of RAAS and its vasoprotective effects.

Conflicts of Interests

The Authors declare that there is no conflict of interests regarding the publication of this study.

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References

1 Brandi C, Aniello CD, Grimaldi L, Bosi B, Dei I, Lattarulo P and Alessandrini C: Carbon dioxide therapy in the treatment of localized adiposities: Clinical study and histopathological correlations. Aesthetic Plast Surg *25*: 170-174, 2001.

2 Sakai Y, Miwa M, Oe K, Ueha T, Koh A, Niikura T, Iwakura T, Lee SY, Tanaka M and Kurosaka M: A novel system for transcutaneous application of carbon dioxide causing an "Artificial Bohr Effect" in the human body. PLoS One *6*: e24137, 2011.

3 Minamiyama M and Yamamoto A: Direct evidence of the vasodilator action of carbon dioxide on subcutaneous microvasculature in rats by use of intra-vital. J Biorheol *24*: 42-46, 2010.

4 Fabry R, Monnet P, Schmidt J, Lusson JR, Carpentier PH, Baguet JC and Dubray C: Clinical and microcirculatory effects of transcutaneous CO_2 therapy in intermittent claudication. Randomized double-blind clinical trial with a parallel design. Vasa *38*: 213-224, 2009.

5 Schmidt J, Monnet P, Normand B and Fabry R: Microcirculatory and clinical effects of serial percutaneous application of carbon dioxide in primary and secondary Raynaud's phenomenon. Vasa *34*: 93-100, 2005.

6 Veselá A and Wilhelm J: The role of carbon dioxide in free radical reactions of the organism. Physiol Res *51*: 335-339, 2002.

7 Cooke JP: Asymmetrical dimethylarginine: The Über Marker? Circulation *109*: 1813-1819, 2004.

8 Németh B, Kustán P, Németh Á, Lenkey Z, Cziráki A, Kiss I, Sulyok E and Ajtay Z: Asymmetric dimethylarginine: Predictor of cardiovascular diseases? Orv Hetil *157(13)*: 483-487, 2016.

9 Willeit P, Freitag DF, Laukkanen JA, Chowdhury S, Gobin R, Mayr M, Di Angelantonio E and Chowdhury R: Asymmetric dimethylarginine and cardiovascular risk: Systematic review and meta-analysis of 22 prospective studies. J Am Heart Assoc *4*: e001833, 2015.

10 Schulze F, Lenzen H, Hanefeld C, Bartling A, Osterziel KJ, Goudeva L, Schmidt-Lucke C, Kusus M, Maas R, Schwedhelm E, Strödter D, Simon BC, Mügge A, Daniel WG, Tillmanns H, Maisch B, Streichert T and Böger RH: Asymmetric dimethylarginine is an independent risk factor for coronary heart disease: Results from the multicenter Coronary Artery Risk Determination investigating the influence of ADMA concentration (CARDIAC) study. Am Heart J *152*: 493.e1-e8, 2006.

11 Cakar M, Bulucu F, Karaman M, Ay SA, Kurt Ö, Balta Ş, Demirkol S3, Sarlak H, Akhan M, Altun B, Yaman H, Arslan E, Demirbaş Ş and Sağlam K: Asymmetric dimethylarginine and augmentation index in newly diagnosed patients with hypertension. Angiology *66*: 43-48, 2015.

12 Furuki K, Adachi H, Enomoto M, Otsuka M, Fukami A, Kumagae S, Matsuoka H, Nanjo Y, Kakuma T and Imaizumi T: Plasma level of asymmetric dimethylarginine (ADMA) as a predictor of carotid intima-media thickness progression: Six-year prospective study using carotid ultrasonography. Hypertens Res *31*: 1185-1189, 2008.

13 Sydow K and Münzel T: ADMA and oxidative stress. Atheroscler *4(Suppl)*: 41-51, 2003.

14 Németh B, Kiss I, Péter I, Ajtay Z, Németh Á, Márk L, Csorba A, Kőszegi T, Mühl D and Kustán P: Monitoring of L-arginine and endogenous dimethylarginines in survivor septic patients - A pilot study. In Vivo *30*: 663-669, 2016.

15 Sibal L, Agarwal SC, Home DP and Boger RH: The role of asymmetric dimethylarginine (ADMA) in endothelial dysfunction and cardiovascular disease. Curr Cardiol Rev *6*: 82-90, 2010.

16 Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T and Cooke JP: Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. Circulation *99*: 3092-3095, 1999.

17 Cziráki A, Ajtay Z, Németh Á, Lenkey Z, Sulyok E, Szabados S, Alotti N, Martens-Lobenhoffer J, Szabó C and Bode-Böger SM: Effects of coronary revascularization with or without cardiopulmonary bypass on plasma levels of asymmetric dimethylarginine. Coron Artery Dis *22*: 245-252, 2011.

18 Bolevich S, Kogan AH, Zivkovic V, Djuric D, Novikov AA, Vorobyev SI and Jakovljevic V: Protective role of carbon dioxide (CO_2) in generation of reactive oxygen species. Mol Cell Biochem *411*: 317-330, 2016.

19 Veresh Z, Debreczeni B, Hamar J, Kaminski PM, Wolin MS and Koller A: Asymmetric dimethylarginine reduces nitric oxide donor-mediated dilation of arterioles by activating the vascular renin-angiotensin system and reactive oxygen species. J Vasc Res *49*: 363-372, 2012.

20 Ito A, Egashira K, Narishige T, Muramatsu K and Takeshita A: Renin-angiotensin system is involved in the mechanism of increased serum asymmetric dimethylarginine in essential hypertension. Jpn Circ J *65*: 775-778, 2001.

21 Napoli C, Sica V, de Nigris F, Pignalosa O, Condorelli M, Ignarro LJ and Liguori A: Sulfhydryl angiotensin-converting enzyme inhibition induces sustained reduction of systemic oxidative stress and improves the nitric oxide pathway in patients with essential hypertension. Am Heart J *148*: e5, 2004.

22 Hartman JC: The role of bradykinin and nitric oxide in the cardioprotective action of ACE inhibitors. Ann Thorac Surg *60(3)*: 789-792, 1995.

23 Zhang X, Xie Y-W, Nasjletti A, Xu X, Wolin MS and Hintze TH: ACE inhibitors promote nitric oxide accumulation to modulate myocardial oxygen consumption. Circulation *95*: 176-182, 1997.

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