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Methodological aspects of dose calculations in transdermal carbon dioxide therapy: estimation of absorbed dose and confirmation of systemic distribution

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ABSTRACT

Carbon dioxide (CO_2) gas is well characterized for medical applications. It is a chemically stable, biocompatible agent that has a long history of traditional use (in natural "mofettes") particularly in Hungary, as a natural infallible remedy. Medical CO_2 gas has been widely used in various area of medicine, mostly in surgical laparoscopy and endoscopic procedures in gastroenterology as an insufflation gas, since it is considered as an inert gas without side effects and the residuals easily absorbed in vascular tissues. On the other hand, the scope of intended use in case of transdermal or intradermal application is substantially different. Based on its primary mechanism of action carbon dioxide along with some other metabolites can cause prompt vasodilation of precapillary sphincters of blood vessels, and consequently a reduction of peripheral resistance and an increase the flow of microcirculation in tissues. Additionally, an increase in perivascular partial oxygen pressure in tissues ($tcPO_2$) has been detected, because of a shift on the oxyhemoglobin dissociation curve, which resulted in an overall enhancement of oxygenation for cells.
Scientific literature revealed some other aspects of physiology during transdermal CO_2 gas administration, mainly in the field of microcirculation and tissue oxygenation. In this report we confirm the rate of absorption and the systemic elimination and highlight the principles of calculation for distribution and dosing utilizing the methodology of stable isotope ratio analysis. All these data may provide at least one further step supporting the use of carbon dioxide as an effective, non-invasive, convenient therapy. For the future this low-cost treatment option can be considered either (i) as a therapy with a reasonable cost-effectiveness ratio; or (ii) as an adjuvant therapy in rehabilitation with significant increase in quality of life for patients, alternatively (iii) as a prevention transdermal CO_2 may provide a safe method for public access with a potential health benefit and reducing the symptoms of arterial and venous disease development.

Keywords: CCA ash, Concrete, Corn Cobs, CO_2, Pozzolanic, ASTM

1. Introduction

There are several platforms of medical reports to investigate the effective and synergic therapies or methods to current pharmacotherapies that can provide significant sustainable efficiency as treatment options. The most referred medical conditions are the group of peripheral arterial diseases (PAD) with a circulatory, atherosclerotic origin that may cause critical limb ischemia (CLI) with ulceration, chronic wound or gangrene. This is a frequent status in neuropathic foot ulcers, but also venous leg ulcers and pressure sores. Of similar significance is the systemic vasculopathy with macrovascular and microvascular changes in progressive chronic medical condition. All of these are characterized as significant and progressive disturbances of local circulation of blood, with a decreased flow and, consequently, a decreased and inefficient oxygenation at the cellular level in tissues in the area.

Most frequent clinical signs of occlusive peripheral PAD are ischemia and relevant pain (intermittent claudication) induced by exercise, most often by walking. Other vascular disorders having autoimmune origin, similar in pathophysiology are less frequent. They include systemic sclerosis (SSc) with symptoms of abnormal vasoreactivity, consequent hypoxia, dysfunction in angiogenesis and vascular repair, and damages of vascular and perivascular cells. Decreased capillary blood flow and all other clinical symptoms may show up, such as pale fingertip in Raynaud's syndrome and ulcers in skin and progressive damages of various other organs, predominantly the lung, kidney, and heart (Müller-Ladner, 2009; Fleming, 2009).

Over the past decades, increasing amount of clinical evidences provided insight to results of new therapeutic options for specific medical conditions: it has been offered as an additional treatment options for diabetic vasculopathy, wound healing, rehabilitation and in general when the enhancement in microcirculation required. The so-called transdermal carbon dioxide (CO_2) therapy (TdCT) is an efficient, convenient, non-invasive natural-based application that delivers the actively absorbed gas into the proximity of vessels resulting in prominent arterial vasodilatation, particularly in muscles and skin. The effect can be achieved either as an immersion of the patient in CO_2-enriched warm-water, as done in balneotherapy (Nishimura *at al*., 2002; Fabry *at al*., 2009; Schmidt *at al*., 1989), or the more convenient application of transdermal administration of gas molecules through the skin (Wollina *et al*., 2004; Fabry *et al*., 2009; Cseh and Dozsa, 2011). A similar effect is achieved as a local treatment by a subcutaneous micro-injections administration of CO_2 to multiple sites in the affected area (Brandi, 2010). This latter is also a subject to topical application in cosmetology for treatment of cellulite, stretch marks and in the rejuvenation of certain skin areas (Eldsouky *et al*., 2018).

Specific and characteristic effects being observed during partial or full immersion of the human body in CO_2-rich water or in dry-CO_2 bath applications, which is similar to natural "mofetta gas-mix" effect that being used in some regions of Central Europe. The main physiological changes are similar in either way of applications: (*i*) elevation of subcutaneous CO_2 tension, leading to extracellular acidosis (Ito *et al*., 1989; Nishimura *et al*., 2002); *(ii*) dilation of precapillary smooth muscles in arterioles that result in elevated cutaneous blood flow (Hartmann *et al*., 1997); (*iii*) a consequent increase of tissue perfusion and oxygenation (tcPO2) due to a right-shift in O_2 dissociation curve (Bohr effect); and (*iv*) an elevation of the thermal sensation score (Nishimura *et al*., 2002). The local "warm" sensation and the observed physiological changes in blood flow appear after a few minutes (Hartmann *et al*., 2009) of exposure to carbon dioxide, applied in either way of exposure.

Though, natural CO_2 bath has been used for over a century in our region for "various indications" as complementary/alternative medical therapy or as an adjuvant medical option, there is no clear therapeutic guideline or dose-range recommendation other than empirical data on how to use this transdermal gas exposure in certain medical conditions. Therefore, the therapy is still lacking a wider acceptance as stated by Pagourelias *et al.* (2011) in a review paper. Over the past two decades, observational or clinical evidences have been gathered in order to elucidate the additional possible therapeutic targets doable by transdermal CO_2 applications. Evidence based on its pathophysiologic mechanisms and the consequent clinical outcome parameters (Verhagen *et al*., 2015; Akahane et al., 2017; Matsumoto *et al.*, 2018; Nemeth *et al.*, 2018; Nikura *et al.*, 2019) provided a scientific support and methodology of research (Flaten *et al*., 2015).

Key issues to understand the action of current and proposed carbon-dioxide-based therapies are the following:

(1) suggested therapeutic applications of CO_2-gas should be aligned with the mechanisms of action in targeted pathophysiology of disorders,

(2) efficacy of medical-grade CO_2 gas alone, as a main active component in natural mofetta-gas is comparable

or potentially better than a CO_2-rich gas-mix in aspects of physiology and safety,

(3) it is important to demonstrate the calculations for proper dosing as a therapeutic intervention for efficacy and safety of CO_2 gas in dry-bath applications for a wide range of medical conditions.

Dosage calculations are, however, discussed only in very few publications and the theory has not yet been elucidated. Methodology based on mathematical modelling and the preliminary results of calculations disclosed here, demonstrating the background for dose calculations to elucidate additional safety aspects. This step is required for the extended scope of applications of transdermal gas therapy (TdCT) in rehabilitation and for preventive medicine in a larger scope for the future.

To understand the proper dosing and the assessments of dose-response efficacy by the mechanism of action, one should calculate the amount of carbon dioxide absorbed through the human skin and estimate the effects of co-factors, such as the treatment conditions; the patient's conditions and the parameters of skin permeability.

Based on the extended therapeutic interest it is necessary to provide the base for optimal dose calculations for efficacy and safety. The goal of our experiments was to assess the accurate values of absorption of carbon dioxide by transdermal application. Carbon dioxide diffuses into the blood and is transported by the aqueous phase to the lung. Then, it is exhaled *via* pulmonary capillaries. In this report we confirm the rate of systemic elimination of CO_2 and discuss the basic principles of distribution and dose calculation by the isotope ratio analysis. All these steps should be considered in the model. All these data may provide a further support for the use of carbon dioxide as an effective, non-invasive, convenient transdermal therapy.

2. Materials and Methods

In our experimental design the measurements were performed on C-isotopes detecting the proportion of stable carbon dioxide gas. To determine the origin of CO_2 (e.g., from CO_2 gas applied transdermal or from the respiratory air-born CO_2) the isotope separation technique was adopted. This isotope ratio analysis was performed at Hertelendi Laboratory of Environmental Studies at the Institute of Nuclear Research of the Hungarian Academy of Sciences (MTA Atomki).

During the test run approximately 60% of the total body surface (~1.2 m^2) was immersed in CO_2 gas (50-90% in gradient) to allow transdermal absorption for 30 minutes. In order to identify the sources of carbon-isotope a separated source of air intake (from deep diving-cylinder) was used. Exhaled air gas samples were collected at specified intervals into a sealed plastic gas container. Next, C-isotope measurements were performed to demonstrate the origin of C-atoms. C-isotopes from exhaled air samples collected during the test were injected through the ion source and the controlled electromagnetic field diverts the isotopes differently. During the ionization phase, molecules with mass numbers of 44, 45, and 46 were formed, depending on whether ^{12}C, ^{13}C, or ^{14}C was bound to oxygen and each detected by the collectors separately. The heavier ones deviate more due to electromagnetic field, the light one deviates less. Detection of the proportions of isotopes of different sources serves as a base of methodology. This sensitive technique can measure ions up to a mass number of 100, thus the test environment is ideal for carbon dioxide molecules. The accuracy is very high, ± 0.1 ‰ for δ13C measurements.

The estimated amount of differences to detect is based on model calculations, according to the $^{14}C/^{12}C$ isotope ratio measurement principles. More precisely, the $^{13}C/^{12}C$ and $^{14}C/^{12}C$ isotope ratios of CO_2 gas entering the skin are significantly different from the $^{13}C/^{12}C$ and $^{14}C/^{12}C$ isotope ratios of CO_2 gas produced in the body during the normal physiological living. Changes of $^{13}C/^{12}C$ and $^{14}C/^{12}C$ isotope ratios should reflect the amount of CO_2 entering the skin, being transported in the body by the blood flow, and exhaled to air *via* alveoli of lung. The preliminary calculations determine the change of the $^{14}C/^{12}C$ isotope ratio in exhaled air over time. A biokinetic physiological mathematical model was developed, which consists of first-order differential equations, as described elsewhere (Segel and Edelstein-Keshet, 2013). The calculation for the rate in body (*via* blood):

$$\frac{d\,{}^{14}_{6}C_b}{dt} = \frac{GR_0}{V_b} - \frac{(G+j_s)}{V_b\,{}^{12}_{6}C_b}\,{}^{14}_{6}C_b \qquad (1)$$

Where the amount of blood is estimated as V_b = 5 L, and js is the rate of CO_2 uptake through the skin. The other parameters are the estimated ratio of ^{14}C ($^{14}C_b$) in mixed venous blood (approx. G = 200 cm^3 min^{-1}) of carbon dioxide, as formed in the body under normal conditions (as the R_0 parameter). Integration of Eq. (1) yields Eq. (2):

$${}^{14}_{6}C_b(\mathrm{t}) = {}^{12}_{6}C_b R_0 \left(1 - \frac{G}{G+j_s}\right) e^{-bt} + \frac{G}{G+j_s}\,{}^{12}_{6}C_b R_0 \qquad (2)$$

The change in concentration of ^{14}C ($^{14}C_A$) in exhaled alveolar air is increasing by the ^{14}C emitted by carbon dioxide escaping from the venous blood into the alveoli and on the other hand ^{14}C it is being reduced by the exhaled air during the test period. Similar calculation in exhaled air:

$$\frac{d^{14}_{6}C_A}{dt} = \frac{G + j_s}{V_A} R_b - \frac{(G + j_s)}{V_A\, {}^{12}_{6}C_A}\, {}^{14}_{6}C_A = \quad (3)$$

and that combined with the rate (Rb) calculated in body from Eq.(2) yields Eq.(4)

$$\frac{G + j_s}{V_A}\left(R_0\left(1 - \frac{G}{G + j_s}\right)e^{-bt} + \frac{G}{G + j_s}R_0\right) - \frac{(G + j_s)}{V_A\, {}^{12}_{6}C_A}\, {}^{14}_{6}C_A \quad (4)$$

Where V_A is denoted here as the average volume of the gas mixture in lungs.

CO_2 content was cryogenically recovered from the gas samples collected during the experiment. The $\delta^{13}C$ shift in exhaled air was measured with a Thermo Finnigan (IRMS) Delta plus XP type stable isotope-ratio mass spectrometer. This instrument is suitable to determine the load of ions passed up to a mass number of 100, which means that it is ideal for detecting nuclei with lower mass number. The $\delta^{13}C$ (delta) stable isotope ratio shifts relative to PDB (Belemnitella Americana PeeDee from the formation) international standard is being used. Delta values are calculated from the intensity peaks of the three different molecules using the software, according to Eq. (5).

$$\delta\,(‰) = \frac{R_m - R_s}{R_s} \cdot 1000, \quad (5)$$

where: R_m and R_s are the isotope ratios of the sample and the standard, measured under the same experimental conditions.

Simultaneously with isotope assessments, online tests for CO_2 concentration were conducted by a QMS spectrometry (QMS 200 M2 Omnistar, Balzers Instruments, Ltd.) to check the concentration gradient of the skin immersed to skin absorption. The detection limit was found equal to LLOQ-reaching the level 6x signal magnitude that exceeds the pattern of natural fluctuations in ^{14}C. Model calculations were performed by means of Mathematica 10.3 version (Wolfram Research, Inc., Champaign, Illinois, USA).

During the immersion test procedures, the temperature of the gas-filled space and, at the same time, the temperature of the body and warmest surface on the skin were detected. Optris PI450 Thermo camera and Optris PIX Connect filter were applied (40 °mK sensitivity). Temperature was recorded throughout the immersion experiment.

3. Results and discussion

Results of gas composition in cabinet (Fig. 1), the shift in C-isotope ratios prior to and during the transdermal exposure (Fig. 2), and changes in body temperature were summarized. Composition of gas used for immersion (i.e., CO_2 mixed with air) in cabinet and samples of exhaled air has been monitored in parallel *via* QMS measurements. Continuous detection of CO_2, N_2, and O_2 was accomplished by using a slow exhaust pump (1 cm^3 min^{-1}). The concentration of CO_2 gas in a factory-filled cylinder was >99.5%. Nevertheless, CO_2 sedimentation provides higher CO_2 concentration in the lower segments of the experimental cabinet, while a proportion of gas mixes with the air present in the upper half of the immersion cabinet. Prior to the test, the open air-filled cabinet at its baseline showed 19.9-20.7% of O_2. During the test, the cabinet was filled with CO_2 gas, in the upper mixed space, such that the residual amount of O_2 did not exceeded 0.6-1.0%. Thus, approximately 65-95% of CO_2 concentration gradient was present in the semi-sealed cabinet. The other molecules in air (H_2, H_2O, O_2, Ar, and CH_4) performed in QMS measurements and the peak of Ar(40) was chosen as the base for normalization. It was concluded that none of the components other than air and CO_2 mix were present during the experimental procedure.

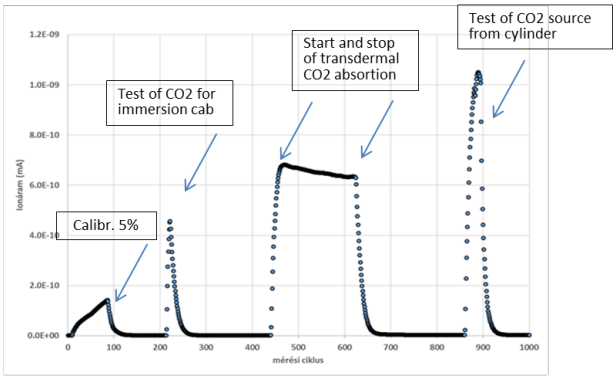

Figure 1. Current magnitudes (mA) of peaks associated with the samples taken from the immersion cabinet during the CO_2 absorption test. Calibration of 5% CO_2 and the direct source from the cylinder served as control.

Continuous thermocamera-recording for environment and the body surface temperatures were performed during tests cycles. Within ambient/room-temperature the cabinet was 21.9 °C at baseline. Data revealed that there was transient drop in cabinet temperature during the CO2 fill-in period, due to the expansion of gas to normal air pressure and recovered close to baseline in few minutes. Simultaneously, the body surface temperature and the warmest point on immersed skin surface (interdigital I-II region of hand) were measured. Baseline temperature of body (29.50 °C) showed a transient decrease to 25 °C during the CO_2 fill for the

first 3 min then gradually augmented to 29.90 °C by t = 10 min, and increased further to 30.30 °C, at t = 15 min which became the steady temperature throughout the test run. Similarly, the warmest point of hand decreased from 36.68 °C (baseline) to 34.52 °C, then increased to 36.73 °C and became steady at 37.39 °C. Both the temperature of body surface and the hand showed an increase because of CO_2 exposure, explained by the enhancement of peripheral circulation.

The shift in C-isotope ratios $\delta^{13}C$ in exhaled air was measured repeatedly at baseline and in 3-5 min (Fig. 2), throughout the immersion test and for an additional after-load period. Considering the detection limits for isotope variants (LLOQ: 6x signal magnitude) the experimental test-results that exceeded the pattern of natural fluctuation are suitable for the detection of CO_2 load by transdermal absorption.

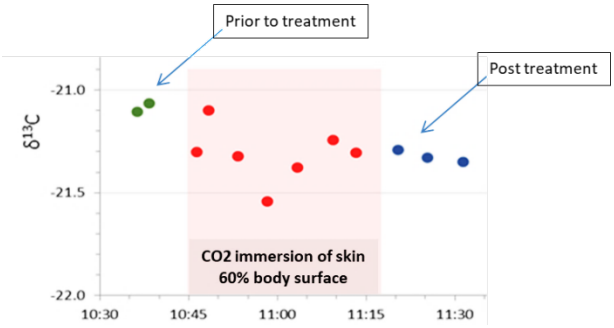

Figure 2. Results of the $\delta^{13}C$ isotope shift ratio in exhaled air before, during, and after CO_2 exposure to skin (the abscissa represents the time at which measurements were made).

Preliminary results of gas uptake through the skin indicate that based on the stationary model the load can be estimated as 7 cm^3 min^{-1}. Considering the uncertainties (at the 68% CI level) the estimated range of transdermal absorption is 4-10 cm^3 min^{-1} m^{-2}, when exposed to 65-95% of CO_2 concentration in the cabinet. This provided a reasonable result for calculations of safe dosing when therapy applied in various indications.

Our experimental design utilizing a dry CO_2-bath in a semi-sealed cabinet (with its upper part partially open) demonstrated that body immersion in non-humidified gas provides efficient transdermal absorption within a few minutes. Efficacy was shown as a local and as a systemic distribution, demonstrated by the thermal effect on skin and the shift in C-isotope ratio of the exhaled air. In this experimental design of a dry CO_2-bath absorption with the environmental conditions ambient room-temperature, normal pressure was used. The temperature of the body surface and the immersed arm and hand underwent an initial transient decrease caused by the expansion of the gas, and then the temperature of body gradually increased by the effect of locally absorbed CO_2 gas, reaching a steady temperature 0.4-0.7 °C higher than the baseline. This is a consequence of the enhanced blood flow in superficial tissues. This finding seems to be controversial to observations reported by Nishimura *et al*. (2009). The authors indicated that CO_2 water-bathing produces a decline in core temperature, because of an increase in cutaneous blood flow. This difference is explained mainly with the environment: we utilize dry CO_2 immersion in room-temperature, compared to their water bath (of fixed temperature at 34°C) during the trial.

Placebo-controlled human investigations with carbonated–water balneotherapy confirmed the direct enhancement on cutaneous microcirculation. Nevertheless, some of the results were inconsistent and, in some aspects even controversial, mainly because of semi-standard experimental conditions or CO_2 load. In our pilot study the applied isotope-shift technique revealed the methodology on how to separate the amount of gas absorbed via skin versus the quantity of CO_2 produced by the body *in vivo*. It has also revealed the estimation on absorption rate of CO_2 from external gas sources, in the range of 4-10 cm^3 min^{-1} m^{-2}. This is lower than values reported by other researchers in a range of 10-80 cm^3 min^{-1} m^{-2} (Schmidt, 1989; Fabry *et al.*, 2009). Differences in environmental conditions can be significant since the mentioned higher estimations were reported in balneotherapy done with CO_2-enriched warm water vs. dry CO_2 bath. There were other influencing factors present, such ambient temperature vs. 30-34 °C, and the individual properties of blood flow and other features of the skin.

In theory the modeling of absorption is a penetration of CO_2 gas into skin layers and permeation through the skin to reach the capillaries. The skin consists of three main layers: epidermis, dermis, and subcutaneous tissue. Although many quantitative and qualitative methods for calculations of penetration exist (Zsiko *et al*., 2019), these are affected by multiple and also individual parameters. It was documented that the locally absorbed CO_2 gas may exert local as well as systemic effects by distribution. Clinical and pre-clinical data confirmed that either carbonated spring water in balneotherapy or dry-CO_2 bath both enhances the cutaneous vasodilation elicited by the CO_2 that diffuses into the subcutaneous tissues through the skin layers and exerts also thermal effects (Komoto *et al*., 1986; Ito et al., 1989; Hartmann *et al*., 1997; Jordan, 1985).

On the other hand, the clinical efficacy is an increase in tissue partial oxygen pressure due to the enhanced oxygen-dissociation, caused by a rightward shift in the O_2 dissociation curve (Bohr-effect) along with the vasodilatation and increased microcirculation. The increased parasympathetic activity could be

concomitantly responsible for peripheral vasodilatation (Hartmann *et al*., 1997; Toriyama *et al*., 2002; Sato *et al*., 2009).

4. Conclusions

The therapeutic effects of absorbed CO_2 are caused by an increase in blood flow and an enhancement of microcirculation. Our study revealed that transdermal absorption of CO_2 can be detected by the shift in $^{13}C/^{12}C$ and $^{14}C/^{12}C$ isotope ratios, since it is sufficiently different from the patient's natural (i.e. biological) live isotope ratios when immersed to concentrated CO_2. The isotope separation methodology applied under the test conditions can be used to detect the absorbed amount of CO_2 from upon different origin (i.e. based on C-isotope ratios).

Estimation of absorption efficiency by the rate of systemic absorbed/ eliminated amount of CO_2, considering parameters as optimal CO_2 concentration of gas-mix, the area of skin immersed plus duration of exposure could provide a useful tool for calculations of effective dose and address properly all the safety concerns. Due to the fact that absorbed CO_2 in circulation changes the pH of blood, resulting in a rightward shift of the O_2–hemoglobin dissociation and resulting in a higher partial oxygen tension in tissues, the carbon-dioxide treatment must be considered as a treatment for systemic effect. It is important to assess the systemic distribution and the concentration of applied CO_2 during the therapy and advise on the duration. The estimation of dosing is essential to establish optimal efficacy in addition to PAD/CLI treatments, immunological disorders (Müller-Ladner et al., 2009) and moreover as suggested for a protective role in scavenging free radicals and suppressing oxidative metabolism (Vesela and Wilhelm, 2002). It can be concluded that comparison of these C-isotope ratios can serve as a suitable tool to measure the rate of transdermal absorption pass into the circulation. The methodology described here could provide one additional step for a more precise base in dose calculations, considering the systemic distribution and excretion rate by exhaled elimination.

These additional data provided confirmation on the effectiveness of dry-CO_2 bath therapies, confirming the rate of transdermal absorption, and given details of the systemic distribution and elimination via exhaled air. More proper dose calculation is also supporting the recent clinical practice of CO_2 therapies, which are intended to provide a long–term sustainable, safe and effective treatment options. The methodology encompasses not just the classical adjuvant PAD/CLI therapy for rehabilitation of microvascular disorders but represents a powerful tool for the future in a broad area of prevention, providing a cost-effective option for public use.

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