Residential ventilation and carcinogenesis.

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INTRODUCTION

The adverse effects of the most widely recognized greenhouse gas and pollutant on the planet, carbon dioxide, are also the most ubiquitously overlooked when present as a (processed) food ingredient, as a (supercritical) solvent and as an excipient. CO_2 is a constituent of carbonated beverages, used as an insufflation gas and to elevate alveolar CO_2 concentration in acute respiratory distress syndrome (ARDS). It is used in endoscopic and dermatological cryotherapy, as a solvent in food, chemical and pharmaceutical supercritical extraction processes, as an inert atmosphere during certain types of active pharmaceutical ingredient (API), excipient and drug product manufacture and in fire extinguishers. Notably, it is also breathed in at ~6 times its concentration in atmospheric air over one third of the lifetime of individuals who dwell in closed mechanically ventilated environments.

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Increased CO_2 concentration (or partial pressure); independent of extracellular pH (1) as well as sensing enzymes associated with the hypoxia inducible factor (HIF) pathway *in vitro*; correlated with a suppression of genes involved the regulation of innate immunity and inflammation (2). This phenomenon, in turn, was largely attributed to altered activity of the nuclear factor-kappa B (NF-kB) family of transcription factors (3). Elevated CO_2 (hypercapnia) inhibited autophagy in the human macrophage independently of acidosis by inducing the expression of antiapoptotic factors Bcl-2 and Bcl-xL (4). Hypercapnia suppressed macrophage synthesis of pro-inflammatory cytokines tumor necrosis factor (TNF) and Interleukin (IL-6), phagocytosis and the generation of reactive oxygen species (ROS) by lung neutrophils (5) independently of intra or extracellular acidosis by mouse and human macrophages *in vitro*. Epithelial cell lines from mice exposed to CO_2 concentrations above 5% resulted in the increased transcription and secretion of proinflammatory cytokines resulting in lung inflammation *in vivo* (6).

Arterial CO_2 is elevated in chronic obstructive pulmonary disease (COPD) and as a consequence of 'permissive hypercapnia'; therapeutic ventilation in acute respiratory distress syndrome (ARDS) to reduce mechanical damage to the lungs (7), lung oxidative stress and alveolar cell apoptosis (8). Therapeutic hypercapnia with 3% inhaled CO_2 was also found to protect against hepatic ischemia reperfusion injury in a murine model (9) by reducing and increasing the levels of pro and anti-inflammatory cytokines respectively and by attenuating both apoptosis and the over-expression of NF-kB. For example, protein phosphatase-2 (PP2A) release and NF-$\varkappa$B nuclear translocation from the pulmonary cells of mice exposed to hypercapnia for one hour had p-values < 0.027 for a CO_2 concentration 5% when compared with that at 0% (6). Similarly, the phagocytic index (PI) and H_2O_2 production of lung neutrophils from *Pseudomonas aeruginosa* infected mice decreased significantly from air-breathing to 10% CO_2 ($p<0.003$ for PI)(5).

There exists a profusion of signaling pathways and transcription factors that can repolarize macrophages either toward a pro-tumor or an anti-tumor phenotype (10). In this context, long-term, consistent intermittently elevated CO_2 would be expected to reprogram macrophages toward an anti-inflammatory, immunosuppressive phenotype.

A common ventilation rate measure for residential buildings is the air changes per hour (ACH), which is the hourly ventilation rate divided by the volume of the space. Today, more rigorous building codes and voluntary labeling programs are requiring builders to test for and meet stricter air leakage (infiltration) limits. The 2009 International Energy Conservation Code (IECC), required homes to have air leakage of 7 or less air changes per hour at 50 Pa pressure (ACH 50) while the 2012 IECC requires homes to meet an airtightness limit of 3 ACH 50 or less in most climates and the Passive House program sets the limit at 0.6 ACH 50. This translates to 0.35 (in 2009), 0.15 (in 2012) and 0.03 ACH at atmospheric pressure (11). The American Society of heating, refrigerating and air-conditioning engineers (ASHRAE) 62.2 standard recommends a minimum ACH of 0.35 but no less than 3 (cubic feet per minute) CFM/100 square feet (15 l/s/100 square meters) plus 7.5 CFM/person (3.5 L/s/person). The ASHRAE standards assume the dwelling to be one continuous ventilated zone. However, since different rooms can be isolated by closable doors with minimal air exchange among them; and given that significantly non-uniform diurnal dweller density exists; the greatest inspired CO_2 levels can be anticipated in a closed bedroom during the night. Since the International residential code (IRC) 2012, only requires whole house mechanical ventilation where the tested air infiltration rate is < 5 ACH50 (< 0.25 ACH at atmospheric pressure) such ventilation systems can be, and frequently are, designed to cycle on and off in tandem with the home's heating, ventilation and air conditioning (HVAC) system (12). These homes are under ventilated since fresh outside air is metered through the intake/return duct only when the thermostat calls for heating/cooling. Consequently, when there is minimum difference between outside and thermostat set temperatures, typically occurring during late evening to early morning, CO_2 levels increase. As builders and codes approach zero energy homes with ever-tighter and efficient insulated building enclosures, ventilation requirements are not regulated or implemented at a pace comparable to those for energy conservation. It is ironic that one of the justifications for recommending tighter standards for residential homes (other than increased energy efficiency) is to decrease

indoor pollutants such as fungi, pollen, mold and bacteria. However, the increase in CO_2 levels may negate these benefits because of its propensity to attenuate resistance to opportunistic microbial infections.

METHODS

The mathematical model in this paper assumes a 16.5 x 16.5 t x 8 feet closed bedroom (61.7 m^3) (13) with 2 persons exposed to the maximum long-term consistently elevated CO_2 levels for 6.6 hours every night. CO_2 exhaled per minute per person was taken as 0.5 g (14). The CO_2 concentration in the room was calculated using Equation 1(15).

$$c = \left[\frac{q}{nv}\right]\left[1 - \left(\frac{1}{e^{nt}}\right)\right] \qquad \text{Eq. 1}$$

Where; c is the CO_2 concentration in the room at time t hours, n is the ACH, q is the concentration of the CO_2 supplied to the room, taken to be 400 ppm, t is the time in hours and v is the volume of the room.

Arterial PCO_2 was calculated from humans breathing air containing different concentrations of CO_2 from data obtained from three studies (16-18) The data fit a sigmoidal relationship between normobaric and 7% atmospheric CO_2 with an $R^2 > 0.999$.

$$y = 285.0536 + \frac{(40.05 \; - \; 285.0536)}{1 \; + \left(\frac{x}{250393.5}\right)^{1.901143}} \qquad \text{Eq. 2}$$

The normobaric CO_2 concentration was taken as 0.03% and the corresponding arterial PCO_2 as 40 mmHg. The solubility of CO_2 in blood was calculated for different atmospheric concentrations by first converting those concentrations to arterial PCO_2 values (from Equation 2 above) and then using the CO_2 solubility coefficient(19) of 0.0308 mMol. L^{-1}. $mmHg^{-1}$ to calculate the solubility at a given arterial PCO_2. The pH was calculated from the Henderson-Hasselbach equation using a value of 6.1 for the pK_a of carbonic acid (Equation 3).

$$pH = 6.1 + \log\left[\frac{HCO_3^{-1}}{0.0308 \text{ x } pCO_2}\right] \qquad \text{Eq. 3}$$

Where, $HCO_{3\text{-}1}$ is the bicarbonate ion concentration in mM. L^{-1} , and pCO_2 is the partial pressure of CO_2 in mmHg.

RESULTS AND DISCUSSION

As shown in Table 1, the indoor CO_2 concentration can increase to as much as 1600 and 2400 ppm over a period of 6 hours with an ACH of 0.35 and 0.15 respectively; representing a 4 and 6 times increase over normobaric atmospheric CO_2 levels. It has been postulated that the metabolic syndrome represents an adaptation to these increased CO_2 levels (20).

Table 1 Concentration of indoor CO_2 over time as a function of ACH

Minutes	ppm CO_2 @ 0.15 ACH	ppm CO_2 @ 0.35 ACH
0	387.00	387.00
20	548.02	542.81
30	625.55	614.16
60	846.87	804.85
80	985.46	914.65
120	1242.70	1099.31
160	1475.45	1245.55
200	1686.06	1361.36
240	1876.63	1453.06
280	2049.07	1525.69
320	2205.09	1583.20
360	2346.27	1628.74
400	2474.02	1664.81

Table 2 shows that even though there is a ~4 fold increase in the partial pressure of CO_2 at 400 minutes, the increased value is negligible when compared with a PCO_2 of ~40 mm Hg in arterial blood.

Table 2 pH of blood as a function of inhaled CO_2 concentration at 0.35 ACH

Minutes	CO_2 air, mm Hg	ppm CO_2	Arterial CO_2 partial pressure, mmHg	Solubility of CO_2 in blood mMol/L	pH of blood
0	0.294	387.00	40.051	1.201	7.363
20	0.413	542.81	40.052	1.201	7.363
30	0.467	614.16	40.053	1.201	7.363
60	0.612	804.85	40.054	1.201	7.363
80	0.695	914.65	40.056	1.201	7.363
120	0.835	1099.31	40.058	1.201	7.363
160	0.947	1245.55	40.060	1.201	7.363
200	1.035	1361.36	40.062	1.201	7.363
240	1.104	1453.06	40.064	1.201	7.363
280	1.160	1525.69	40.065	1.201	7.363
320	1.203	1583.20	40.066	1.202	7.363
360	1.238	1628.74	40.067	1.202	7.363
400	1.265	1664.81	40.068	1.202	7.363

This explains the insignificant increase in arterial PCO_2 (and solubility) at a CO_2 concentration of 1664 ppm; in turn explaining the constant blood pH. This is consistent with the Davenport diagram which predicts a pH change of only 0.15 units when the arterial PCO_2 is increased to 60 mm Hg (21), corresponding to an atmospheric CO_2 concentration of 7% as calculated from the equation. Other studies reported an even lesser effect of arterial PCO_2 on pH; of the order of decreasing 0.014 pH units for every 10 mm Hg increase of PCO_2, even in hypercapnic COPD patients (22). It can be readily seen that elevated indoor CO_2 concentrations in under ventilated dwellings are an order of magnitude lesser than arterial PCO_2 as well as (an order of magnitude) less than concentrations that could cause an increase in arterial PCO_2. *In vitro* studies that reported significant effects of increased PCO_2 on inflammation and immunity used atmospheric PCO_2 greater than arterial PCO_2 (> 5%); a situation that would never be expected to occur even with the elevated CO_2 levels (~0.5%) in under ventilated homes. In the absence of a positive concentration differential between atmospheric and arterial PCO_2 levels in under ventilated homes, it is not possible to attribute physiological effects using the classical model of a concentration gradient or acidosis; consequently such published models (23) must be viewed with skepticism (24).

On the other hand, there are significant changes in the allostatic load, breathing rate; increased prevalence of acute health symptoms such as mucosal irritation and headache (25); slower work performance and a decrement in decision-making performance (26); at inhaled CO_2 concentrations > 600 ppm suggesting that a positive concentration gradient between atmospheric CO_2 and arterial CO_2 (such as that employed in *in vitro* studies) is not necessary to cause CO_2 related physiological effects. Since physiological effects rarely manifest without concomitant modulation of the underlying molecular machinery, an elevation of level to one order of magnitude greater than that existing in atmospheric air (such as that found in under-ventilated homes) may be sufficient to cause re-programming of signaling pathways and/or transcription factors upon chronic exposure. There is no statistical method to correlate the (statistical significance) of the observed change in a few measured effectors

from acute studies that result from large perturbations to the independent variable with chronic exposure studies that involve mildly perturbed variables. However, retrospective analysis of pathway data that probes multiple genes and pathways simultaneously (27) suggests that the effect of interconnected signaling pathways is to change (the expression of) observed pathways or genes to either approach or achieve statistical significance in chronic exposure studies by virtue of their known mechanistic involvement in the disease process. In this case, statistically significant changes in the expression levels of downstream bio-effectors are not obligatory. This explains why statistical significance in acute studies is not necessarily correlated to clinical outcomes in chronic studies (28). It is therefore possible that long-term exposure to intermittent moderately elevated CO_2 levels may cause clinical outcomes that cannot be detected or predicted by acute large-magnitude exposures.

Allostatic load, as quantitated by measuring the DNA lesion oxidized nucleoside, 8-hydroxydeoxyguanosine (8-OHdG) and the pro-inflammatory peptides IL-6 and TNF-α, was significantly correlated with the difference between indoor (median 664 ppm) and outdoor CO_2 levels in sick buildings (29). Hypercapnia can increase the antigenicity and pathogenicity of opportunistic bacteria (30) residing in the airway, thereby increasing the potential to cause allergenic (31), inflammatory and infectious disease (32). Chronic low-grade upregulation of pro-inflammatory cytokine and chemokine network pathways has been known to be associated with the etiology and progression of cancer (33).

As a first approximation, regulatory agencies calculate the acceptable daily intake (ADI) of a chemical by dividing the no-observed-adverse-effect-level (NOAEL) by an uncertainty factor. The uncertainty factor is usually 100, a factor of 10 compensating for interspecies variation and another factor of 10 compensating for intraspecies susceptibility or variation of response (34). Taking 1% CO_2 as the NOAEL above which alteration of gene expression, modulation of immunological and inflammatory pathways and physiological adaptive changes (35) were evident in sub-chronic studies, and correcting only for the interspecies variability, a dose of 0.1% (1000 ppm) CO_2 per day may be considered analogous to the ADI.

There have been no long-term studies performed to date to attribute consistently intermittent moderately elevated CO_2 levels to the modulation of carcinogenesis, thus, there is no evidence linking the two. Studies on astronauts, while legitimately focusing on radiation as being the major causative factor (36), do not even list elevated CO_2 levels in spacecraft (7000 ppm) as a confounding variable (37). However, routine reports of toxicity at these levels has prompted re-evaluation of long-term exposure. Studies on submariners adopt the Occupational Safety and Health Administration (OSHA) standard of a maximum permissible level of 5000 ppm (38). Of all the studies that have investigated the effects of carbonated beverages on cancer risk, only one mentions CO_2 as a probable mechanistic ingredient (39). The others concentrate on high fructose corn syrup, sugar substitutes or carcinogenic compounds that either spill over into the final product from the manufacturing process or are minor components of the ingredients themselves.

CONCLUSIONS

Recent and emerging research suggests that acute exposure to CO_2 levels as low as 1% alters inflammatory and immune signaling pathways independent of extra or intracellular acidosis; including those effectors that are known to be implicated in the modulation of carcinogenesis. Acute exposure to CO_2 levels as low as 600 ppm, which are commonly

exceeded in indoor air, causes increased allostatic load and adverse physiological effects. Are decreased utility bills a sufficient tradeoff to compensate for a, as yet undetermined, long-term risk of carcinogenesis? Will the increased rate of CO_2 greenhouse gas generation caused by burning fossil fuels to keep homes from being under-ventilated offset the, as yet undetermined, long-term risk of carcinogenesis? The building code today takes into account the probability of electrocution by mandating location specific ground fault circuit interrupters (GFCI). Although unaware of the less-evident yet potentially far more insidious risk of carcinogenesis arising from increased CO_2 levels in under-ventilated homes, ASHRAE standard 62.1-2004 does make allowance for demand controlled ventilation (DCV), wherein, CO_2-DCV can be implemented with a view to reducing energy use (40). Although there is no definitive evidence linking increased indoor CO_2 levels to carcinogenesis, enough allostatic and physiological data exists to warrant further research, especially in the hypercapnia associated COPD population, in chronic exposure animal studies and *in vitro* models. Given the well-established role of oxygen sensing pathways in cancer, and the recent acidosis independent role of CO_2 in modulating immune and inflammation linking pathways, it seems obligatory to validate (or not) the effects of long-term inspired elevated CO_2 on the modulation of carcinogenesis.

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