Influence of methazolamide on the human control of breathing: a comparison to acetazolamide

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Abstract

Acetazolamide is used to prevent/treat acute mountain sickness and both central and obstructive sleep apnoea. Methazolamide, like acetazolamide reduces hypoxic pulmonary vasoconstriction, but has fewer side effects including less skeletal muscle function impairment. Since methazolamide's effects on respiratory control in humans are unknown, we (1) compared the effects of oral methazolamide and acetazolamide on ventilatory control, and (2) determined the ventilation-log PO_2 relationship in humans. In a double blind, placebo-controlled, randomized cross-over design, we studied the effects of acetazolamide (250 mg tid), methazolamide (100 mg bid) and placebo in fourteen young male subjects who were exposed to 7 minutes of normoxic hypercapnia and to three levels of eucapnia and hypercapnic hypoxia. With placebo, methazolamide, and acetazolamide, the CO_2 sensitivities were 2.39 ± 1.29, 3.27 ± 1.82 and 2.62 ± 1.79 l/min/mmHg (NS) and estimated apnoeic thresholds were 32 ± 3, 28 ± 3 and 26 ± 3 mmHg, respectively ($P < 0.001$, placebo *vs* methazolamide and acetazolamide). The relationship between ventilation ($\dot{V}_I$) and log PO_2 (using arterialized venous PO_2 in hypoxia) was linear, while neither agent influenced the relationship between hypoxic sensitivity ($\Delta\dot{V}_I/\Delta\log PO_2$) and arterial $[H^+]$. Using $\Delta\dot{V}_I/\Delta\log PO_2$ rather than $\Delta\dot{V}_I/\Delta SaO_2$ enables a more accurate estimation of oxygenation and ventilatory control in metabolic acidosis/alkalosis when right- or left-ward shifts of the oxyhaemoglobin saturation curve occur. Since acetazolamide and methazolamide has similar effects on ventilatory control, methazolamide may be preferred for indications requiring the use of a carbonic anhydrase inhibitor, avoiding some of the negative side-effects of acetazolamide.

Key words: carbonic anhydrase inhibitors, respiration, ventilation, hypoxia, hypercapnia, altitude sickness.

New Findings

What is the central question of this study?

Acetazolamide and methazolamide both reduce hypoxic pulmonary vasoconstriction equally, but methazolamide does not impair skeletal muscle function. The effects of methazolamide on respiratory control in humans is not yet known.

What is the main finding and its importance?

Similar to acetazolamide after chronic oral administration, methazolamide causes a metabolic acidosis and shifts the ventilatory CO_2 response curve leftwards without reducing O_2 sensitivity. The change in ventilation over the change in log PO_2 provides a more accurate measure of hypoxic sensitivity than the change in ventilation over the change in SaO_2.

Introduction

Acetazolamide (AZ) has been used to stimulate ventilation in patients with chronic obstructive pulmonary disease (COPD), to correct metabolic alkalosis (Adamson & Swenson, 2017), to treat sleep disordered breathing (Edwards *et al.*, 2012; Javaheri *et al.*, 2014) and in the prophylaxis of acute mountain sickness [AMS; (Leaf & Goldfarb, 2007; Kayser *et al.*, 2012)]. Another property of AZ is its ability to reduce pulmonary vasoconstriction upon acute exposure to hypoxia (Teppema *et al.*, 2007; Ke *et al.*, 2013). The rationale for the use of AZ in COPD patients and in correcting metabolic alkalosis, however, is limited and disputed (Adamson & Swenson, 2017). A recent study showed no effect of intravenous AZ on liberation from mechanical ventilation in patients with COPD and hypercapnic respiratory failure (Faisy *et al.*, 2016). Weaning failure is more associated with respiratory muscle weakness and airway resistance rather than reduced respiratory drive (Adamson & Swenson, 2017). In rabbits, low-dose AZ causes neuromuscular impairment of the diaphragm (Kiwull-Schone *et al.*, 2001). In humans, AZ impairs respiratory muscle function at rest and after moderate-intensity cycling exercise or respiratory muscle loading (Gonzales & Scheuermann, 2013; Dominelli *et al.*, 2018). In both animals and humans, an acute low intravenous dose of AZ reduces the acute hypoxic ventilatory response (AHVR) (Teppema & Dahan, 2004; Teppema *et al.*, 2006*b*), but the usual chronic oral administration in humans does not share this depressant effect (Swenson & Hughes, 1993; Teppema *et al.*, 2010).

In animals, the depressant effects of acute intravenous AZ on respiratory muscles and AHVR are not shared by methazolamide (MZ), an analogue of AZ with a similar potency in inhibiting carbonic anhydrase isozymes (Teppema *et al.*, 2006*a*; Kiwull-Schone *et al.*, 2009). In AMS and glaucoma, MZ is effective at lower doses than AZ, with fewer side effects (Dahlen *et*

al., 1978; Forster, 1982; Wright *et al.*, 1983). In humans, MZ (100 mg bid) results in less diaphragm and dorsiflexor neuromuscular fatigue than AZ (250 mg tid), but reduces hypoxic pulmonary vasoconstriction (HPV) equally (Dominelli *et al.*, 2018; Boulet *et al.*, 2018). MZ might thus be an alternative for AZ in preventing AMS and other situations where stimulation of ventilation and moderation of hypoxic pulmonary vasoconstriction has benefits, provided it improves oxygenation equally while lacking depressant effects on respiratory control and respiratory muscle strength. In humans, the effects of MZ on respiratory control are unknown and our aim was to measure its actions on ventilatory O_2 and CO_2 sensitivities, and to compare these with those of AZ. Metabolic acidosis shifts the oxyhaemoglobin dissociation curve rightwards, leading to an underestimation of hypoxic sensitivity when using oxyhaemoglobin saturation (SaO_2 or SpO_2) as the independent variable, since the peripheral chemoreceptors sense PaO_2 and not SaO_2. Therefore, our second aim was to examine if a linear relationship exists between ventilation and logPO_2 by exposing subjects to three levels of inspired hypoxia. AZ and MZ appeared to have similar effects on ventilatory control and oxygenation. For the first time we show a linear relationship between ventilation and log PO_2 in humans. Consequently, by this relationship, under- or over-estimation of hypoxic sensitivity in metabolic acidosis and alkalosis, respectively, can be avoided.

Methods

All subjects provided written informed consent and the study conformed to the latest revision of the declaration of Helsinki; the procedures were approved by the Clinical Research Ethics Board of the University of British Columbia (H16-00028) and the trial registered (Clinicaltrials.gov; NCT02760121).

Fourteen healthy young men (mean age 25 ± 3 yrs; BMI 25.2 ± 2.0 kg/m^2) were recruited for three testing sessions separated by ≥ 10 days (AZ, MZ, and placebo). All participants completed ventilatory testing to (1) eucapnic hypoxia, (2) hypercapnia, and (3) hypercapnic hypoxia to determine O_2 and CO_2 ventilatory sensitivity and their interaction. This study was part of a series of two separate studies in the same subjects. The first study addressing pulmonary vascular responses to one hour of poikilocapnic hypoxia (Boulet *et al.*, 2018) and occurred at least one hour after the control of breathing assessments made in this study to avoid carry-over effects.

Drug administration. An independent pharmacy provided randomized and blinded medication in identical blister packages containing gel capsules without identification. AZ (250 mg/dose) and placebo (microcrystalline cellulose) were ingested 3x per day (every 8 hours) for three days; whereas, MZ (100 mg/dose; three days) was ingested 2x per day but separated by a placebo capsule to maintain a similar ingestion schedule to AZ and placebo.

Experimental design. A double blind, placebo-controlled, randomized cross-over study design was utilized. One hour after the last dose was taken, testing was performed in a climate-controlled laboratory at 344 m above sea level. Participants were asked to avoid exercise and caffeine on each experimental day and to arrive at the lab 2-hours post-prandial. Following 10 minutes of supine rest, arterial blood was acquired from a radial arterial puncture and analysed

for PO_2, PCO_2, SaO_2, electrolytes, $[H^+]$, $[HCO_3^-]$, haemoglobin and haematocrit. Thereafter, participants rested for 15 minutes before determination of their eucapnic AHVR by exposing them to three, 3-minute step reductions in end-tidal PO_2 ($P_{ET}O_2$; 65, 57, and 47 mmHg) while maintaining isocapnia with titrated addition of small amounts of CO_2 into the inspired gas. Following 10-minutes of recovery, the end-tidal PCO_2 ($P_{ET}CO_2$) was elevated 6 mmHg from baseline for seven minutes while maintaining $P_{ET}O_2$ at baseline levels to determine the hypercapnic ventilatory response (HCVR), then $P_{ET}O_2$ was again reduced in 3-minute steps to 65, 57, and 47 mmHg while maintaining hypercapnia to determine the hypercapnic AHVR. End-tidal gas control was achieved using a custom built end-tidal forcing system (Tymko *et al.*, 2015, 2016). Throughout testing, minute ventilation (Pneumotach, 800L, Hans Rudolph, Shawnee, KS), end-tidal gases ($P_{ET}O_2$ and $P_{ET}CO_2$, ML206, AD Instruments, Colorado Springs, CO), beat-by-beat blood pressure (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands), and heart rate (lead II, FE132, AD Instruments) were recorded continuously. Arterialized venous blood samples were collected from a dorsally placed anterograde intravenous catheter in the hand continuously heated to 45 °C at each stage and immediately analysed for PO_2, PCO_2, oxyhaemoglobin saturation (SO_2), $[H^+]$ and $[HCO_3^-]$ by a blood gas analyser (ABL 90 Flex, Radiometer, Copenhagen, Denmark). In two subjects after AZ treatment a catheter was inserted into a radial artery to compare arterial blood gases directly with those obtained from arterialized venous blood. In addition, in all subjects we compared baseline arterial blood gases from arterial punctures with those obtained from arterialized venous samples. As expected, venous PO_2 values greatly underestimated PaO_2 by 30-34 mmHg at baseline, but this underestimation was minimal (2-3 mmHg) during hypoxia. For this reason, PaO_2 was used at baseline while arterialized venous PO_2 was used as an estimate of PaO_2 in

hypoxia. To avoid misunderstandings when both arterial and arterialized venous PO_2 are presented together, we express data as PO_2 only (eg. log PO_2).

Analysis & statistics. Breath-by-breath and beat-by-beat data were averaged over the last 30 seconds of each stage of gas manipulation. Data are presented as means ± SD. Statistics were performed using R statistical language (V3.5.0, R Foundation for Statistical Computing, Vienna, Austria). For baseline variables, a one-way repeated measures ANOVA was used to identify differences among treatments. When significant F ratios were detected Tukey's post-hoc analysis was conducted. To determine the influence of each drug on responses to eucapnic hypoxia, hypercapnia, and hypercapnic hypoxia, a mixed effect linear model was constructed to detect significant differences in the slope and intercept of the relationships between ventilation, and imposed stimuli (i.e. logPO_2, PCO_2, $[H^+]$, or SO_2). Drug condition was entered as a fixed factor along with the relevant stimuli as a continuous predictor. Subject was entered as a random factor to account for correlation within subjects. Statistical significance was set at the $P < 0.05$ level.

Results

Participants.

Three subjects were excluded from data analysis for lack of adherence to the medication schedule (n = 1), or unstable and irregular breathing patterns (n = 2). The eleven participants included in the primary data analysis had a mean age of 25 ± 3 years, a body mass index of 25.1 ± 2.0 kg/m_2 and were normotensive (MAP = 89 ± 10 mmHg) with normal pulmonary function (FEV_1 = 102 ± 17 % of predicted; FVC = 115 ± 10 % of predicted; FEV_1/FVC = 0.76 ± 0.17).

Effects of methazolamide and acetazolamide on resting blood and exhaled gas parameters.

MZ and AZ induced a hyperchloraemic metabolic acidosis, accompanied by a fall in $PaCO_2$. Resting arterial blood gases, haemtaology, acid-base and electrolyte status is provided in Table 1. Both MZ and AZ increased $[H^+]$ and base excess compared to placebo, however the effect was significantly greater for AZ ($P < 0.01$). With AZ, there was a small but significant reduction in plasma $[K^+]$. There were no differences in plasma osmolality or $[Na^+]$, $[lactate^-]$, and [glucose] across all treatments.

Table 2 summarizes baseline end-tidal gases, arterial oxyhaemoglobin saturation, ventilatory, and circulatory parameters. Compared to placebo, $P_{ET}CO_2$ was lower and $P_{ET}O_2$ higher after MZ and AZ. Mean arterial blood pressure was similar across all treatments.

Effects of methazolamide and acetazolamide on respiratory control

Figure 1 illustrates the eucapnic and hypercapnic AHVR for all subjects after MZ, AZ, and placebo. The eucapnic AHVR is expressed as the slope of the relationship between minute ventilation during the last 30 seconds of the exposure and log PO_2. Similar to its relationship

with SaO_2, the relationship between ventilation and log PO_2 is linear (mixed model R^2, eucapnic AHVR = 0.76; hypercapnic AHVR = 0.91).

SaO_2 vs. log PO_2. The percent change in AHVR slope between eucapnic and hypercapnic conditions tended to be underestimated in the presence of metabolic acidosis when expressed as $\Delta\dot{V}_I/\Delta SaO_2$. In placebo, the difference in the percent change in AHVR slope calculated using log PO_2 and SaO_2 was -9 ± 51 % (P = 0.57), but was 48 ± 69 % in MZ (P = 0.04) and 47 ± 77 % (P = 0.07) in AZ. Since metabolic acidosis causes a rightward shift of the oxyhaemoglobin saturation curve, we prefer log PO_2 over SaO_2 for assessing hypoxic sensitivity (PaO_2 rather than saturation is the actual carotid body stimulus; PO_2 in this study was obtained from arterialized venous samples). Regardless of drug condition, the hypercapnic hypoxia response lines are shifted upwards (P < 0.001) and have a steeper slope compared with the eucapnic AHVR (P < 0.001). In eucapnia, the AHVR (not its sensitivity to changes in $[H^+]$) was slightly but significantly reduced by AZ compared with placebo (P < 0.01) but not MZ (P = 0.13). Conversely, the hypercapnic AHVR was similar across all drug treatments (P = 0.32; figure 1). Figure 2A illustrates the interaction between the AHVR and arterial $[H^+]$. The relationship between AHVR and arterial $[H^+]$ remained intact (*i.e.* slopes remain parallel, P = 0.77) indicating that the O_2/H^+ interaction at the level of ventilation was unaffected by MZ and AZ. Figure 2B displays the HCVR and shows a leftwards shift of the relationship between ventilation and $P_{ET}CO_2$ by MZ and AZ, without significant effects on the slope (P = 0.25).

Discussion

AZ and MZ cause a hyperchloraemic metabolic acidosis and about equal decreases (5-6 mmHg) in $P_{ET}CO_2$ and increases in $P_{ET}O_2$ (4-5 mmHg). Our data show that both agents caused equivalent parallel leftward shifts of the HCVR. In addition, for the first time we show a linear relationship between ventilation and log PO_2 in humans. In eucapnia, AZ led to a small but significant reduction in AHVR while placebo and MZ were similar. In hypercapnia, AHVR was similar for both drugs. Finally, neither drug altered the ventilatory interaction between O_2 and $[H^+]$. Our results suggest that oral doses of AZ and MZ as studied herein result in similar effects on the control breathing.

Slope of the HCVR and the apnoeic threshold

In placebo, the slope of the mixed effect linear model expressing the group HCVR (2.39 ± 1.33 l/min/mmHg) and the range of the individual HCVR (0.98 - 4.75 l/min/mmHg) was similar as previously reported for male subjects (Hirshman *et al.*, 1975). AZ and MZ produced about equal decreases in the (estimated) apnoeic threshold but did not affect the HCVR slope (figure 2B).

Due to differences in methodology, study design, oxygen levels, dose regimen, route of administration, magnitude of the metabolic acidosis and between-day variance, the reported effects of AZ on the HCVR are ambiguous. Usually, oral AZ causes an increase in the HCVR determined with Read rebreathing in hyperoxia (Tojima *et al.*, 1986; Javaheri *et al.*, 2014). In agreement with the known parallel leftward shift of the HCVR in metabolic acidosis, AZ usually has no influence on the steady state *normoxic* HCVR slope (Swenson & Hughes, 1993; Teppema

& Dahan, 1999), but a small increase in the *hyperoxic* slope is not unusual (Teppema *et al.*, 2010; Teppema & Dahan, 2010).

Expressing the acute hypoxic ventilatory response

Usually, the AHVR is expressed as $\Delta\dot{V}_I/\Delta SpO_2$, because the $\dot{V}_I$-SpO_2 relationship is linear and SpO_2 is easy to measure using pulse oximetry - although at saturation levels < 80%, SpO_2 is a less reliable measure of arterial oxyhaemoglobin saturation and the linearity of the relationship depends on the experimental conditions (Teppema & Dahan, 2010).

Metabolic acidosis shifts the oxyhaemoglobin dissociation curve rightward and changes its shape, resulting in underestimation of hypoxic sensitivity if oxyhaemoglobin saturation is used as the independent variable, and vice versa for metabolic alkalosis since the actual stimulus is PaO_2, not saturation. We were not able to measure P_{50} values; in a previous study with the same AZ dose we found a significantly lower hypoxic sensitivity (after AZ, but not after placebo) when using saturation instead of log PO_2 (Teppema *et al.*, 2010). By exposing subjects to four different oxygenation levels, we show that, similar to the cat (Riedstra, 1963), a linear $\dot{V}_I$-logPO_2 relation also exists in humans, with correlation coefficients similar to and regularly even somewhat greater than those of the $\dot{V}_I$-SaO_2 relationship. We are therefore confident that expressing hypoxic sensitivity as $\Delta\dot{V}_I/\Delta\log PO_2$ is a more accurate measure of the AHVR in metabolic acidosis (see also (Teppema *et al.*, 2010)).

Hypoxic sensitivity, arterial [H^+] and central PCO_2

Figure 1 shows the ventilatory O_2-CO_2/H^+ interaction, resulting in up-ward shifts and steeper $\dot{V}_I$-logPO_2 lines at higher PCO_2. This overall ventilatory O_2-H^+ interaction results from

an O_2-H^+ interaction in the carotid bodies on the one hand, and hyper-additivity between the peripheral and central chemoreceptors on the other (Wilson & Teppema, 2016). Figure 2A shows about equal slopes of the relation between hypoxic sensitivity and $[H^+]$ with placebo, MZ and AZ, indicating that both agents neither directly inhibit the peripheral chemoreceptors nor affect the peripheral-central interaction (Teppema *et al.*, 2010). Taking the inert action of CO_2 on the carotid bodies into account (changes in arterial $[H^+]$ were mediated via increases in PCO_2), we suggest that, via a hyper-additive interaction between peripheral and central chemoreceptors (Teppema *et al.*, 2010; Wilson & Teppema, 2016), the resulting lower *central* PCO_2 must be responsible for the similar hypoxic sensitivities at rest with placebo and MZ and the slightly lower sensitivity after AZ compared to placebo (Figure 2A). The slightly reduced $[K^+]$ (see Table 1) observed in AZ, owing to its diuretic effect, may also be a contributing factor (McLoughlin *et al.*, 1995; Paterson, 1997). Figure 2A shows the considerable impact of the *central* PCO_2 on the AHVR (*cf.* isohydric points in the plot; note that the lower eucapnic AHVR after AZ is due to the lower prevailing $PaCO_2$). In contrast to clinical oral doses, in humans a single acute intravenous dose of AZ, which when studied before full development of a metabolic acidosis, inhibits the AHVR (Swenson & Hughes, 1993; Teppema *et al.*, 2006*b*). In rabbits, even high dose MZ does not share the neuromuscular impairment of the diaphragm by low intravenous dose AZ (Kiwull-Schone *et al.*, 2009), suggesting that AZ acts independently of carbonic anhydrase inhibition to induce this effect.

AZ, MZ and high altitude

It has been suggested that chronic oral AZ increases central chemoreceptor output while inhibiting the carotid bodies, resulting overall in a rise in ventilation (Leaf & Goldfarb, 2007). The scenario is probably more complex.

At sea level, chronic oral AZ and MZ do *not* inhibit the carotid bodies, while central CO_2 sensitivity remains unaltered (Teppema & Dahan, 1999), and due to its low penetration rate into the CNS direct effects of AZ on the central chemoreceptors are unlikely.

At high altitude, AZ and MZ increase arterial $[H^+]$, tending to *increase* carotid body output and sensitivity. However, the rise in ventilation improves oxygenation and lowers the $PaCO_2$, tending to *decrease* carotid body output and gain of the peripheral chemoreflex, respectively - note that the peripheral chemoreflex gain depends on both the H^+/O_2 interaction in the carotid bodies and the peripheral-central interaction. The overall effects are increases in ventilation and hypoxic sensitivity, both induced by the lower pH but accompanied by a *lower* PCO_2 (figure 2); note that the metabolic hyperbola for CO_2 dictates that with already high ventilation, at altitude the drop in PCO_2 after AZ and MZ is less than at sea level (see (Wright *et al.*, 1983)) preventing a net decrease in hypoxic sensitivity. AZ and MZ have about equal effects on blood gases at high altitude (Wright *et al.*, 1983).

Combined with the present findings on respiratory control, our previous data on the equal reduction in HPV but the superior effect of MZ on muscle performance suggest that future field (including dose-response) studies are warranted to investigate whether MZ may provide an equal or potentially even better benefit in AMS prophylaxis than AZ (Kiwull-Schone *et al.*, 2001, 2009; Teppema *et al.*, 2007; Dominelli *et al.*, 2018; Boulet *et al.*, 2018). In these studies, potential confounding factors such as different ascent rates and timing of drug intake (before, during or even after ascent) should be avoided.

AZ in sleep and metabolic alkalosis

Sleep. We detected no differences in effect on ventilation, oxygenation and chemosensitivities between both agents. Both caused about equal decreases in $PaCO_2$, and leftwards shifts of the HCVR. Both effects underlie the increase in breathing stability during sleep as shown for AZ: the fall in PCO_2 causes a decrease in the plant gain of the ventilatory control system (Edwards *et al.*, 2012; Javaheri *et al.*, 2014) and the leftwards shift results in an increase in the CO_2 reserve (*i.e.* a greater difference between the prevailing PCO_2 and the PCO_2 at which apnoea ensues). Effects of MZ on breathing during sleep are unknown, but we suggest that it may have equally beneficial effects.

Metabolic alkalosis. The linear relationship between ventilation and log PO_2 may enable clinicians, by using log PO_2 rather than saturation, to avoid a potentially dangerous overestimation of the oxygen status and hypoxic sensitivity due to a leftward shift of the oxyhaemoglobin dissociation curve. This overestimation is due to the fact that the PaO_2 rather than SaO_2 is the actual stimulus to the carotid bodies. In the ICU, intravenous AZ is effective in correcting the metabolic alkalosis in patients nonresponsive to fluid and potassium repletion (Mazur *et al.*, 1999), albeit not without exceptions (Bar *et al.*, 2015). However, this does not necessarily imply a reduction in weaning time (Rialp Cervera *et al.*, 2017) and studies are warranted to investigate if MZ, with lesser side effects and no inhibitory actions on the AHVR and diaphragm, may be a better alternative to AZ for this purpose. Apart from the question whether MZ may be less harmful than AZ in ICU patients, we suggest it is more important to carefully consider whether the use of respiratory stimulants in general in patients with poor lung function is justified.

Limitations

Generally, the between-day variances of the HCVR and AHVR are considerably higher than the within-day variance (Sahn *et al.*, 1977) and this could be a confounding factor in our study. In fact, seven subjects showed steeper CO_2 response slopes after MZ and/or AZ, but taking potential between-day variability into account, we are cautious in interpreting individual responses. Due to the large variation in CO_2 sensitivities, no significant changes could be detected. In theory, both agents should effectively inhibit CA IV located at the luminal endothelial surface of brain capillaries leading to some impairment of CO_2 clearance from the brain and a less steep tissue-to arterial PCO_2 gradient with increasing $PaCO_2$ explaining an increase in the HCVR slope after MZ and AZ in individual cases. Taken together, we cannot exclude that (at least in some subjects) MZ and AZ increase ventilatory CO_2 sensitivity. A larger sample size and a protocol taking the between-day variance into account are necessary to study this.

We cannot exclude that changes in water balance after MZ or AZ influenced responses to CO_2 and O_2. AZ has complex effects on volume status of the body: an increase in intracellular but decrease in extracellular and total body water without changing osmolality (Brechue *et al.*, 1990) that may also occur with MZ, since both drugs inhibit renal CA and cause diuresis. AZ and MZ did not alter osmolality but caused small significant increases in haematocrit indicative of a small loss of extracellular volume. Assuming a plasma volume of 5 l, we estimate a volume depletion of 4.5-5.5 % by MZ and AZ (Brechue *et al.*, 1990).

We did not use equal dosing of the drugs based on the following rationale. Both agents show different pharmacokinetics, lipophilicity (MZ >> AZ) and plasma protein binding (MZ<< AZ) so we did not consider administering equal doses of both.

A meta-analysis on effective AZ doses in AMS prophylaxis published in 2012 showed that doses of 750, 500 and 250 mg/day were all effective in preventing AMS (Low *et al.*, 2012). According to a recent report, even lower doses (125 and 62.5 mg/day) reduced the incidence of AMS (McIntosh *et al.*, 2019). It is likely, therefore, that we used a higher AZ dose than necessary, although 250 mg tid is not unusual in clinical practice (Adamson & Swenson, 2017) and in AMS prophylaxis (Kayser *et al.*, 2012). Owing to the fact that acetazolamide and likely methazolamide inhibit HPV (Teppema *et al.*, 2007; Ke *et al.*, 2013; Boulet *et al.*, 2018) by a mechanism not dependent on CA inhibition, it is highly probable that the pharmacokinetics for CA inhibition may be different from that of HPV inhibition by these drugs.

As to the MZ dose, this agent is effective at about one-third of an equipotential acetazolamide dose in glaucoma (Dahlen *et al.*, 1978). MZ 200 mg/day is within the range used clinically, and in most patients with glaucoma achieves a reasonable balance between desired and undesired effects (Dahlen *et al.*, 1978). In addition, due to its relatively easier permeation into red cells, a higher MZ dose could result in a confounding effect of CO_2 retention and a CO_2 disequilibrium between blood and alveoli on the one hand, and tissue and blood on the other.

To prevent confounding factors with respect to potential gender differences of the AHVR (Teppema & Dahan, 2010) and in the effects of AZ (Caravita *et al.*, 2015) we performed our study in men only. Future studies in women are necessary. In addition, future studies are warranted with either higher MZ dosing or lower AZ dosing to achieve equivalent metabolic acidosis and hypokalemia.

Conclusion

In summary, we have shown that the effects of AZ on ventilatory control considered to underlie the beneficial effects in AMS prophylaxis and sleep are shared by MZ. However, compared to AZ, MZ seems to lack adverse effects on the diaphragm and skeletal muscle (Dominelli *et al.*, 2018), while both agents equally reduce HPV (Boulet *et al.*, 2018). Collectively, our findings raise the impression that MZ may provide better protection against AMS, however comparative dose-response studies at high altitude are warranted to confirm this. Realising that our data collected in this and previous studies (Dominelli *et al.*, 2018; Boulet *et al.*, 2018) do not provide a robust justification, we note that with regard to the use of AZ in respiratory- and sleep-related diseases, especially in cases where it is administered intravenously (ICU), the use of AZ could be reconsidered. Finally, we have shown for the first time a linear relationship between ventilation and log PO_2 in humans that may be of particular clinical relevance in metabolic alkalosis.

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Figure Legends

Figure 1. Eucapnic and hypercapnic AHVR for (A) placebo, (B) methazolamide, and (C) acetazolamide (N = 11). Regression equations describe the relationship between minute ventilation ($\dot{V}_I$) and log PO_2 and are based on mixed effect linear models which account for correlation within subjects. Data are presented as PO_2 to reflect the fact that actual PaO_2 was determined from arterial punctures in normoxia only, while in hypoxia arterialized venous samples were collected. *P < 0.01, slope differs from placebo. †P < 0.05, slope differs from eucapnia. In placebo, mean isocapnic $PaCO_2$ values were 37.2 ± 4.3 and 41.2 ± 4.3 mmHg in eucapnia and hypercapnia, respectively; after methazolamide these values were 32.9 ± 2.3 and 38.7 ± 3.3 mmHg and after acetazolamide 31.4 ± 2.7 and 36.6 ± 3.6, respectively. Data points are means ± SD.

Figure 2. The interaction between (A) hypoxic ventilatory sensitivity and $[H^+]$, and (B) the hypercapnic ventilatory response for placebo, methazolamide, and acetazolamide (N = 11). *P < 0.01, compared with placebo. †P < 0.001, leftward shift for AZ and MZ response lines compared with placebo. Hypoxic sensitivity is measured as the slope of the mixed effect model for ventilation versus log PaO_2. In panel B, the regression equations describe the relationship between minute ventilation ($\dot{V}_I$) and end-tidal PCO_2 ($P_{ET}CO_2$), according to: $\dot{V}_I = S(P_{ET}CO_2 - B)$, where S = CO_2 sensitivity and B = apneic threshold. Regression equations are based on mixed effect linear modelling that accounts for correlation within subjects. Data points are means ± SD. AZ: acetazolamide; MZ: methazolamide.

Tables

Table 1. Resting arterial blood gases, haematology, and acid-base and electrolyte status in subjects (n=11) following placebo, methazolamide, and acetazolamide.

Variable, unit	Placebo	Methazolamide	Acetazolamide
PaO_2, mmHg	102.3 ± 16.0	103.8 ± 8.3*	104.7 ± 8.0*
$PaCO_2$, mmHg	38.1 ± 3.3	33.4 ± 2.7*	32.0 ± 2.3*
$[H^+]$, nM	37.4 ± 2.0	42.1 ± 1.7*	46.4 ± 1.0*,†
Base Excess, meq/l	0.9 ± 1.3	-5.0 ± 1.3*	-8.0 ± 1.0*,†
$[K^+]$, mM	3.9 ±0.3	3.8 ± 0.3	3.6 ± 0.3*
$[Cl^-]$, mEq/l	107.2 ± 1.7	112.7 ± 2.0*	114.5 ± 1.7*,†
[lactate$^-$], mM	0.87 ±0.3	0.62 ±0.3	0.65 ± 0.3
$[Na^+]$, mM	140.4 ± 1.7	139.9 ± 1.0	139.5 ± 1.7
[Glucose], mM	5.7 ± 1.0	5.9 ± 0.7	5.7 ± 0.7
Osmolality, mosm/kg H_2O	286.2 ± 2.7	285.6 ± 1.7	285.1 ± 2.7
Hct, %	45.2 ± 2.0	47.3 ± 3.3*	47.8 ± 2.7*
Hb, g/dl	14.8 ± 0.7	15.4 ± 1.0*	15.6 ± 1.0*

Values are means ± SD. *P < 0.05 vs. Placebo; †P<0.05 vs. Methazolamide; PaO_2, partial pressure of arterial O_2; $PaCO_2$, partial pressure of arterial CO_2; $[H^+]$, concentration of hydrogen

ions, [K^+], concentration of potassium ions; [Cl^-], concentration of chloride ions; [Na^+], concentration of sodium ions; Hct, haematocrit; Hb, haemoglobin.

Table 2. Resting end-tidal gases, minute ventilation ($\dot{V}_I$), oxyhemoglobin saturation (S_aO_2), and mean arterial pressure (MAP) in subjects (n=11) following methazolamide, acetazolamide, and placebo.

Variable, unit	Placebo	Methazolamide	Acetazolamide
$P_{ET}CO_2$, mmHg	38.9 ± 3.0	33.1 ± 3.3*	32.7 ± 3.0*
$P_{ET}O_2$, mmHg	98.1 ± 2.3	102.3 ± 5.6†	103.2 ± 2.7*
S_aO_2, %	98.0 ± 0.7	98.0 ± 1.3	97.9 ± 0.3
$\dot{V}_I$, l/min	12.2 ± 1.3	12.6 ± 2.7	11.1 ± 5.3
MAP, mmHg	80 ± 10	81 ± 10	83 ± 13

Values are means ± SD. *P < 0.001 vs. Placebo; †P<0.01 *vs*. Placebo. No differences in end-tidal gases between MZ and AZ. $P_{ET}CO_2$, end-tidal partial pressure of CO_2; $P_{ET}O_2$, end-tidal partial pressure of O_2; SaO_2, arterial oxyhaemoglobin saturation; $\dot{V}_I$, minute ventilation; MAP, mean arterial pressure.

Figure 1.

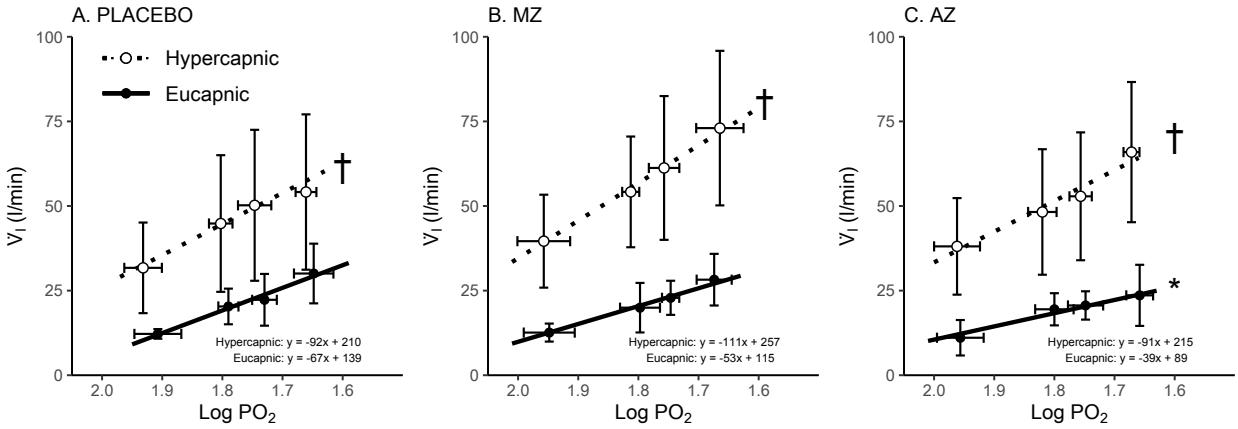
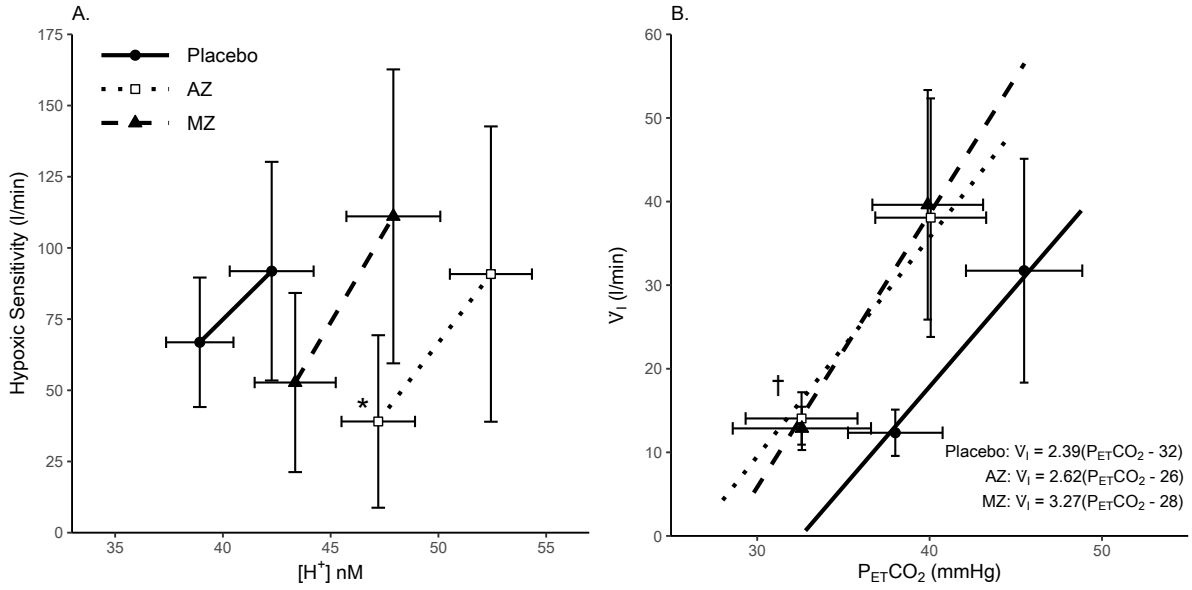

A. PLACEBO
Hypercapnic
Eucapnic
100
75
50
25
0
V_I (l/min)
†
Hypercapnic: y = -92x + 210
Eucapnic: y = -67x + 139
2.0
1.9
1.8
1.7
1.6
Log PO_2
B. MZ
100
75
50
25
0
V_I (l/min)
†
Hypercapnic: y = -111x + 257
Eucapnic: y = -53x + 115
2.0
1.9
1.8
1.7
1.6
Log PO_2
C. AZ
100
75
50
25
0
V_I (l/min)
†
*
Hypercapnic: y = -91x + 215
Eucapnic: y = -39x + 89
2.0
1.9
1.8
1.7
1.6
Log PO_2

Figure 2.


A.
Placebo
AZ
MZ
Hypoxic Sensitivity (l/min)
[H+] nM
*
B.
†
Placebo: V̇I = 2.39(PETCO2 - 32)
AZ: V̇I = 2.62(PETCO2 - 26)
MZ: V̇I = 3.27(PETCO2 - 28)
V̇I (l/min)
PETCO2 (mmHg)