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IMPROVEMENT OF BRAIN TISSUE OXYGENATION BY INHALATION OF CARBOGEN

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Abstract—Hyperoxic therapy for cerebral ischemia is suspected to reduce cerebral blood flow (CBF), due to the vasoconstrictive effect of oxygen on cerebral arterioles. We hypothesized that vasodilation predominates when 5% CO_2 is added to the inhaled oxygen (carbogen). Therefore, we used positron emission tomography (PET) to measure CBF and cerebral metabolic rate of oxygen ($CMRO_2$) during inhalation of test gases (O_2, CO_2, carbogen and atmospheric air) in 10 healthy volunteers. Arterial blood gases were recorded during administration of each gas. The data were analyzed with volume-of-interest and voxel-based statistical methods. Inhalation of CO_2 or carbogen significantly increased global CBF, whereas pure oxygen decreased global CBF. The $CMRO_2$ generally remained unchanged, except in white matter during oxygen inhalation relative to condition of atmospheric air inhalation. The volume-of-interest results were confirmed by statistical cluster analysis. Oxygen and carbogen were equally potent in increasing oxygen saturation of arterial blood (Sa_{O2}). The present data demonstrate that inhalation of carbogen increases both CBF and Sa_{O2} in healthy adults. In conclusion we speculate that carbogen inhalation is sufficient for optimal oxygenation of healthy brain tissue, whereas carbogen induces concomitant increases of CBF and Sa_{O2}.

Key words: oxygen, carbogen, brain tissue oxygenation, CBF, OEF.

Under normal steady-state circumstances, about 90% of the brain's glucose consumption precedes via oxidative phosphorylation to carbon dioxide (CO_2), with generation of stoichiometric amounts of water (Gjedde et al., 2002). Un-interrupted supply of oxygen is essential to brain function, such that disruption of oxygen delivery to the brain leads to loss of consciousness within seconds (Hansen, 1985). The therapeutic effect of oxygen therapy in patients with ischemic–hypoxic brain lesions can be explained by the occurrence of negligible mitochondrial oxygen tension such that even minimal increases of the arterial oxygen tension facilitate oxygen diffusion to the intracellular space (Hempel et al., 1977). The potential neuroprotective effect of oxygen therapy (hyperoxia) in patients with cerebral ischemia has been the focus of investigation for the past years. Different trials tested the applicability of normobaric (Flynn and Auer, 2002; Singhal et al., 2002; Kim et al., 2005; Singhal, 2007) and hyperbaric (Badr et al., 2001; Lou et al., 2004; Schabitz et al., 2004) oxygen therapy in the salvaging of ischemic brain. However, these trials have shown different and conflicting results, calling into question the efficacy of oxygen treatment of patients with cerebral ischemia. Indeed, some reports have claimed that hyperoxic ventilation is harmful, actually worsening the clinical outcome of stroke (Mickel et al., 1987; Bromont et al., 1989; Rusyniak et al., 2003).

One of the strongest arguments against oxygen therapy is the observation that breathing of pure oxygen leads to declining cerebral blood flow (CBF) both directly and indirectly via hyperoxia-induced hypocapnia (Floyd et al., 2003). The tissue oxygenation depends on the oxygen content of the blood as well as the blood flow to the tissue. Delivery of CO_2 along with medical oxygen (carbogen) relaxes the tone of the smooth muscles of the arteriolar resistance vessels, and thus effectively counteracts the tendency of pure oxygen to decrease perfusion. In addition to blocking hyperoxia-induced vasoconstriction, artificially elevating partial tension of carbon dioxide in arterial blood (P_{aCO2}) causes a rightward shift of the oxygen–hemoglobin dissociation curve, which favors the deposition of oxygen in brain tissue. Previous animal studies showed an inverse relationship between oxygen extraction fraction (OEF) and arterial P_{aCO2} such that cerebral metabolic rate of oxygen ($CMRO_2$) remains within normal limits (Rhodes et al., 1981). In the present study, we address the question of whether carbogen also improves the oxygenation of brain tissue. On the basis of the above arguments, we predicted that increased brain tissue oxygenation would be more readily achievable with carbogen than with oxygen alone. We used positron emission tomography (PET) to measure CBF and $CMRO_2$ changes in healthy brain tissue during inhalation of carbogen, oxygen, and CO_2. We tested this prediction in a group of healthy volunteers, in order to substantiate the rationale for conducting further

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Abbreviations: CBF, cerebral blood flow; $CMRO_2$, cerebral metabolic rate of oxygen; FWHM, full width half maximum; GM, gray matter; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; OEF, oxygen extraction fraction; Pa_{CO2}, partial tension of carbon dioxide in arterial blood; Pa_{O2}, partial tension of oxygen in arterial blood; PET, positron emission tomography; Sa_{O2}, oxygen saturation of arterial blood; VOI, volume of interest; WM, white matter.

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clinical investigation of the use of carbogen inhalation to improve the oxygenation of post-ischemic brain tissue.

EXPERIMENTAL PROCEDURES

Subjects

Ten healthy volunteers (five men and five women), aged between 40 and 70 (mean and S.D.: 53.4±9.4 years) were recruited by advertisement in a local newspaper. Exclusion criteria included history of stroke, current cardiovascular, pulmonary, or hematological disease, smoking, pregnancy, breast-feeding, and contraindications to magnetic resonance imaging (MRI) (pacemaker, metal prosthesis, claustrophobia etc.). All subjects underwent medical (including ECG) and neurological examinations before and after participation in the project. National Institutes of Health Stroke Scale (NIHSS) rating was performed and only subjects with an NIHSS=0 were included. MRI scans were evaluated by neuroradiologists to exclude the presence of overt brain abnormalities. Written informed consent was obtained from all participants in the study, as approved by the Research Ethics Committee of County Aarhus.

PET measurements

PET scans were acquired in 3D mode with the ECAT EXACT HR 47 (CTI/Siemens, Knoxville, TN, USA) whole-body tomograph, with a transverse resolution of 3.6–7.4 mm and an axial resolution of 4.0–6.7 mm. Images were reconstructed as 128×128 matrices of 2×2 mm pixels using filtered back-projection with a 0.5 cycles^{-1} ramp filter full width half maximum (FWHM), followed by a 6 mm gaussian filter, resulting in an isotropic resolution of 7 mm. The reconstructed images were corrected for random and scattered events, detector efficiency variations, and dead time. Tissue attenuation scans were performed using a rotating ^{68}Ge source.

Subjects rested in a supine position on the scanner bed, with their head comfortably immobilized near the center of the tomograph's filed of view using a customized head-holder (Vac-Lock; MED-TECH). Catheters were inserted in the left radial artery for arterial blood sampling and right cubital vein for tracer injection. The arterial blood radioactivity was measured by an automated blood sampling system and then corrected for external delay and dispersion caused by bolus distortion in the sampling catheter. Dynamic 3-min emission recordings consisting of 21 frames were initiated upon bolus i.v. injection of [^{15}O]-H_2O (500 MBq), or inhalation of [^{15}O]-O_2 (1000 MBq).

Study design

One session of PET measurements consisted of four [^{15}O]-H_2O scans and four [^{15}O]-O_2 scans. The scans for each tracer consisted of one baseline where subjects inhaled normal atmospheric air followed by three consecutive measurements, in which subjects inspired one of the test gases (100% O_2, 5% CO_2+95% O_2, 5% CO_2+95% atmospheric air) in a random order. During the entire experiment the subjects were breathing via a mask connected to a gas administration unit. PET scans were obtained at intervals of 10 min. Ninety seconds prior to each PET scan, the subjects started breathing the test gas which was delivered to the mask by the gas application unit. Recordings were obtained with eyes open and the subjects were instructed to fixate their gaze on a crosshair during the recordings.

In order to compare the potencies of oxygen and carbogen to improve the oxygen content of the blood, we measured the steady state oxygen saturation (Sa_{O2}) and oxygen tension (P_{O2}) in arterial blood samples during application of each gas.

MRI measurements

MRI scans of the head of each subject were obtained on a separate day. A high resolution T1-weighted MR (for registration purpose) was acquired for each subject with a 3.0 T Signa Excite GE Magnet using a 3D-IR-FSPGR sequence (256×256, TE1=min full, TI=750, slice thickness=1.2 mm). T2-weighted MRI (TE/TR=102/3500ms, slice thickness 5 mm) and T2-FLAIR (TE/TR/TI=120/865/2250 ms, slice thickness 5 mm) scans were performed and evaluated by a neuroradiologist to rule out cerebral pathology.

Subject monitoring

Standard biochemical parameters were measured prior to the study and arterial blood gas samples analyzed during administration of each gas. Blood pressure, pulse, and blood oxygen saturation were monitored and recorded throughout the experiment.

Data analysis

The T1-weighted MRI images of the brain of each subject were co-registered to an MR template defined by the brain of 85 young adults in Talairach space, using a combination of linear and non-linear transformations (Grabner et al., 2006). Each summed PET emission recording was linearly co-registered to the corresponding MR image using automated algorithms by first registering the initial summed PET recording to MR and then each sequential summed PET recording to the first PET. PET images were subsequently re-sampled to standard stereotaxic space.

Using the delay- and dispersion-corrected arterial inputs, parametric maps of CBF and $CMRO_2$ were calculated with the single step, two-compartment, three-weighted-integration method with corrections for internal dispersion and delay (Ohta et al., 1992, 1996). The corrections apply a global adjustment of the timing difference between the measured arterial curve and the PET scan.

Volumes of interest (VOI) analysis

VOIs containing total gray matter (GM) and white matter (WM) were defined automatically in Talairach space (Zijdenbos et al., 2002) and applied to the corresponding co-registered parametric maps, extracting averaged physiological parameters within these structures. We investigated absolute magnitude of CBF and $CMRO_2$ within non-normalized volumes. To analyze the global changes of CBF and $CMRO_2$ we used a VOI encompassing the entire GM and WM. We also calculated OEF values for the individual VOIs ($OEF=CMRO_2\times CBF^{-1}\times Ca_{O2}{}^{-1}$), where Ca_{O2} is the arterial oxygen concentration. Finally, we calculated GM/WM ratios for CBF and $CMRO_2$ in the individual subjects. Global changes in CBF and $CMRO_2$ and the corresponding GM/WM ratios were tested by means of two-sided Student's paired *t*-tests.

Voxel-based analysis

For the purpose of this analysis, reconstructed CBF and $CMRO_2$ maps were transformed into stereotaxic coordinates and blurred with a Gaussian filter (FWHM: 14×14×14 mm^3) to correct for inter-individual gyral variation and to improve signal-to-noise ratio (SNR). We converted mean subtracted-image volumes of "gas" minus baseline states to *t*-statistic volumes by the FMRISTAT package (available at www.math.mcgill.ca/keith/fmristat). In the analysis, we used the mixed effect model analysis method (Worsley et al., 2002), with spatial smoothing of the standard deviation image to increase the degrees of freedom. To determine the global effect of oxygen versus carbogen on brain CBF and metabolism we performed voxel-based cluster analysis of the data. In contrast to the voxel-based peak value analysis, this method reveals more subtle changes (with lower peak *t*-values) as signif-