**Effect of 5 weeks of oral acetazolamide on patients with pulmonary vascular disease: a randomized, double-blind, cross-over trial**

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**Assessments**

All assessments were performed during baseline examination and at the end of each 5-week treatment period. The 6- minute walk test (6MWT) and questionnaires for QoL and cognitive performance tests were additionally performed after the washout before the start of treatment phase 2.

A complete medical history and physical examination was obtained at the beginning of the study. The 6MWT was performed according to standard procedures.1 WHO functional class (WHO-FC) was assessed. QoL was measured by the Minnesota living with heart failure questionnaire (MLHF=living with PH).2 Cognitive performance was tested by the TRAIL MAKING-Test and the 5PT-Test.3-5 Arterial blood gases were drawn from a radial artery and immediately analysed (ABL 90 Flex-blood analyser, Radiometer GmbH).

Echocardiographic recordings were obtained with a real-time, phased array sector scanner (CX 50, Philips, Philips Respironics, Zofingen, Switzerland) with an integrated color Doppler system and a transducer containing crystal sets for imaging (1-5 MHz) and for continuous wave (CW) and pulsed-wave (PW) Doppler as described previously.6 Recording and analysis were performed according to guidelines of the European Society of Echocardiography.7 Maximal tricuspid regurgitation pressure gradient (TRPG) was calculated from maximal tricuspid regurgitation velocity (TRV) obtained with CW-Doppler using the modified Bernoulli equation: Δpressure= 4 × TRVmax2. Right atrial pressure (RAP) was estimated by the diameter of the inferior vena cava and its variation during inspiration. Systolic PAP (sPAP) was calculated as TRPG + RAP. Mean PAP (mPAP) was calculated as mPAP = 0.61× sPAP + 2 mmHg.28 Right ventricle (RV) area was manually traced, fractional area change (FAC) of the RV was calculated as (end-diastolic RV area ‒ end-systolic RV area) / end-diastolic RV area. Tricuspid annular plane systolic excursion (TAPSE) was measured in M-mode. Cardiac output (Q) was estimated by the Doppler velocity time integral method from the left ventricular outflow tract 8 and stroke volume (SV) as SV = Q/HR.

**References**

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**Supplementary table 1: Quality of life**

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| --- | --- | --- | --- |
|  | **Acetazolamide** | **Placebo** | **Treatment effect** |
| **Per protocol** | Start | End | Mean diff. 95%CI | p-value | Start | End | Mean diff. 95%CI | p-value | **Mean diff. 95%CI** | p-value |
| LHFQ general | 22± 3 | 27 ± 3 | 4.8 (0.5 to 9.1) | 0.029 | 21 ± 3 | 23 ± 3 | 2.2 (-2.1 to 6.5) | 0.317 | 2.6 (-3.5 to 8.7) | 0.402 |
| LHFQ physical | 13 ± 1 | 14 ± 1 | 0.9 (-0.9 to 2.6) | 0.323 | 12 ± 1 | 12 ± 1 | 0.8 (-0.9 to 2.5) | 0.370 | 0.1 (-2.4 to 2.5) | 0.950 |
| LHFQ emotional | 4 ± 1 | 7 ± 1 | 2.5 (1.0 to 4.0) | 0.001 | 4 ± 1 | 5 ± 1 | 0.5 (-1.0 to 2.1) | 0.491 | 2.0 (-0.2 to 4.1) | 0.076 |
| Data are presented as mean ± standard deviation or mean difference with 95%-Confidence interval. LHFQ: the Minnesota living with heart failure questionnaire  |

**Supplementary table 2: Side effects with acetazolamide and placebo**

|  |  |  |  |
| --- | --- | --- | --- |
| **Side effect** | **Acetazolamide, n=27** | **Placebo, n=26** | **p-value**  |
| Change of taste | 6 (22%) | 0 | <0.001 |
| Paraesthesia | 10 (37%) | 1 (4%) | <0.001 |
| Gastrointestinal distress | 4 (15%) | 1 (4%) | 0.008 |
| Nausea | 1 (4%) | 0 | 0.174 |
| Headache | 4 (15%) | 1 (4%) | 0.008 |
| Dizziness | 3 (11%) | 2 (8%) | 0.469 |
| Chest pain | 1 (4%) | 1 (4%) | 1.000 |
| Dyspnoea | 7 (26%) | 1 (4%) | <0.001 |
| Fatigue | 4 (15%) | 0 | <0.001 |
| Respiratory tract infection | 0 (0%) | 1 (4%) | 0.174 |
| Data are shown as numbers (%) for acetazolamide and placebo respectfully.  |

**Supplementary table 3: Echocardiography**

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| --- | --- | --- | --- | --- |
| **Characteristic** | **Acetazolamide** | **Placebo** | **Mean difference (95% CI)** | **p-value** |
| **Right ventricular and atrial indices and function** |  |  |  |  |
| Tricuspid regurgitation pressure gradient, mmHg | 39 ± 16 | 41 ± 18 | -0.3 (-4.5 to 4.0) | 0.900 |
| Tricuspid regurgitation velocity, m/sec | 3 ± 1 | 3 ± 1 | -0.0 (-0.2 to 0.1) | 0.770 |
| Systolic pulmonary artery pressure, mmHg | 43 ± 17 | 44 ± 18 | 0.6 (-3.9 to 5.1) | 0.800 |
| Mean pulmonary artery pressure, mmHg | 27 ± 5 | 30 ± 7 | -2.8 (-6.2 to 0.6) | 0.100 |
| Stroke volume, ml | 66 ± 20 | 66 ± 24 | -1.3 (-7.7 to 5.1) | 0.680 |
| Stroke volume index, ml/m2 | 34 ± 9 | 34 ± 10 | - 0.5 (-3.9 to 2.8) | 0.740 |
| Cardiac output, l/min | 4.9 ± 1.1 | 4.9 ± 1.7 | 0.2 (-0.9 to 0.5) | 0.600 |
| Cardiac index, l/min/m2 | 2.6 ± 0.6 | 2.4 ± 0.9 | 0.2 (-0.4 to 0.8) | 0.590 |
| Right arterial pressure, mmHg | 3.4 ± 1.1 | 3.0 ± 0 | 0.4 (-0.1 to 0.9) | 0.120 |
| Right atrial area, cm2 | 17 ± 7 | 18 ± 7 | 0.2 (-1.9 to 2.3) | 0.850 |
| Right ventricle end-diastolic area A4C, cm2 | 26 ± 8 | 24 ± 7 | 1.9 (-0.6 to 4.4) | 0.120 |
| Right ventricle end-systolic area A4C, cm2 | 17 ± 6 | 16 ± 5 | -0.3 (-1.5 to 2.0) | 0.760 |
| Right ventricle fractional area change, % | 36 ± 9 | 34 ± 10 | 2.0 (-3.2 to 0.7) | 0.430 |
| Eccentricity Index end-diastolic | 1.2 ± 0.3 | 1.2 ± 0.2 | 0.1 (-0.1 to 0.2) | 0.500 |
| Eccentricity Index end-systolic | 1.4 ± 0.5 | 1.3 ± 0.3 | 0.2 (-0.0 to 0.4) | 0.090 |
| Right ventricle anterior wall diameter, cm | 0.6 ± 0.2 | 0.5 ±0.1 | 0.0 (-0.1 to 0.2) | 0.920 |
| Right ventricle diameter end-diastolic, cm | 3.5 ± 0.7 | 3.3 ± 0.9 | 0.4 (-0.0 to 0.8) | 0.046 |
| Tricuspid annular plane systolic excursion, cm | 1.7 ± 0.4 | 2.3 ± 2.6 | -0.5 (-1.7 to 0.6) | 0.360 |
| RV systolic excursion velocity s′, cm/s | 12.1 ± 3.6 | 12.1 ± 3.1 | -0.4 (-1.7 to 1.0) | 0.590 |
| **Left ventricle and left atrium indices** |  |  |  |  |
| Left ventricle end-diastolic area A4C, cm | 4.6 ± 0.7 | 4.4 ± 0.6 | 0.1 (-0.3 to 0.4) | 0.750 |
| Septal wall thickness, cm  | 0.9 ± 0.2 | 0.9 ± 0.2 | -0.0 (-0.1 to 0.1) | 0.750 |
| LV end-diastolic dimension, cm | 4.8 ± 0.6 | 4.8 ± 0.6 | 0.0 (-0.2 to 0.2) | 0.640 |
| LV end-diastolic posterior wall, cm | 0.8 ± 0.2 | 0.8 ± 0.2 | -0.0 (-0.2 to 0.2) | 0.930 |
| LV end-systolic dimension, cm | 3.2 ± 0.7 | 3.0± 0.5 | 0.2 (-0.1 to 0.5) | 0.200 |
| Left ventricular outflow tract diameter, cm  | 2.0 ± 0.2 | 2.0 ± 0.3 | 0.0 (-0.1 to 0.1) | 0.740 |
| Left atrium area A2C, cm2 | 17 ± 6.6 | 16 ± 4.8 | 0.1 (-2.7 to 2.9) | 0.930 |
| Left atrium area A4C, cm2 | 17 ± 6.6 | 16 ± 7.0 | 1.1 (-0.9 to 3.1) | 0.280 |
| Left atrium length, cm | 4.8 ± 0.9 | 4.8 ± 0.9 | -0.1 (-0.5 to 0.3) | 0.600 |
| Left atrial volume index, cm2/m2 | 24 ± 13 | 23 ± 9 | 1.5 (-4.8 to 7.8) | 0.620 |
| Ejection Fraction biplane, % | 64 ± 8 | 62 ± 6 | 1.8 (-2.0 to 5.7) | 0.340 |
| Left ventricular outflow tract velocity time integral  | 20 ± 3 | 20 ± 4 | 0.7 (-0.5 to 2.0) | 0.240 |
| Mitral A wave, m/s | 62 ± 20 | 66 ± 21 | -3.2 (-12.0 to 6.1) | 0.480 |
| Mitral E wave, m/s | 59 ± 16 | 63 ± 20 | -3.9 (-11.0 to 3.4) | 0.280 |
| Mitral E/A ratio | 1.1 ± 0.5 | 1.1 ± 0.3 | 0.0 (-0.1 to 0.2) | 0.680 |
| Lateral mitral annulus e′ wave, cm/s | 13 ± 4 | 13 ± 4 | 0.2 (-1.0 to 1.4) | 0.760 |
| Lateral mitral annulus a′ wave, cm/s | 11 ± 4 | 11 ±3 | -0.2 (-1.5 to 1.1) | 0.760 |
| Septal mitral annulus e′ wave, cm/s | 7.7 ± 2.1 | 8.3 ± 1.9 | - 0.5 (-1.4 to 0.5) | 0.290 |
| Septal mitral annulus a′ wave, cm/s | 9.1 ± 2.0 | 8.7 ± 2.5 | 0.1 (-0.9 to 1.1) | 0.900 |
| Lateral E/e′ ratio | 5.3 ± 2.6 | 5.9 ± 2.7 | - 0.7 (-1.6 to 0.2) | 0.100 |
| Septal E/e′ ratio | 8.0 ± 2.2 | 7.8 ± 2.4 | 0.1 (-0.8 to 0.9) | 0.880 |
| Lateral e′/a′ ratio | 1.4 ± 0.6 | 1.5 ± 0.9 | - 0.1 (-0.5 to 0.3) | 0.610 |
| Septal e′/a′ ratio | 0.9 ± 0.3 | 1.1 ± 0.5 | - 0.1 (-0.3 to 0.1) | 0.330 |
| Data are shown as mean ± standard deviation or as mean difference (95% confidence interval). A4C: apical four chamber view, A2C: apical two chamber view, RV: right ventricle, LV: left ventricle. |

**Supplementary table 4: Cognitive function tests**

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|  | **Acetazolamide** | **Placebo** | **Treatment effect** |
| **Per protocol** | Start | End | Mean diff. 95%CI | p-value | Start | End | Mean diff. 95%CI | p-value | **Mean diff. 95%CI** |  |
| Fig 5Pt, total | 36.7 (3.0) | 37.3 (3.0) | 0.6 (-4.5 to 5.8) | 0.806 | 39.2 (3.0) | 42.0 (3.0) | 2.8 (-2.2 to 7.8) | 0.279 | -2.1 (-9.3 to 5.1) | 0.562 |
| Fig 5Pt, invalid | 2.4 (0.6) | 2.1 (0.6) | -0.3 (-1.6 to 1.0) | 0.653 | 3.7 (0.6) | 3.5 (0.6) | -0.3 (-1.6 to 1.0) | 0.691 | -0.1 (-1.9 to 1.8) | 0.951 |
| TMT A, time | 35.1 (3.4) | 36.8 (3.4) | 1.7 (-3.4 o 6.7) | 0.518 | 35.3 (3.4) | 38.1 (3.4) | 2.8 (-2.2 to 7.7) | 0.273 | -1.1 (8.2 to 5.9) | 0.759 |
| TMT A, errors | 0.1 (0.1) | 0.1 (0.1) | 0.0 (-0.1 to 0.1) | 0.983 | 0.1 (0.1) | 0.1 (0.1) | -0.0 (-0.2 to 0.1) | 0.517 | 0.0 (-0.1 to 0.2) | 0.638 |
| TMT B, time | 100.5 (13.9) | 101.2 (13.9) | 0.8 (-13.2 to 14.7) | 0.916 | 95.9 (13.9) | 87.7 (13.9) | -8.2 (-21.9 to 5.4) | 0.238 | 9.0 (10.6 to -28.6) | 0.367 |
| TMT B, errors | 0.9 (0.2) | 0.8 (0.2) | -0.0 (-0.6 to 0.5) | 0.880 | 0.1 (0.2) | 0.3 (0.2) | 0.1 (-0.4 to 0.7) | 0.580 | -0.2 (-0.9 to 0.6) | 0.620 |
| Data are presented as mean (Standard deviation) or mean difference with 95% Confidence interval. Significant p-values are shown in **bold** (p<0.05).Fig 5pt: Figure of 5, TMT A and B: Trail making test A and B |