Adenosine Challenge Procedure

UF Asthma Research Lab

A. Equipment

1. Spirometry and challenge are performed with a KoKo Digidoser (Ferraris Respiratory, Louisville, CO). The adenosine challenge is a modification of the ATS five breath dosimeter method[1] and the procedure of Taylor et al.[2]
2. Aerosol is generated with a ”characterized” DeVilbiss 646 Nebulizer (#492249, Ferraris Respiratory). Nebulizers are numbered and the same nebulizer is used for all challenges for a given subject. The nebulizer is designed to deliver particles with an aerodynamic mass median diameter of 1.8 µm at a constant air pressure of 30 PSI and a constant inspiratory flow (approximately 0.5 L/S). The Digidoser is connected to a compressed air cylinder through an adjustable pressure valve. The output (in ml/min), inscribed on the nebulizer, is entered into the challenge software program. The software is set to deliver the dose over 0.6 sec. With this system, the output of the nebulizer cannot be determined accurately nor calibrated according to ATS Guidelines.[3]
3. Spirometer calibration is performed with a standard 3 liter syringe. The KoKo filter inlet is attached to the outlet on the syringe and the syringe handle is pulled all the way out. The procedure begins by allowing the Pneumotach to register baseline airflow when lying on the table without movement (nulling the device). During the calibration process, at least 3 syringe push/pull efforts are performed. When prompted by the KoKo software, the syringe handle is pushed all the way

in at a low air flow (approximately 2L) and the syringe handle is pulled out. The above step is repeated for medium (4 L/sec) and high flow (8 L/sec). The software notifies the operator if the calibration is acceptable or asks for the procedure to be repeated. The calibration report is printed and saved.

B. Solutions

1. Lyophilized adenosine 5' monophosphate disodium salt is acquired from Merck Biosciences AG (Weidenmattweg4 CH-4448 Läufelfingen, Switzerland). It is provided in sterile vials containing 3.8 gm of active AMP·2 Na. The adenosine product is obtained under FDA Investigator-Sponsored IND #70,241.
2. AMP solutions for bronchoprovocation are prepared by the Investigational Drug Unit of Shands Hospital Pharmacy Department under sterile conditions, by injecting 8 ml of sterile saline into a 3.8 gm AMP vial producing a stock solution of 380 mg/ml. Further dilutions are then made to produce the following concentrations: 200, 100, 50, 25, 12.8, 6.4, 3.2, 1.6, 0.8, and 0.4 mg/ml.
3. Each concentration is contained in a final volume of 2 ml, dispensed in unit dose syringes and stored at 4°C in the Asthma Research Lab, until use or 5 months, whichever occurs first.[4]

C. Baseline Spirometry

1. Prior to the AMP challenge, baseline spirometry is performed with the subject wearing a nose clip. The subject may be seated or standing as long as position is kept constant for each challenge in the same subject. Spirometric efforts will be repeated (up to five times) until at least 3 ATS acceptable efforts[5] have been obtained with the goal of the two highest values not differing by more than 0.1 L.

- ♦ ATS criteria judged by the KoKo software:
  - back extrapolation <5% of FVC or <0.15 liter (whichever is greater)
  - no early termination of exhalation (should have at least 1 second of plateau, or at least 6 seconds of exhalation time)
- ♦ ATS criteria that need to be judged by the technician:
  - no cough during the first second of exhalation
  - no hesitation during the effort which results in a cessation of airflow
  - no glottic closure or variable effort
  - no leak
  - no mouthpiece obstruction (mechanical, tongue, or teeth)

2. If there is a progressive fall from the first maneuver but the last two (of the 5) stabilize, the higher of the last two is selected as the stabilized baseline FEV_1. If the last two differ by more than 0.1L, additional efforts, up to a total of eight, are performed until two consecutive FEV_1 values are within 0.1L. The higher of the two is then selected as the stabilized baseline. If the goal of two values within 0.1L is not achieved, the higher of the last two efforts is accepted as the stabilized baseline.
3. If the stabilized baseline FEV_1 is ≥60% of predicted, the challenge is commenced. If not, the subject is rescheduled once. If this criterion is not met during the second visit, the subject is discontinued from the study.

D. Challenge

1. Two ml of diluent is added to the nebulizer bowl through a 0.22 micron Millipore filter and the top is screwed on tightly with the ball facing the mouthpiece.
2. The subject holds the nebulizer by the Digidoser handle but not by the bowl and wears a nose clip. The mouthpiece is placed in the subject's mouth, and the subject performs any number of normal tidal breaths. For the dosing breaths, the technician triggers the dosimeter during exhalation into the mouthpiece by pressing the spacebar. The Digidoser automatically discharges during the next inhalation. The subject is coached to inhale slowly and deeply to total lung capacity (TLC). At TLC, the subject holds his/her breath for 5 seconds and then exhales slowly into the mouthpiece for 5 seconds. This will be repeated 4 more times for a total of 5 breaths. These breaths do not need to be consecutive (i.e. 1 or 2 non-nebulized breaths may be taken in between).
3. The FEV_1 is measured at 90 and 150 sec after the fifth exhalation. At least one acceptable FEV1 must be obtained. If an acceptable FEV_1 is not obtained one or two additional attempts are made (a max of 3-4 attempts). The highest acceptable FEV_1 is accepted as the post-diluent FEV_1. It should not take more than 2 minutes to perform these maneuvers. If after a total of 4 attempts an acceptable value is not obtained, the challenge is discontinued.
4. AMP challenges are performed only if the post-diluent FEV_1 falls <10%. If it falls by ≥10% below the stabilized baseline value, the test is rescheduled. If this happens again during the rescheduled visit, the subject is not challenged with adenosine and discontinued from the study.

5. Five minutes after the beginning of the first inhalation, the first AMP concentration (0.4 mg/ml) is administered as described above.
6. 90 and 150 sec after the end of the fifth exhalation, spirometry is performed. One acceptable FEV_1 must be obtained. If an acceptable FEV_1 is not obtained, one or two additional attempts are made (a max of 3-4 attempts).
7. If the FEV_1 has not fallen 20% compared to the diluent FEV_1, and the technician feels that it is safe to proceed to the next dose level, the next concentration of AMP is administered 5 minutes after the beginning of the first inhalation of the previous dose. The higher FEV_1 of the two will be recorded for analysis.
8. Doubling concentrations of AMP are administered at 5 minute intervals until a 20% fall in FEV_1 occurs compared to the diluent FEV_1 or the maximum concentration is administered. During screening the maximum is usually 100 mg/ml, while during studies with drugs expected to increase PC_{20} the maximum is 200 mg/ml.

E. Safety Precautions

1. The challenge is not performed if a screening 12 lead ECG demonstrates a PR interval >200 msec or QRS interval >120 msec.
2. Once the AMP challenge is completed, 4 puffs of albuterol from a MDI attached to a valved-holding chamber are administered. This dose is repeated at 15 minute intervals, if necessary. The subject is discharged when his/her FEV_1 has returned to ≥90% of that day's initial baseline value, and the subject is judged to be clinically stable.

3. During the adenosine challenge, all standard precautions for patient safety are followed. A physician is in the building and immediately available by beeper. Medication to treat severe bronchospasm and oxygen is immediately available. Patients are not left unattended during the procedure once the administration of adenosine begins.

F. Data Analysis

1. The following formula is used to calculate the percent decrease:

$$\% \text{ decrease in } FEV_1 = \frac{(\text{best diluent } FEV_1) - (\text{best } FEV_1 \text{ after adenosine})}{(\text{best diluent } FEV_1)} \times 100\%$$

2. The KoKo Software uses the following equation to calculate the PC_{20}:

$$PC_{20} \text{ (mg/ml)} = \text{antilog}\left[\log C1 + \frac{(\log C2 - \log C1)(20 - R1)}{(R2 - R1)}\right]$$

C1 = next to the last concentration (<20% FEV_1 fall)

C2 = last concentration (>20% FEV_1 fall)

R1 = % fall FEV_1 after C1, and R2 = % fall FEV_1 after C2

G. Sanitizing Procedure

1. After each challenge the nebulizer is disassembled, washed in dish soap water, and rinsed.
2. The nebulizer is then placed in isopropyl alcohol for 10 minutes, rinsed and allowed to air dry. Chlorox and Cidex must not be used as these solutions can affect the welded straw and baffle of the nebulizer and thus, alter performance.
3. The nebulizer bowl and body are inscribed with an ID #. Care is taken to make sure that the matching parts are assembled with the ball facing the mouthpiece.

References

1. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG et al. Guidelines for methacholine and exercise challenge testing –1999. Am J Respir Crit Care Med 2000;161:309-29.

2. Taylor DA, Jensen MW, Kanabar V, Engelstätter R, Steinijans VW, Barnes PJ, et al. A dose-dependent effect of the novel inhaled corticosteroid ciclesonide on airway responsiveness to adenosine-5'-monophosphate in asthmatic patients. Am J Respir Crit Care Med 1999;160;237-43.

3. Kahn YR, McDonough P, Cockcroft DW, David BE, Hendeles L. Nebulizer output for methacholine challenges with the KoKo Digidoser (letter). J Allergy Clin Immunol 2005;116:924-6.

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5. American Thoracic Society. Standardization of spirometry:1994 update. Am J Respir Crit Care Med 1995;152:1107-36.