HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use PROVOCHOLINE® safely and effectively. See full prescribing information for PROVOCHOLINE®.

PROVOCHOLINE® (methacholine chloride) for inhalation solution, for oral inhalation use
PROVOCHOLINE® (methacholine chloride) inhalation solution, for oral inhalation use
Initial U.S. Approval: 1986

WARNING: SEVERE BRONCHOCONSTRICTION
See full prescribing information for complete boxed warning.

- **Severe bronchoconstriction can result from Provocholine administration (including the lowest dose) (5.1)**
- **Use of Provocholine is contraindicated in pediatric and adult patients with baseline FEV_1 < 60% predicted or adults with FEV_1 < 1.5 L (5.1)**
- **Use of Provocholine is not recommended in patients with clinically apparent asthma or wheezing (5.1)**
- **If severe bronchoconstriction occurs, reverse immediately with a rapid-acting inhaled bronchodilator agent (β-Agonist) (5.1)**

----------RECENT MAJOR CHANGES----------
Dosage and Administration (2.1, 2.2, 2.3, 2.4, 2.5) 06/2026

----------INDICATIONS AND USAGE----------
Provocholine, a cholinergic agonist used in a methacholine challenge test, is indicated for the diagnosis of bronchial airway hyperreactivity in adults and pediatric patients five years of age and older who do not have clinically apparent asthma (1)

----------DOSAGE AND ADMINISTRATION----------
- The methacholine challenge test should be conducted in a pulmonary function laboratory or clinic, by adequately trained personnel, for safety and accuracy (2.1)
- Determine baseline FEV_1 values to assess whether a patient is able to undergo the methacholine challenge test (2.1)
- See the Full Prescribing Information for the required reconstitution and dilution procedures prior to use (2.2).
- Recommended dosage(s) of Provocholine in the Methacholine Challenge Test is administration of increasing strengths of Provocholine solution via nebulization (2.3)
- Administer using either the 5-Breath Dosimeter Dosing Method or the 1-Minute Tidal Breathing Dosing Method with the doubling or quadrupling stepwise protocols (2.4, 2.5)
- See the full Prescribing Information for administration of inhaled β-agonist after Methacholine Challenge Test (2.6)
- See the Full Prescribing Information for the interpretation of the results and calculation of hyperresponsiveness (2.7)

----------DOSAGE FORMS AND STRENGTHS----------
- For inhalation solution: 100 mg of methacholine chloride powder in amber glass vial (3)
- Inhalation solutions:
  - Ready-to-use-kit: base solution (contains no methacholine chloride), 0.0625 mg/mL (0.1875 mg/3 mL), 0.25 mg/mL (0.75 mg/3 mL), 1 mg/mL (3 mg/ 3mL), 4 mg/mL (12 mg/3 mL), and 16 mg/mL (48 mg/3 mL) of methacholine chloride (3)
  - Single-dose vial: 16 mg/mL (48 mg/3 mL) of methacholine chloride (3)

----------CONTRAINDICATIONS----------
- Hypersensitivity to methacholine chloride or other parasympathomimetic agents (4)
- Baseline FEV_1 < 60% predicted (adults or pediatric patients) or < 1.5 L (adults) (4)

----------WARNINGS AND PRECAUTIONS----------
- *Risk of Severe Bronchoconstriction:* Severe bronchoconstriction can result from Provocholine administration. Do not use Provocholine in pediatric and adult patients with baseline FEV1 <60% predicted or adults with FEV1 <1.5 L. Not recommended in patients with clinically apparent asthma or wheezing. If severe bronchoconstriction occurs, reverse with administration of rapid-acting inhaled β_2-agonist. (5.1)
- *Bronchoconstriction from Inadvertent Exposure*: Healthcare provider and any other personnel involved in the administration of the methacholine challenge test should take precautions to avoid inadvertent inhalation of Provocholine powder and nebulized aerosol (5.2)
- *Risk in Patients with Coexisting Diseases and Conditions*: Provocholine not recommended for patients with uncontrolled hypertension, aortic aneurysm, or history of myocardial infarction or stroke diseases. Consider the benefits and potential risk for use of Provocholine in patients with conditions that could be adversely affected by a cholinergic agent. (5.3)

----------ADVERSE REACTIONS----------
Adverse reactions are bronchoconstriction, headache, throat irritation, light-headedness, and itching (6)

To report SUSPECTED ADVERSE REACTIONS, contact Methapharm at 1-866-701-4636 or call FDA at 1-800-FDA-1088 or visit www.fda.gov/medwatch

----------DRUG INTERACTIONS----------
- *Beta-Adrenergic Blockers:* May impair reversal of Provocholine-caused bronchoconstriction (7)
- *Beta-Agonists, Anticholinergics, and Theophylline:* Inhibit response to Provocholine; therefore, hold these drugs prior to Provocholine use (7)
- *Oral or Inhaled Corticosteroids, and Inhaled Cromoglycate:* May decrease response to Provocholine (7)

----------USE IN SPECIFIC POPULATIONS----------
Pregnancy: Provocholine is not recommended (8.1)

See 17 for PATIENT COUNSELING INFORMATION

Revised: 06/2026

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed

FULL PRESCRIBING INFORMATION

WARNING: SEVERE BRONCHOCONSTRICTION

Severe bronchoconstriction can result from Provocholine administration (including the lowest dose). The use of Provocholine is contraindicated in pediatric and adult patients with baseline FEV_1 < 60% predicted or adults with FEV_1 < 1.5 L. Because of the potential for severe bronchoconstriction, the use of Provocholine in patients with clinically apparent asthma or wheezing is not recommended *[see Warnings and Precautions (5.1)]*.

Emergency equipment and medication should be immediately available to treat acute respiratory distress. If severe bronchoconstriction occurs, reverse immediately with a rapid-acting inhaled bronchodilator agent (β-agonist) *[see Warnings and Precautions (5.1)].*

If baseline spirometry is not performed or is measured inaccurately, the initial FEV1 may be underestimated. In this situation, decreases in FEV1 may not be detected after administration of escalating Provocholine doses, which may result in administration of unnecessary higher doses and an increased risk for excessive bronchoconstriction *[see Warnings and Precautions (5.1)].*

1 INDICATIONS AND USAGE

Provocholine, used in a methacholine challenge test, is indicated for the diagnosis of bronchial airway hyperreactivity in adults and pediatric patients five years of age and older who do not have clinically apparent asthma.

2 DOSAGE AND ADMINISTRATION

2.1 Preparation and Administration Overview

- Provocholine powder for inhalation solution requires reconstitution and dilution prior to use *[see Dosage and Administration (2.2)].*
- Provocholine inhalation solution in a ready-to-use kit does not require reconstitution and/or dilution prior to use.
- Provocholine inhalation solution 16 mg/mL in a single-dose vial requires dilution prior to use *[see Dosage and Administration (2.2)].*

- For safety and accuracy, administer Provocholine during a methacholine challenge test in a pulmonary function laboratory or clinic, by an adequately trained personnel. Only healthcare providers trained in and thoroughly familiar with all aspects of the test's technique and management of respiratory distress should perform the test. Emergency medication and equipment should be immediately available to treat acute respiratory distress.

- Consider Provocholine use in patients on chronic asthma drugs only if the accuracy of the asthma diagnosis is in doubt. In these patients, administer Provocholine only if spirometry is normal after supervised withdrawal of the asthma drugs.

- Provocholine is not recommended for use in patients with clinically apparent asthma or wheezing.

- Before starting a methacholine challenge test, baseline spirometry must be performed. For a patient to be able to undergo the test, he or she must present with baseline FEV_1 (Forced Expiratory Volume in 1 second) greater than or equal to 60% of the predicted value (in adults and pediatric patients) and greater than or equal to 1.5 L (in adults).

- Provocholine is contraindicated in adult and pediatric patients with baseline FEV_1 < 60% predicted or in adults with FEV_1 < 1.5 L *[see Contraindications (4) and Warnings and Precautions (5.1)].*

- At commencement of the methacholine challenge test and prior to administration of Provocholine, a post-diluent FEV_1 must be measured following nebulization of the diluent or base solution (contains no methacholine chloride) *[see Dosage and Administration (2.4, 2.5)].*

- Administer Provocholine by oral inhalation, with or without food, using either the 5-Breath Dosimeter Dosing Method or the 1-Minute Tidal Breathing Dosing Method with the dose doubling or quadrupling protocols *[see Dosage and Administration (2.3, 2.4, 2.5)].*

- Discard any unused solution from the nebulizer after each administration.

- The methacholine challenge test is considered positive if there is a reduction in FEV_1 of 20% or more from the post-diluent FEV_1. The test should be stopped at this point. Calculate and record the FEV_1 reduction value before starting the test *[see Dosage and Administration (2.7)].*

- After the methacholine challenge test with Provocholine, administer an inhaled β-agonist to expedite the return of the FEV_1 to baseline and to relieve any discomfort the patient may experience. Most patients revert to normal pulmonary function within 10 to 20 minutes following administration of an inhaled β-agonist *[see Dosage and Administration (2.6)].*

2.2 Reconstitution and/or Dilution Instructions for Provocholine Powder for Inhalation Solution and Provocholine Inhalation Solution 16 mg/mL (in Single-Dose Vial)

Reconstitution and Dilution of Provocholine Powder for Inhalation Solution

1. Reconstitute Provocholine Powder for Inhalation Solution by adding 6.25 mL of 0.9% Sodium Chloride Injection to the supplied vial(s) containing 100 mg of Provocholine powder**.** Shake the vial to obtain a clear solution.

2. Dilute the reconstituted Provocholine solution, using sterile, empty USP Type 1 borosilicate glass vials. Dilute the reconstituted Provocholine solution with 0.9% Sodium Chloride Injection based on Table 1 to obtain doubling strengths or Table 2 to obtain quadrupling strengths. After adding the 0.9% Sodium Chloride Injection, shake each vial to obtain a clear solution.

3. If not used immediately, refrigerate the reconstituted and diluted solutions at 36˚F to 46˚F (2˚C to 8˚C) for up to 2 weeks. Since the temperature of the solution affects nebulizer output, take the diluted solutions out of the refrigerator and allow them to equilibrate to room temperature (approximately 30 minutes) before use.

Table 1: Reconstitution and Dilution Instructions for Provocholine Powder for Inhalation Solution to Obtain Doubling Strengths

TAKE	ADD 0.9% Sodium Chloride Injection	Provocholine Solution Strength after reconstitution or dilution
100 mg of Provocholine Powder in one single-dose vial	6.25 mL	16 mg/mL
3 mL of 16 mg/mL Provocholine Solution	3 mL	8 mg/mL
3 mL of 8 mg/mL Provocholine Solution	3 mL	4 mg/mL
3 mL of 4 mg/mL Provocholine Solution	3 mL	2 mg/mL
3 mL of 2 mg/mL Provocholine Solution	3 mL	1 mg/mL
3 mL of 1 mg/mL Provocholine Solution	3 mL	0.5 mg/mL
3 mL of 0.5 mg/mL Provocholine Solution	3 mL	0.25 mg/mL
3 mL of 0.25 mg/mL Provocholine Solution	3 mL	0.125 mg/mL
3 mL of 0.125 mg/mL Provocholine Solution	3 mL	0.0625 mg/mL

Table 2: Reconstitution and Dilution Instructions for Provocholine Powder for Inhalation Solution to Obtain Quadrupling Strengths

TAKE	ADD 0.9% Sodium Chloride Injection	Provocholine Solution Strength after reconstitution or dilution
100 mg of Provocholine Powder in one single-dose vial	6.25 mL	16 mg/mL
3 mL of 16 mg/mL Provocholine Solution	9 mL	4 mg/mL
3 mL of 4 mg/mL Provocholine Solution	9 mL	1 mg/mL
3 mL of 1 mg/mL Provocholine Solution	9 mL	0.25 mg/mL
3 mL of 0.25 mg/mL Provocholine Solution	9 mL	0.0625 mg/mL

Dilution of Provocholine Inhalation Solution 16 mg/mL (in a Single-Dose Vial)

1. Transfer the contents of the specified number of 3 mL vials (as per Tables 3 or 4) of Provocholine inhalation solution 16 mg/mL (in Single-Dose Vial) into a sterile, empty USP Type 1 borosilicate glass vial. Swirl the glass vial gently to mix.

2. Dilute the solution with 0.9% Sodium Chloride Injection based on Table 3 (to obtain doubling strengths) or Table 4 (to obtain quadrupling strengths). After adding 0.9% Sodium Chloride Injection, shake each vial to obtain a clear solution.

3. If not used immediately, refrigerate the diluted solutions at 36°F to 46°F (2°C to 8°C) for up to 2 weeks. Since the temperature of the solution affects nebulizer output, take the diluted solutions out of the refrigerator and allow them to equilibrate to room temperature (approximately 30 minutes) before use.

Table 3: Dilution Instructions for Provocholine Inhalation Solution 16 mg/mL (in Single-Dose Vial) to Obtain Doubling Strengths

TAKE	ADD 0.9% Sodium Chloride Injection	Provocholine Solution Strength after dilution
Provocholine Inhalation Solution 16 mg/mL (2 x 3 mL vial)	None	16 mg/mL
3 mL of 16 mg/mL Provocholine Solution	3 mL	8 mg/mL
3 mL of 8 mg/mL Provocholine Solution	3 mL	4 mg/mL
3 mL of 4 mg/mL Provocholine Solution	3 mL	2 mg/mL
3 mL of 2 mg/mL Provocholine Solution	3 mL	1 mg/mL
3 mL of 1 mg/mL Provocholine Solution	3 mL	0.5 mg/mL
3 mL of 0.5 mg/mL Provocholine Solution	3 mL	0.25 mg/mL
3 mL of 0.25 mg/mL Provocholine Solution	3 mL	0.125 mg/mL
3 mL of 0.125 mg/mL Provocholine Solution	3 mL	0.0625 mg/mL

Table 4: Dilution Instructions for Provocholine Inhalation Solution 16 mg/mL (in Single-Dose Vial) to Obtain Quadrupling Strengths

TAKE	ADD 0.9% Sodium Chloride Injection	Provocholine Solution Strength after dilution
Provocholine Inhalation Solution 16 mg/mL (1 x 3 mL vial)	None	16 mg/mL
1 mL of 16 mg/mL Provocholine Solution	3 mL	4 mg/mL
1 mL of 4 mg/mL Provocholine Solution	3 mL	1 mg/mL
1 mL of 1 mg/mL Provocholine Solution	3 mL	0.25 mg/mL
1 mL of 0.25 mg/mL Provocholine Solution	3 mL	0.0625 mg/mL

2.3 Recommended Provocholine Doses for Methacholine Challenge Test

The recommended doses of Provocholine for oral inhalation for the diagnosis of bronchial airway hyperreactivity in adults and pediatric patients five years of age and older during a methacholine challenge test, based on the dose doubling or quadrupling protocol, are provided in Table 5.

Table 5: Recommended Provocholine Doses for Methacholine Challenge Test Based on Dose Doubling or Quadrupling Dilution

Doubling Protocol		
Provocholine Strength	**Recommended Volume**	**Provocholine Dose***
0.0625 mg/mL	At least 2 mL	1.484 mcg
0.125 mg/mL		2.969 mcg
0.25 mg/mL		5.938 mcg
0.5 mg/mL		11.875 mcg
1 mg/mL		23.75 mcg
2 mg/mL		47.5 mcg
4 mg/mL		95 mcg
8 mg/mL		190 mcg
16 mg/mL		380 mcg
Quadrupling Protocol or Ready-to-Use Kit		
Provocholine Strength	**Recommended Volume**	**Provocholine Dose***
0.0625 mg/mL	At least 2 mL	1.484 mcg
0.25 mg/mL		5.938 mcg
1 mg/mL		23.75 mcg
4 mg/mL		95 mcg
16 mg/mL		380 mcg

***** Provocholine dose was based on the Hudson RCI® MicroMist® Small Volume Nebulizer using dry compressed air to power the nebulizer with a pressure regulator set to 50 lb/in^2 (psi) and flow controller set to flow rate of 4.5 LPM (liters per minute) with a nebulization time of one (1) minute. Under these conditions, the device output was within 10% of 0.13 mL/min (or g/min) (measured gravimetrically), the measured MMD was found to be 3.4 µm, i.e. within the acceptable range of MMD of 1.0 – 3.6 µm. The delivered dose of the respirable fraction for the Provocholine 16 mg/mL inhalation solution was about 380 mcg.

2.4 Determination of Post-Diluent FEV_1 and Administration of Provocholine Using the 5-Breath Dosimeter Dosing Method

Determination of Post-Diluent FEV_1 Prior to Administration of Provocholine

Prior to administration of Provocholine, determine the post-diluent FEV_1 value required for the methacholine challenge test.

1. Instill diluent (sterile 0.9% Sodium Chloride Injection) or base solution into the nebulizer.

   - If using Provocholine powder for inhalation solution or Provocholine inhalation solution 16 mg/mL (in single-dose vial), using a 3 mL syringe and needle, draw up 2 to 3 mL of

diluent (sterile 0.9% Sodium Chloride Injection), and instill the 0.9% Sodium Chloride Injection into the nebulizer using a sterile bacterial-retentive filter (porosity 0.22 µm).

- If using the Provocholine inhalation solution ready-to-use kit, instill the contents of the base solution (contains no methacholine chloride) into the nebulizer.

2. Instruct the patient to hold the nebulizer upright with the mouthpiece in his/her mouth. The patient should wear a nose clip while inhaling from the nebulizer.

3. At the end of exhalation during tidal breathing (functional residual capacity), instruct the patient to inhale slowly and deeply through the mouthpiece. Trigger the dosimeter soon after oral inhalation begins. Encourage the patient to continue inhaling slowly (about 5 seconds to complete the inhalation) and to hold the breath at total lung capacity (TLC) for another 5 seconds.

4. Repeat Step 3 for a total of five inspiratory capacity inhalations. Take no more than 2 minutes to perform these 5 inhalations.

5. Perform spirometry and measure the FEV_1 30 and 90 seconds after the fifth inhalation from the nebulizer to obtain the post-diluent FEV_1 value. These values may be left at ambient (spirometer) temperature pressure saturated (ATPS).

   - If the FEV_1 value is not of acceptable quality, repeat the procedure.

   - If the post-diluent FEV_1 falls by ≥ 20% from baseline FEV_1, do not proceed with administration with Provocholine and administer an inhaled β-agonist *[see Dosage and Administration (2.6)].*

   - If the post-diluent FEV_1 falls by< 20% from baseline FEV_1, proceed to Step 6.

<u>Administration of Provocholine Using the 5-Breath Dosimeter Dosing Method</u>

6. Instill Provocholine solution into the nebulizer.

   - If using Provocholine powder for inhalation solution or Provocholine inhalation solution 16 mg/mL (in single-dose vial), using a 3 mL syringe and needle, draw up at least 2 mL of the recommended Provocholine strength based on the dose doubling or quadrupling protocol in Table 5 *[see Dosage and Administration (2.3)]* and instill into the nebulizer using a sterile bacterial-retentive filter (porosity 0.22 µm). Refer to Tables 1, 2, 3 or 4 for preparation of the doubling or quadrupling strengths of Provocholine *[see Dosage and Administration (2.2)]*.

   - If using the Provocholine inhalation solution ready-to-use kit, instill at least 2 mL of the recommended Provocholine strength based on the dose quadrupling protocol in Table 5 *[see Dosage and Administration (2.3)]* into the nebulizer.

7. Repeat Steps 2 through 5 for each Provocholine strength, emptying the nebulizer between each strength. To keep the cumulative effect of Provocholine relatively constant, the time interval between the end of one strength and beginning of the subsequent strength should not be more than 5 minutes.

8. Stop dosing if the FEV_1 has fallen by ≥ 20% from the post-diluent FEV_1, or the highest Provocholine strength (16 mg/mL) has been administered (whichever comes first). Do not administer additional Provocholine doses if severe bronchoconstriction occurs *[see Warnings and Precautions (5.1)]*. After the test is completed, administer an inhaled β-agonist to the patient *[see Dosage and Administration (2.6)].*

9. Wash and clean reusable nebulizers thoroughly according to manufacturer's recommendations.

2.5 Determination of Post-Diluent FEV_1 and Administration of Provocholine Using the 1-Minute Tidal Breathing Dosing Method

Determination of Post-Diluent FEV_1 Prior to Administration of Provocholine

Prior to administration of Provocholine, determine the post-diluent FEV_1 required for the methacholine challenge test.

1. Instill diluent (sterile 0.9% Sodium Chloride Injection) or base solution into the nebulizer.

   - If using Provocholine powder for inhalation solution or Provocholine inhalation solution 16 mg/mL (in single-dose vial), using a 3 mL syringe and needle, draw up 2 to 3 mL of diluent (sterile 0.9% Sodium Chloride Injection). Instill the 0.9% Sodium Chloride Injection into the nebulizer using a sterile bacterial-retentive filter (porosity 0.22 µm). Attach the nebulizer and necessary tubing to the dry compressed air source.

   - If using the Provocholine inhalation solution ready-to-use kit, instill the contents of the base solution (contains no methacholine chloride) into the nebulizer.

2. Place the face mask loosely over the nose and mouth or the mouthpiece in the mouth (with a nose clip) of the patient. Instruct the patient to hold the nebulizer to avoid warming the solution. Keep the nebulizer upright and vertical.

3. Set the pressure regulator to 50 lb/in^2 (psi) and start the nebulizer by setting the flow controller to a flow rate of 4.5 LPM. Start the stopwatch immediately.

4. Instruct the patient to relax and breath the aerosol quietly (tidal breathing) for 1 minute of inhalation time.

5. After exactly 1 minute of tidal breathing, turn off the nebulizer and flow meter, remove the face mask (or the mouthpiece from the mouth), and discard any remaining solution.

6. Perform spirometry and measure the FEV_1 30 and 90 seconds after the end of 1-minute tidal breathing to obtain the post-diluent FEV_1. These values may be left at ambient (spirometer) temperature pressure saturated (ATPS).

   - If the FEV_1 value is not of acceptable quality, repeat the procedure.
   - If the post-diluent FEV_1 falls by ≥ 20% from baseline FEV_1, do not proceed with administration of Provocholine and administer an inhaled β-agonist to the patient *[see Dosage and Administration (2.6)].*
   - If the post-diluent FEV_1 falls by < 20% from baseline FEV_1, proceed to Step 7.

Administration of Provocholine Using the 1-Minute Tidal Breathing Dosing Method

7. Instill Provocholine solution into the nebulizer.

   - If using Provocholine powder for inhalation solution or Provocholine inhalation solution 16 mg/mL (in single-dose vial), using a 3 mL syringe and needle, draw up at least 2 mL of the recommended Provocholine strength based on the dose doubling or quadrupling protocol in Table 5 *[see Dosage and Administration (2.3)]* and instill into the nebulizer using a sterile bacterial-retentive filter (porosity 0.22 µm). Refer to Tables 1, 2, 3 and 4 for preparation of the doubling and quadrupling strengths of Provocholine *[see Dosage and Administration (2.2)].*

   - If using the Provocholine inhalation solution ready-to-use kit, instill at least 2 mL of the recommended Provocholine strength, based on the dose quadrupling protocol in Table 5 *[see Dosage and Administration (2.3)]* into the nebulizer.

8. Repeat steps 2 through 6 for each Provocholine strength, emptying the nebulizer between each strength or dose.

   However, stop dosing if the FEV_1 has fallen by ≥ 20% from the post-diluent FEV_1 or the highest Provocholine strength (16 mg/mL) has been administered (whichever comes first). Do not administer additional Provocholine doses if severe bronchoconstriction occurs *[see Warnings and Precautions (5.1)]*.

9. After the test is completed, administer an inhaled β-agonist to the patient *[see Dosage and Administration (2.6)].*

10. Wash and clean reusable nebulizers thoroughly according to manufacturer's recommendations and discard disposable nebulizers appropriately.

2.6 Recommended Administration of Inhaled β-agonist after Methacholine Challenge Test

After the methacholine challenge test is completed, administer an inhaled β-agonist to the patient to expedite the return of the FEV_1 to within 90% of baseline and to relieve any discomfort (the majority of patients revert to normal pulmonary function within 10 to 20 minutes after β-agonist administration; in contrast the majority of patients revert to normal pulmonary function within 30-45 minutes without β-agonist administration). Wait 10 minutes and measure the FEV_1 and Vital Capacity. Do not allow patients to leave the laboratory or clinic until their FEV_1 has returned to within 90% of baseline.

2.7 Interpretation of Methacholine Challenge Test Results and Calculation of Airway Hyperresponsiveness

Positive Methacholine Challenge Test

A positive methacholine challenge test is a ≥ 20% reduction in the FEV_1 (after Provocholine oral inhalation) compared to the mean post-diluent FEV_1. The test should be stopped at this point. Record the post-diluent FEV_1 value and calculate the ≥ 20% FEV_1 reduction value before starting the methacholine challenge test.

If asthma drugs are discontinued prior to the methacholine challenge test, consider the possibility of rebound airway hyperreactivity in the interpretation of the test results. The methacholine challenge test may occasionally be falsely positive after an influenza infection or upper respiratory infection, immunizations, in very young or very old patients, in patients with chronic lung disease (e.g. cystic fibrosis, sarcoidosis, tuberculosis, chronic obstructive pulmonary disease), in patients with allergic rhinitis without asthma symptoms, in smokers, or in patients after exposure to air pollutants.

If the 5-breath dosimeter dosing method is used, calculate airway hyperresponsiveness (PC_{20}) based on the provocative Provocholine strength (mg/mL) that results in a fall in FEV_1 of ≥ 20%.

If the 1-minute tidal breathing dosing method is used, calculate airway hyperresponsiveness (PD_{20}) based on the provocative Provocholine dose (mcg) that results in a fall in FEV_1 of ≥ 20%.

Calculation of PC_{20} (5-breath dosimeter dosing method)

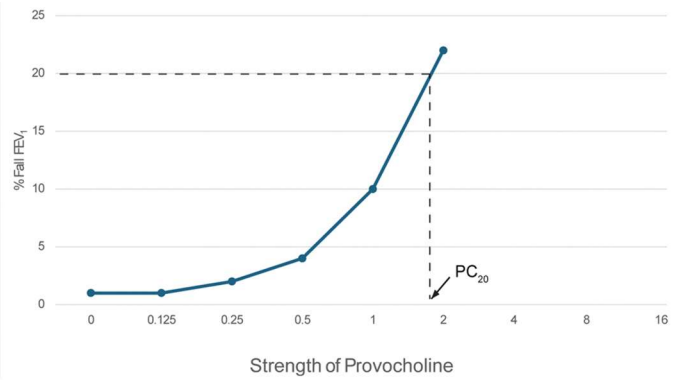
1. Determine the percent decrease [was fall] in FEV_1 using the mean post-diluent FEV_1 and the lowest FEV_1 post- Provocholine (post-dose), as shown below:

$$\% \text{ fall in } FEV_1 = \frac{\text{mean post-diluent } FEV_1 - \text{lowest } FEV_1 \text{ post-Provocholine}}{\text{mean post-diluent } FEV_1} \times 100$$

2. Calculate PC_{20} using one of the following methods:

   Method #1: Plot the percent decrease in FEV_1 against the increasing strength of Provocholine using a log scale and obtain the PC_{20} by linear interpolation between the last two points, as shown in Figure 1.

Figure 1: Calculation of PC_{20}



Method #2: Calculate the PC_{20} using the following equation:

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

Where:

- C1 = second last Provocholine strength in mg/mL (< 20% FEV_1 decrease)
- C2 = last Provocholine strength in mg/mL (≥ 20% FEV_1 decrease)
- R1 = % fall FEV_1 after C1
- R2 = % fall FEV_1 after C2

<u>Calculation of PD_{20} (1-minute tidal breathing dosing method)</u>

Calculate the PD_{20} using the following equation:

$$PD_{20} = \text{antilog}\left[\log D1 + \frac{(\log D2 - \log D1)(20 - R1)}{(R2 - R1)}\right]$$

Where:

- D1 = second last Provocholine dose in mcg (< 20% FEV_1 decrease)
- D2 = last Provocholine dose in mcg (≥ 20% FEV_1 decrease)
- R1 = % FEV_1 decrease after D1
- R2 = % FEV_1 decrease after D2

Negative Methacholine Challenge Test

A negative (normal) methacholine challenge result is defined as FEV_1 reduction of < 20% after all the doses, as part of the dose doubling or quadrupling protocol in Table 5, have been administered *[See Dosage and Administration (2.3)].*

3 DOSAGE FORMS AND STRENGTHS

- For inhalation solution: 100 mg of methacholine chloride crystalline powder, white to off-white in color in amber glass vials (powder is reconstituted and then diluted prior to administration)

- Inhalation solution ready-to-use kit (sterile): Contain the following strengths of methacholine chloride in a clear, colorless solution.
  i. 3 mL base solution (contains no methacholine chloride)
  ii. 0.0625 mg/mL (0.1875 mg/3 mL) of methacholine chloride
  iii. 0.25 mg/mL (0.75 mg/3 mL) of methacholine chloride
  iv. 1 mg/mL (3 mg/3 mL) of methacholine chloride
  v. 4 mg/mL (12 mg/3 mL) of methacholine chloride
  vi. 16 mg/mL (48 mg/3 mL) of methacholine chloride

- Inhalation solution (in a single-dose vial): 16 mg/mL (48 mg/3 mL) of methacholine chloride in a clear, colorless solution in a 3 mL plastic single-dose vial with a twist-off cap

4 CONTRAINDICATIONS

Provocholine is contraindicated in patients with:

- Hypersensitivity to methacholine or other parasympathomimetic agents. Reactions have included rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.

- Baseline FEV_1 < 60% predicted (adults or pediatric patients) or < 1.5 L (adults).

5 WARNINGS AND PRECAUTIONS

5.1 Risk of Severe Bronchoconstriction

Severe bronchoconstriction can result from Provocholine administration (including the lowest dose). The use of Provocholine is contraindicated in pediatric and adult patients with baseline FEV_1 < 60% predicted or adults with FEV_1 < 1.5 L. Emergency equipment and medication should be immediately available to treat acute respiratory distress. Because of the potential for severe bronchoconstriction, the use of Provocholine in patients with clinically apparent asthma or wheezing is not recommended. If severe bronchoconstriction occurs, reverse immediately by the administration of a rapid-acting inhaled β_2-agonist.

If baseline spirometry is not performed or is measured inaccurately, the initial FEV_1 may be underestimated. In this situation, decreases in FEV_1 may not be detected after escalating Provocholine doses, which may result in administration of unnecessary higher doses and an increase in the risk for excessive bronchoconstriction.

5.2 Bronchoconstriction from Inadvertent Exposure to Healthcare Providers Administering Provocholine

The supplied Provocholine powder or the Provocholine nebulized aerosol may cause bronchoconstriction in healthcare providers administering Provocholine in a methacholine challenge test. Healthcare providers and any other personnel involved in the administration of Provocholine should take the following precautionary steps:

- Do not inhale the supplied Provocholine powder
- Do not handle the Provocholine powder if you have asthma or hay fever
- Apply a low resistance filter to expiratory ports of dosing apparatus, as necessary, to prevent Provocholine release in the room air

5.3 Risk in Patients with Coexisting Diseases and Conditions

Provocholine is not recommended for patients with uncontrolled hypertension, aortic aneurysm, or history of myocardial infarction or stroke diseases. Patients with epilepsy, vagotonia, peptic ulcer disease, thyroid disease, urinary tract obstruction or other condition that could be adversely affected by a cholinergic agent should undergo methacholine challenge only if the healthcare provider considers the benefit to the individual outweighs the potential risks.

6 ADVERSE REACTIONS

The following adverse reactions associated with the use of Provocholine were identified in clinical studies or post marketing reports. Because some of these reactions were reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Respiratory System: Bronchospasm (includes symptoms such as chest tightness, cough, or wheezing).

Less Common Adverse Reactions: Headache, throat irritation, light-headedness, and itching.

7 DRUG INTERACTIONS

Beta-Adrenergic Blockers
The use of beta-adrenergic blockers may impair reversal of Provocholine-caused bronchoconstriction.

Beta-Agonists, Anticholinergics, and Theophylline
Beta-agonists, anticholinergics, and theophylline inhibit the response of airways to Provocholine; therefore, hold these drugs before Provocholine use for the following duration:

- Short-acting β-agonists (e.g., albuterol): 6 hours
- Long-acting β-agonists (e.g., salmeterol): 36 hours
- Short-acting anti-cholinergics (e.g., ipratropium): 12 hours
- Long-acting anti-cholinergics (e.g., tiotropium): ≥168 hours
- Oral theophylline: 12-48 hours

Oral or Inhaled Corticosteroids, and Inhaled Cromoglycate
Regular use of oral or inhaled corticosteroids and inhaled cromoglycate may acutely decrease bronchial responsiveness to Provocholine. However, these drugs may be continued with Provocholine use.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary
The available data from published literature on Provocholine use in pregnant women are insufficient to evaluate for a drug-associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes. Animal reproduction studies evaluating effects of methacholine chloride on embryofetal development have not been conducted. Diagnosis of bronchial airway hyperreactivity with bronchoprovocation challenge is not recommended for pregnant women because of the potential for hypoxia in the fetus. If bronchial airway hyperreactivity is suspected, consider trial of empiric treatment.

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the United States general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively.

8.2 Lactation

Risk Summary
There are no available data on the presence of methacholine chloride in human milk, the effect on the breastfed infant, or the effect on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Provocholine and any potential adverse effects on the breastfed infant from Provocholine or from the underlying maternal condition.

8.4 Pediatric Use

The safety and effectiveness of Provocholine used in a methacholine challenge test, has been established for the diagnosis of bronchial airway hyperreactivity in pediatric patients 5 years of age and older who do not have clinically apparent asthma.

The safety and effectiveness of Provocholine have not been established in pediatric patients younger than 5 years of age.

8.5 Geriatric Use

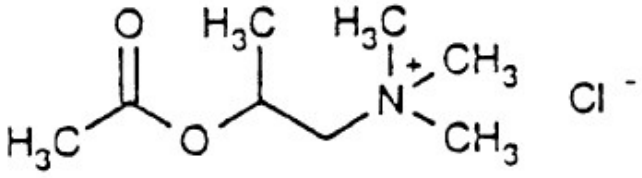
The diagnosis of bronchial airway hyperreactivity is largely performed in pediatric and younger adult patients. Clinical studies of Provocholine did not include patients 65 years of age or older.

11 DESCRIPTION

Methacholine chloride, the active ingredient of Provocholine, is a parasympathomimetic (cholinergic) bronchoconstrictor agent. Provocholine is administered by oral inhalation.

Chemically, methacholine chloride is 1-propanaminium, 2-(acetyloxy)-N,N,N,-trimethyl-, chloride. It is a white to practically white deliquescent compound, soluble in water, alcohol, and chloroform and insoluble in ether. Aqueous solutions are neutral to litmus.

Methacholine chloride has an empirical formula of $C_8H_{18}ClNO_2$, a molecular weight of 195.69, and the following structural formula:



Provocholine (methacholine chloride) for Inhalation Solution:
Each vial of Provocholine contains 100 mg of methacholine chloride powder.

Provocholine (methacholine chloride) Inhalation Solution:

- Ready-to-use kit (sterile): Plastic vials with twist-off cap containing 3 mL of the following strengths of methacholine chloride solution. Each solution also contains the following inactive ingredients: sodium acetate trihydrate, sodium chloride, water for injection. Glacial acetic acid is added to adjust pH.

  a) base solution (contains no methacholine chloride)
  b) 0.0625 mg/mL (0.1875 mg/3 mL)
  c) 0.25 mg/mL (0.75 mg/3 mL)
  d) 1 mg/mL (3 mg/3 mL)
  e) 4 mg/mL (12 mg/3 mL)
  f) 16 mg/mL (48 mg/3 mL)

- Single-dose plastic vial with twist off cap containing 16 mg/mL (48 mg/3mL) of methacholine chloride solution. Solution also contains the following inactive ingredients: sodium acetate trihydrate, sodium chloride, and water for injection. Glacial acetic acid is added to adjust pH.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Methacholine chloride is a cholinergic agonist. Bronchial smooth muscle contains significant parasympathetic (cholinergic) innervation. Methacholine chloride agonizes the muscarinic receptors which eventually induce bronchoconstriction.

12.2 Pharmacodynamics

After oral inhalation of Provocholine, patients with asthma are more sensitive to Provocholine-induced bronchoconstriction than are healthy subjects. This difference in response is the pharmacological basis for Provocholine in the methacholine challenge test.

12.3 Pharmacokinetics

There are no metabolic and pharmacokinetic data available on methacholine chloride.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

There have been no studies with methacholine chloride that would permit an evaluation of its carcinogenic or mutagenic potential or of its effect on fertility.

16 HOW SUPPLIED/STORAGE AND HANDLING

Provocholine (methacholine chloride) for Inhalation Solution:

- Powder: 100 mg of methacholine chloride as a white to off-white powder in amber glass vial. Each carton contains six vials (NDC 64281-100-06).
  - Store the supplied powder at 59°F to 86°F (15°C to 30°C).

Provocholine (methacholine chloride) Inhalation Solution:

- Ready-to-use kit: Each carton contains six sterile ready-to-use kits (NDC: 64281-110-06). Each sterile ready-to-use kit (NDC 64281-110-05) contains six plastic vials with twist-off cap. Each vial contains 3 mL of different strengths of methacholine chloride in a clear, colorless solution as follows:

  a) base solution (contains no methacholine chloride) (NDC 64281-111-00)
  b) 0.0625 mg/mL (0.1875 mg/3 mL) (NDC 64281-112-00)
  c) 0.25 mg/mL (0.75 mg/3 mL) (NDC 64281-113-00)
  d) 1 mg/mL (3 mg/3 mL) (NDC 64281-114-00)

e) 4 mg/mL (12 mg/3 mL) (NDC 64281-115-00)
f) 16 mg/mL (48 mg/3 mL) (NDC 64281-116-00)

- Single-dose vial: 16 mg/mL of methacholine chloride in a clear, colorless solution in a 3 mL plastic single-dose vial with twist-off cap. Each carton contains two inner cartons (NDC 64281-110-12). Each inner carton contains six vials of 16 mg/mL solution (NDC 64281-116-06).

  - Store kits and single-dose vials between 15°C to 25°C (59°F to 77°F). Use immediately upon opening the vial.

17 PATIENT COUNSELING INFORMATION

Risk of Severe Bronchoconstriction

Inform the patient or caregiver that severe bronchoconstriction can result from Provocholine administration *[see Warnings and Precautions (5.1)].*

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