Bronchoprovocation Research Kits

Inclusion/Exclusion Criteria
Epidemiological Studies
Clinical Studies

Customized Bronchoprovocation Research Kits for Single or Multi-Centre Clinical Trials

Methapharm's clinical services team is pleased to offer customized bronchoprovocation research kits with Provocholine (methacholine chloride USP) for use in single or multi-center clinical trials.

Our customized research kits are designed to provide uniformity and consistency across your multiple research sites. Our clinical services team is available to consult with you to design kits that are specific to your research protocol's methacholine challenge testing.

We invite you to contact us at:

Tel: (+1) 519-751-3602 Ext. 7223
e-mail: clinicalservices@methapharm.com

Provocholine®
(methacholine chloride)

Kit contents are based on your protocol

A sample research kit may contain:

Product	Quantity
Provocholine® 100 mg/vial	One
Provocholine® Product Insert	One
10 mL Sterile Empty Vials with Stoppers	Ten
10 cc Syringe	One
3 cc Syringe	One
20 Gauge 1” Needle	Two
0.22 µm Syringe Filter	One
Alcohol Pads	Ten
100 mL Sodium Chloride with 0.4% Phenol	One
Set of Customized Labels	One

For further information or to discuss your study needs contact us at:
Tel: (+1) 519-751-3602 Ext. 7223 | e-mail: clinicalservices@methapharm.com

Provocholine®
(methacholine chloride)

methapharm
Specialty Pharmaceuticals

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PRV-INT-CUST-2-06-15

Provocholine®
(methacholine chloride)

THERAPEUTIC CLASSIFICATION
Cholinergic / Diagnostic Aid (Bronchial Asthma)

SUMMARY PRODUCT INFORMATION

Route of Administration	Dosage Form/Strength	Non-Medicinal Ingredients
Inhalation	Powder 100 mg	• N/A

INDICATIONS AND CLINICAL USE
Provocholine® is indicated for:
• Diagnosis of asthma (bronchial airway hyperresponsiveness).
Provocholine® (methacholine chloride USP) is indicated for the diagnosis of bronchial airway hyperresponsiveness in subjects suspected of having asthma. The methacholine challenge test with Provocholine® provides a measure of the severity of asthma. The methacholine challenge test with Provocholine® may be used to confirm occupational asthma. The product should be administered under the supervision of a qualified health professional who is experienced in the use of inhalation agents and in the management of patients experiencing severe bronchoconstriction. Appropriate management of therapy and complications is only possible when adequate diagnostic and treatment facilities are readily available.
Geriatrics: No data is available.
Pediatrics (<5 years of age): The safety and efficacy of methacholine challenge tests with Provocholine® have not been established in children below the age of 5 years.

CONTRAINDICATIONS
- Provocholine® (methacholine chloride USP) is contraindicated in patients with known hypersensitivity to this drug or to other parasympathomimetic agents.
- A repeat challenge test on the same day is contraindicated.
- ß-agonists, anticholinergics and theophylline may be contraindicative. (See DRUG INTERACTIONS).

WARNINGS AND PRECAUTIONS

Serious Warnings and Precautions

- Provocholine® is to be administered only by inhalation. **See Warnings and Precautions – General**
- Provocholine® is a bronchoconstrictor agent for diagnostic purposes only, and should not be used as a therapeutic agent. **See Warnings and Precautions – General**
- Patients with severe hyperresponsivness of airways can experience bronchoconstriction at the lowest dosages or with the diluent alone. **See Warnings and Precautions - Respiratory**
- Test should not be performed on any patient with baseline FEV_1 of less than 1.5 litres or 70% of predicted value. **See Warnings and Precautions - Respiratory**
- When administered orally or by injection, Provocholine® is associated with nausea, vomiting, substernal pain or pressure, hypotension, fainting and transient complete heart block. **See Adverse Reactions**
- When administered orally or by injection overdosage can result in a syncopal reaction, with cardiac arrest and loss of consciousness. **See Overdosage**
- Baseline spirometry must be accurate, if not, the initial FEV_1 may be underestimated, and subsequent falls after inhaling Provocholine® solutions may not be detected, resulting in too high a dose and excessive bronchoconstriction. **See Warnings and Precautions - General**

General Provocholine® (methacholine chloride USP) is to be administered only by inhalation. Provocholine® is a bronchoconstrictor agent for diagnostic purposes only, and should not be used as a therapeutic agent. Administration of Provocholine® to patients with epilepsy, cardiovascular disease accompanied by bradycardia, vagotonia, peptic ulcer disease, thyroid disease, urinary tract obstruction or other condition that could be adversely affected by a cholinergic agent should be undertaken only if the physician feels the benefit to the individual outweighs the potential risks. It is essential that the baseline spirometry is accurate. If the baseline spirometry is not performed or measured accurately, and the initial FEV_1 is underestimated, subsequent falls after inhaling Provocholine® solutions may not be detected, resulting in too high a dose and excessive bronchoconstriction. Methacholine challenge test with Provocholine® should be performed only under the supervision of a physician trained in and thoroughly familiar with all aspects of the technique of methacholine challenge, all contraindications, warnings and precautions, and the management of respiratory distress. A physician responsible for the tests must be present in the building when tests are carried out, and available to be contacted quickly if necessary. If the physician is performing the test, another person must be available in the building to give assistance if required. The patient must never be left unattended during the test.
Emergency medication and equipment should be immediately available to treat acute respiratory distress.
Carcinogenesis, Mutagenesis and 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.
Cardiovascular Administration of Provocholine® to patients with cardiovascular disease accompanied by bradycardia, which could be adversely affected by a cholinergic agent, should be undertaken only if the physician feels benefit to the individual outweighs the potential risks.
Endocrine and Metabolism Administration of Provocholine® to patients with thyroid disease, which could be adversely affected by a cholinergic agent, should be undertaken only if the physician feels benefit to the individual outweighs the potential risks.
Gastrointestinal Administration of Provocholine® to patients with peptic ulcer disease, which could be adversely affected by a cholinergic agent, should be undertaken only if the physician feels benefit to the individual outweighs the potential risks.
Genitourinary Administration of Provocholine® to patients with urinary tract obstruction, which could be adversely affected by a cholinergic agent, should be undertaken only if the physician feels benefit to the individual outweighs the potential risks.
Neurological Administration of Provocholine® to patients with epilepsy, which could be adversely affected by a cholinergic agent, should be undertaken only if the physician feels benefit to the individual outweighs the potential risks.
Respiratory Severe bronchoconstriction can result from the administration of Provocholine®, if guidelines for careful administration are not followed. Patients with severe hyperresponsiveness of the airways can experience bronchoconstriction at the lowest dosages of Provocholine®, or with the diluent alone. If severe bronchoconstriction occurs, it should be reversed immediately by the administration of a rapid-acting inhaled ß-agonist. Because of the potential for severe bronchoconstriction, Provocholine® challenge should not be performed in any patient with low baseline FEV_1 of less than 1.5 litres or less than 70% of the predicted value. Please consult standard nomograms for predicted values.[1]
Special Populations
Pregnancy: Teratogenic Effects - Animal reproduction studies have not been conducted with methacholine chloride. It is not known whether methacholine chloride can cause fetal harm when administered to a pregnant patient or affect reproductive capacity. Provocholine® should be given to a pregnant woman only when the benefits clearly outweigh the risks.
Nursing Mothers: It is not known if methacholine chloride when inhaled is excreted in breast milk. Methacholine challenge test with Provocholine® should be administered to nursing mothers only when the benefits clearly outweigh the risks.
Pediatric Use: The safety and efficacy of methacholine challenge tests with Provocholine® have not been established in children below the age of 5 years.
Laboratory Personnel: Provocholine® aerosol may cause bronchoconstriction in laboratory personnel and others in the same room as the patient undergoing the test. Laboratory personnel with asthma or hay fever should take appropriate precautions when handling the material. (See SPECIAL HANDLING INSTRUCTIONS)

Information to be Provided to the Patient
To assure the safe and effective use of the methacholine challenge test with Provocholine®, the following instructions and information should be given to patients:
• Patients should be educated on the symptoms that may occur as a result of the test, and instructed to alert the test administrator of these symptoms so that the test can be stopped before pulmonary function is reduced to less than 1.5 litres.
• Women of child-bearing age should be questioned on the possibility of pregnancy (See Special Populations - Pregnancy).

ADVERSE REACTIONS
Adverse reactions associated with inhaled methacholine challenge tests are rare, and include incidences of headache, throat irritation, light-headedness and itching.
A positive reaction to methacholine challenge may produce symptoms of bronchospasm, such as chest tightness, cough or wheezing. Incidences of severe bronchoconstriction can be avoided by limiting the challenge test to cases of potentially mild asthma, in those patients with normal or near normal FEV_1, and by cautiously increasing the dosage.
Provocholine® (methacholine chloride USP) is to be administered only by inhalation. When administered orally or by injection, Provocholine® is reported to be associated with nausea and vomiting, substernal pain or pressure, hypotension, fainting and transient complete heart block. (See OVERDOSAGE)

DRUG INTERACTIONS
Overview Provocholine® (methacholine chloride USP) is a parasympathomimetic (cholinergic) bronchoconstrictor agent to be administered in solution only, by inhalation. Methacholine chloride is the ß-methyl homolog of acetylcholine, is slowly hydrolysed by acetylcholinesterase and almost totally resistant to inactivation by non-specific cholinesterase or pseudocholinesterase.
Drug-Drug Interactions Precaution should be taken when the inhalation challenge is performed in patients receiving any ß-adrenergic blocking agents, as it is possible that bronchoconstriction may not reverse as readily.
The following asthma and hayfever medications inhibit the response of airways to Provocholine®, and should be withheld before the test, for their duration of action: ß-agonists, anticholinergics and theophylline. Corticosteroids, cromoglycate and nedocromil, after regular use, may alter Provocholine® responsiveness but they do not do this acutely; thus, they may be continued in their regular dose before any test. The effects of other newer medications have not been investigated.
Drug-Food Interactions Methacholine chloride can be administered without regards to timing of meals.
Drug-Herb Interactions The interactions of methacholine chloride with herbal medications or supplements have not been established.
Drug-Laboratory Test Interactions The interactions of methacholine chloride with laboratory tests have not been established.

DOSAGE AND ADMINISTRATION
Recommended Dose and Dosage Adjustments

For Provocholine® (methacholine chloride USP), adults and children (5 years or older) are exposed to the following increasing concentrations: 0.03, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 mg/mL. (See Table 1)
Preparation of Dilutions:
Provocholine® (methacholine chloride USP) requires dilution before use. All dilutions using Provocholine® should be made with either 0.9% sodium chloride solution for injection (saline) or 0.9% sodium chloride solution with 0.4% phenol (saline with 0.4% phenol)or 0.9% sodium chloride solution for injection containing 0.9% benzyl alcohol (saline with 0.4% benzyl), as suggested in Table 1 for Provocholine® 100 mg/vial. After adding the chosen diluent, shake each vial to obtain a clear solution. Check the date of preparation or expiry before using dilutions that are not freshly prepared.
Provocholine® solutions prepared from powder and using aseptic technique may be stored in a refrigerator (2° to 8°C) for up to 2 weeks. After this time, discard the vials and prepare new dilutions. Freezing does not affect the stability of the dilutions. Since the temperature of the solution affects nebulizer output, solutions should be taken out of the refrigerator and allowed to equilibrate to room temperature (approximately 30 minutes) before use.
Table 1 describes methods of producing appropriate dilutions, using a single vial of Provocholine®.
When preparing dilutions using Provocholine®, a sterile bacterial-retentive filter (porosity 0.22 µm) should be used when transferring a solution from each vial (at least 2 mL) to a nebulizer.

Table 1: Preparation of Serial Dilutions Using a Single 100 mg Vial of Provocholine® (methacholine chloride USP)

TAKE	ADD 0.9% saline or 0.9% saline with 0.4% phenol or 0.9% saline with 0.9% benzyl	OBTAIN DILUTION
100 mg Provocholine®	6.25 mL	16 mg/mL (A)
3 mL of dilution A	3 mL	8 mg/mL (B)
3 mL of dilution B	3 mL	4 mg/mL (C)
3 mL of dilution C	3 mL	2 mg/mL (D)
3 mL of dilution D	3 mL	1 mg/mL (E)
3 mL of dilution E	3 mL	0.5 mg/mL (F)
3 mL of dilution F	3 mL	0.25 mg/mL (G)
3 mL of dilution G	3 mL	0.125 mg/mL (H)
3 mL of dilution H	3 mL	0.0625 mg/mL (I)
3 mL of dilution I	3 mL	0.03 mg/mL (J)

Administration
General Procedures:
The challenge test must be conducted in a pulmonary function laboratory or clinic, by adequately trained personnel, for safety and accuracy.
The FEV_1 value should be established before and after diluent inhalation. After determination of the post-diluent baseline pulmonary function, the predicted value of a positive response is then calculated from the mean before diluent inhalation.
The methacholine challenge is performed by giving a subject increasing serial concentrations of Provocholine®, after determining baseline FEV_1. When using Provocholine®, baseline FEV_1 is determined with inhaled normal saline control or normal saline control with 0.4% phenol or normal saline control 0.9% benzyl **(Note: Use the same diluent that the Provocholine® has been reconstituted with).** A subject to be challenged must have an FEV_1 of at least 70% of the predicted value. A common error giving inaccurate results is caused by not taking a full inspiratory breath prior to baseline FEV_1 determination. **Consult a physician if the FEV_1 falls below 1.5 litres. Do not leave the patient unattended at any time.**
An inhaled ß-agonist must be administered after a methacholine challenge test with Provocholine® to expedite the return of the FEV_1 to baseline and to relieve any discomfort of the subject. Most patients revert to normal pulmonary function within 10 to 20 minutes following administration of a ß-agonist.
In order to produce interpretable results, it is important to calibrate nebulizers to produce a standard output, and validate the reproducibility of the delivery system. Suitable nebulizers and standard settings are discussed in published sources.
Two methods of administration of the methacholine challenge test with Provocholine® have been widely used in current clinical practice; the tidal breathing method and the dosimeter method. The tidal breathing technique requires the patient to breathe normally, over a two-minute period, a constantly generated aerosol of Provocholine®. By contrast, the dosimeter method requires the patient to take five full breaths of Provocholine® aerosol generated by an appropriate dosimeter to produce a specific dose per breath. Additional delivery devices and methods have been described in the literature. Approved manufacturer's instructions should be followed when using these devices. With all techniques, the test is stopped if the FEV_1 falls by 20% or more from the mean baseline FEV_1. The dose concentration and the percent fall in FEV_1 are then used to calculate either the provocative concentration to cause a fall in FEV_1 of 20% (PC_{20}), or the provocative dose (PD_{20}).
Tidal Breathing Method:
The following method is based on the use of the Wright nebulizer. If using other nebulizer models, consult published sources on methacholine challenge tests for the appropriate operation of alternate nebulizers.
1. Using a 3 mL syringe and needle, draw up 2-3 mL of the diluent (0.9% saline or 0.9% saline with 0.4% phenol or 0.9% saline with 0.9% benzyl) and place it in the nebulizer vial. Attach the nebulizer and necessary tubing to an appropriate compressed gas source.
2. At this time, the subject should be told that subsequent aerosols may produce mild cough, chest tightness or shortness of breath. Tell the subject that if these symptoms become uncomfortable, to remove the face mask or mouthpiece and to stop inhaling the aerosol immediately. Try to avoid suggesting that these symptoms will definitely develop, as suggestion alone can lower the FEV_1. Remember that perception of airway narrowing can vary considerably between subjects, making it advisable to watch and listen for other signs such as wheeze and an altered pattern of breathing. Instructions to cease inhaling the aerosol if symptoms become troublesome should be repeated before every dose.
3. Instruct the patient to relax and breathe the aerosol quietly (tidal breathing) for 2 minutes.
4. Keeping the nebulizer well away from the patient, adjust the flow meter so that the nebulizer is operating at the calibrated output (0.13 mL/min for the Wright nebulizer).
5. Apply a nose clip and place the face mask loosely over the nose and mouth (or the mouthpiece in the mouth). Start the stopwatch immediately. The nebulizer should be kept vertical. The patient should hold the nebulizer so as to avoid warming the solution, and subsequently altering the output.
6. After exactly two minutes, remove the nebulizer from the patient's mouth, turn off the flow meter, and discard the solution.
7. Measure the FEV_1 30 and 90 seconds after the end of the inhalation. These values may be left at ATPS. If the FEV_1 at 90 seconds is the same or lower than that at 30 seconds, the measurement must be repeated at 3 minutes and, if needed, at 2 minute intervals until the FEV_1 starts to rise. To avoid tiring the patient, the FEV_1 should only be measured once on each occasion. If it is not technically satisfactory, it should be repeated after 10 seconds.
8. If the FEV_1 falls by 20% or more from the mean baseline FEV_1 (ATPS) or to less than 1.0 litre, no further inhalations are given. (A physician should be consulted if the FEV_1 falls below 1.5 litres.) If the FEV_1 has fallen by 16% or more from baseline, it is unwise to give further doses. The PC_{20} may be extrapolated from the last two points of the dose response curve.
9. For Provocholine®, the concentration of the first aerosol of Provocholine® is 0.03 mg/mL. Subsequent doses are given at approximately 5-minute intervals in doubling concentrations. (0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg/mL).
10. Repeat steps 1 through 8 with each increasing concentration of Provocholine® until the FEV_1 has fallen by 20% or more from baseline, or the FEV_1 is 1.5 litres or less, or the highest concentration has been given. Do not give any further aerosols of Provocholine®.
11. After the test is completed, give the patient 2 puffs of a ß-agonist. Wait 10 minutes and measure the FEV_1 and VC. Patients should not be allowed to leave the laboratory until their FEV_1 has returned to within 90% of baseline.
12. After the test, reusable nebulizers should be sterilized according to manufacturer's recommendations. Disposable nebulizers should be discarded appropriately.

Dosimeter Method:
The following method is based on the use of a DeVilbiss jet nebulizer attached to a Rosenthal-French dosimeter operating at 20 psi and a period of 0.6 seconds per actuation. If using other nebulizers or dosimeters, consult manufacturer's instructions and published sources on methacholine inhalation challenge for the appropriate operation of alternate nebulizers and dosimeters. The dosimeter should be calibrated to ensure accurate dose delivery and re-calibrated whenever the length of the tubing is changed.
All solutions are delivered from functional residual capacity (FRC) to total lung capacity (TLC). Factors that influence the response to inhalation challenge, and which should be consistent, are nebulizer output and inspiratory time.
The FEV_1 value should be established before and after diluent inhalation. After determination of the post-diluent baseline pulmonary function, the predicted value of a positive response is then calculated from the mean before diluent inhalation.
1. Solution is put in the nebulizer, and the necessary tubing attached to the dosimeter. The aerosol is generated by the compressed air delivered at 20 psi through the nebulizer. The output is controlled by a solenoid valve that is triggered by the inspiration and is kept open for 0.6 seconds. A nose clip is used. The subjects are instructed to inhale slowly from the functional residual capacity (FRC) to total lung capacity (TLC). During the inhalation, the vent of the nebulizer should be kept open.

2. Baseline pulmonary function is established with five inhalations of diluent for Provocholine® (0.9% saline or 0.9% saline with 0.4% phenol or 0.9% saline with 0.9% benzyl), and the baseline FEV_1 noted. A subject to be challenged must have an FEV_1 of at least 70% of the predicted value, when tested with the diluent. Spirometry is measured within 5 minutes of the fifth inspiration of the diluent.
3. At this time, the subject should be told that subsequent aerosols may produce mild cough, chest tightness or shortness of breath. Tell the subject that if these symptoms become uncomfortable, to remove the mouthpiece immediately. Try to avoid suggesting that these symptoms will definitely develop, as suggestion alone can lower the FEV_1. Remember that perception of airway narrowing can vary considerably between subjects, making it advisable to watch and listen for other signs such as wheeze and an altered pattern of breathing. Instructions to cease inhaling the aerosol if symptoms become troublesome should be repeated before every step up in concentration.
4. As with the tidal breathing technique, serial concentrations of Provocholine® are administered. Five inhalations of each concentration are taken, followed by measurement of FEV_1 within 5 minutes of the last inhalation at each dosage. One inhalation unit is defined as one inhalation of a solution of Provocholine® containing 1 mg/mL. Because doses are taken in rapid succession, the units are expressed as cumulative units, as shown is Table 2 below.

Table 2: Cumulative Inhalation Units

Serial Concentration	Number of Breaths	Cumulative Units per Concentration	Total Cumulative Units
0.03 mg/mL	5	0.15	0.15
0.0625 mg/mL	5	0.3	0.45
0.125 mg/mL	5	0.625	1.08
0.25 mg/mL	5	1.25	2.33
0.5 mg/mL	5	2.5	4.83
1 mg/mL	5	5	9.83
2 mg/mL	5	10	19.83
4 mg/mL	5	20	39.83
8 mg/mL	5	40	79.83
16 mg/mL	5	80	159.83

5. If the FEV_1 falls by 20% or more from the mean baseline FEV_1 (ATPS) or to less than 1.0 litre, no further inhalations are given. (A physician should be consulted if the FEV_1 falls below 1.5 litres.) Partial doses (fewer than 5 inhalations) may be given if the FEV_1 is between 15% and 20% less than baseline control, in order to protect against an excessive fall in pulmonary function.
6. After the test is completed, give the patient 2 puffs of a ß-agonist. Wait 10 minutes and measure the FEV_1. Patients should not be allowed to leave the laboratory until their FEV_1 has returned to within 90% of baseline.
7. After the test, reusable nebulizers should be sterilized according to manufacturer's recommendations. Disposable nebulizers should be discarded appropriately.

Shortening the Test Procedure:
Technicians should be well versed on the longer procedure before attempting a shorter version. Shortening the test does run the risk of inadvertently giving the patient too high a dose; always err on the side of safety and give a lower dose when in doubt. If clinical history suggests that the patient may not have asthma or that their asthma is very mild, then the lowest concentration may be omitted, as described below:
1. Starting Concentrations in Adults
As a guide, the first concentration of Provocholine® can be based on the following criteria:
a) If FEV_1/VC >80% AND FEV_1 >70% predicted AND FEV_1 falls <10% after the diluent inhalation AND the patient's symptoms are **well controlled** on the following medications, use these starting concentrations:

Medication	Starting Concentration
Inhaled or ingested corticosteroids	0.125 mg/mL
Daily bronchodilators	0.25 mg/mL
Occasional bronchodilators (< once/day)	1.0 mg/mL
No medications	2.0 mg/mL

b) If FEV_1/VC <80% OR FEV_1 <70% predicted AND FEV_1 falls <10% after the diluent inhalation AND the patient's symptoms are well controlled on the following medications, use these starting concentrations:

Medication	Starting Concentration
Inhaled or ingested corticosteroids	0.03 mg/mL
Other or no medications	0.125 mg/mL

c) If a patient's FEV_1 falls by 10% or more after the diluent inhalation, or if asthma symptoms do not appear to be well controlled, **DO NOT** omit any concentrations, and start patient at 0.03 mg/mL.

2. Starting Concentrations in Children
a) If FEV_1/VC >80% AND the child's symptoms are **well controlled** on the following medications, use these starting concentrations:

Medication	Starting Concentration
Inhaled or ingested corticosteroids	0.03 mg/mL
Daily or occasional bronchodilators	0.0625 mg/mL
No medications	0.25 mg/mL

b) If FEV_1/VC <80% OR if asthma symptoms do not appear to be well controlled, **DO NOT** omit any concentrations, and start patient at 0.03 mg/mL.

3. Omission of Concentrations
If, after the first concentration of Provocholine®, there has been no evidence of any significant fall in the FEV_1 (less than 5% from mean baseline) and there is **NO** clinical evidence of any bronchoconstriction (chest tightness, cough or wheezing), the next dose may be omitted. As soon as there is any evidence of symptoms or a fall greater than 5% from mean baseline FEV_1, **DO NOT** omit any further concentrations. If a concentration is omitted, it is important to stress before every subsequent inhalation that the subject should remove the face mask/mouthpiece as soon as they experience any breathing or chest discomfort.

Calculation and Interpretation of Results:
Either the provocative concentration or the provocative dose causing a 20% fall in FEV_1 (PC_{20} or PD_{20}) may be calculated as described below:

1. Calculation of PC_{20}

With either the tidal breathing method or the dosimeter method, airway responsiveness may be expressed as that concentration of Provocholine® provoking a fall in FEV_1 of 20% (PC_{20}). The percent fall in FEV_1 can be calculated using the mean baseline FEV_1, as shown below:

$$\% \text{ fall in } FEV_1 = \frac{\text{mean baseline } FEV_1 - \text{lowest } FEV_1 \text{ post-Provocholine® x 100}}{\text{mean baseline } FEV_1}$$

The percent fall in is then plotted against the rising concentration of Provocholine® (log scale). The PC_{20} is obtained by linear interpolation between the last two points, as shown in Figure 1.

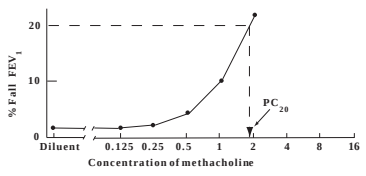

Figure 1: Calculation of PC_{20}

Alternatively, the PC_{20} may be calculated as follows:

$$PC_{20} = \text{antilog}\left[\log C_1 + \frac{(\log C_2 - \log C_1)(20 - R_1)}{(R_2 - R_1)}\right]$$

Where:
C_1 = second last concentration (<20% FEV_1 fall)
C_2 = last concentration (>20% FEV_1 fall)
R_1 = % fall FEV_1 after C_1
R_2 = % fall FEV_1 after C_2

2. Calculation of PD_{20}

The FEV_1 from the best spirogram at each dose is plotted on semilog paper (see example Figure 2, below) and a dose response curve constructed. The dose is expressed as cumulative units, either µmoles or breath units, where 1 mg/mL is equal to 0.5 µmoles or 10 breath units. The curve starts at 100%, and the last data point should be at 80% of saline control or lower. From this curve, the PD_{20}, the provocation dose of agonist necessary for a 20% drop in FEV_1, can be interpolated. The PD_{20} is the measure of the sensitivity to Provocholine®. Patients who do not respond to five inhalations of Provocholine® at the 16 mg/mL concentration can be said to have a negative challenge.

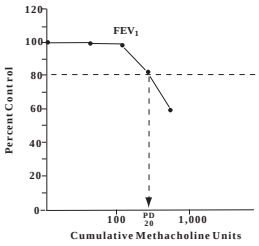

Figure 2: Airway responsiveness to Provocholine® (PD_{20}), expressed as cumulative units (either µmoles or breath units)

3. Interpretation of Results
In clinical trials, most asthmatics had a positive response at the 10 mg/mL concentration or less. Results can be interpreted with respect to the presence or absence of asthma only if the initial FEV_1/VC is >70%. The cut-off point between normal and increased responsiveness is a PC_{20} of 8 mg/mL, or a PD_{20} of 4 cumulative µmoles or 80 cumulative breath units (Figure 3). Increased responsiveness is arbitrarily graded as borderline if between 4 and 8 mg/mL (2 and 4 µmoles or 40 and 80 breath units), as mild between 2 and <4 mg/mL (1 and <2 µmoles or 20 and 40 breath units), as moderate if between 0.25 and <2 mg/mL (0.125 and <1 µmoles or 5 and <20 breath units), and as severe if <0.25 mg/mL (<0.125 µmoles or <2.5 breath units). Patients with a PC_{20} >16 mg/mL (or a PD_{20}>8 µmoles or >160 cumulative breath units) are unlikely to have current symptoms due to asthma. When the PC_{20} is between 2 and 16 mg/mL, or the PD_{20} is between 1 and 8 µmoles or 20 and 160 cumulative breath units, current symptoms due to asthma are likely to be mild, infrequent or absent. Current symptoms of asthma are usual when the PC_{20} is <2 mg/mL, or the PD_{20} is <1 µmoles or <20 cumulative breath units.

NOTE: When using a single dose automatic provocation device system to administer Provocholine®, the equivalent of the above values will need to be calculated, as appropriate.

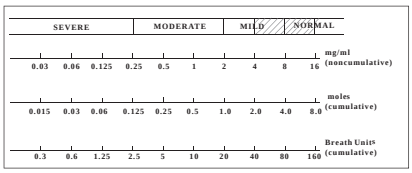

Figure 3: Comparison of Provocholine® airway responsiveness expressed as PC_{20} (mg/mL), using the tidal breathing method, and expressed as PD_{20} (cumulative µmoles and cumulative breath units) using the dosimeter method.

OVERDOSAGE

Provocholine® (methacholine chloride) is to be administered only by inhalation. When administered orally or by injection, overdosage with Provocholine® can result in a syncopal reaction, with cardiac arrest and loss of consciousness. Serious toxic reactions should be treated with 0.5 mg to 1 mg of atropine sulfate, administered IM or IV.

ACTION AND CLINICAL PHARMACOLOGY

Mechanism of Action
Provocholine® (methacholine chloride) is a parasympathomimetic (cholinergic) bronchoconstrictor agent to be administered in solution only, by inhalation, for diagnostic purposes.
Methacholine chloride is the ß-methyl homolog of acetylcholine and differs from the latter primarily in its greater duration and selectivity of action. Bronchial smooth muscle contains significant parasympathetic (cholinergic) innervation. Bronchoconstriction occurs when the vagus nerve is stimulated and acetylcholine is released from the nerve endings. Muscle constriction is essentially confined to the local site of release because acetylcholine is rapidly inactivated by acetylcholinesterase.
Compared with acetylcholine, methacholine chloride is more slowly hydrolysed by acetylcholinesterase and is almost totally resistant to inactivation by non-specific cholinesterase or pseudocholinesterase.
When a solution containing Provocholine® is inhaled, subjects with current asthma are more sensitive to methacholine and bronchoconstrict at lower doses than healthy subjects. This difference in response is the pharmacologic basis for the Provocholine® inhalation diagnostic challenge. The test is most useful diagnostically when there are current symptoms consistent with asthma and when the forced expiratory volume at one second (FEV_1) is normal at >70% predicted. A normal result excludes current asthma (variable airflow limitation), but does not exclude past asthma.

Pharmacodynamics
When there is chronic airflow limitation with an FEV_1/VC of <70%, the test can be abnormal due to other pathophysiological causes such as smoker's bronchitis, emphysema or cystic fibrosis. The challenge may also be positive in patients with allergic rhinitis without symptoms of asthma, or in patients who have had or will in the future develop asthma symptoms. Certain drugs can affect the pharmacodynamic response to Provocholine®. (See Drug-Drug Interactions)

STORAGE AND STABILITY

Temperature:
• Store unopened vials of Provocholine® at room temperature (15° to 30°C).
Reconstituted Solutions:
• Freezing does not affect the stability of dilutions made with Provocholine® and 0.9% saline or 0.9% saline with 0.4% phenol or 0.9% saline with 0.9% benzyl.
• Provocholine® reconstituted with 0.9% saline or 0.9% saline with 0.4% phenol or 0.9% saline with 0.9% benzyl, using aseptic technique, may be stored under refrigeration (2° to 8°C) for up to 2 weeks.

SPECIAL HANDLING INSTRUCTIONS

Provocholine® is a potent bronchoconstrictor. Do not inhale the powder. Do not handle this material if you have asthma or hay fever. A low resistance filter should be applied to an expiratory port of any dosing apparatus, as necessary, to prevent Provocholine® aerosol from being released into the air of the room.

When using Provocholine® any unused solution should be discarded from the nebulizer after each concentration.

DOSAGE FORMS, COMPOSITION AND PACKAGING

Provocholine®:
• 100 mg – in 20 mL and 50 mL amber glass vials in boxes of 6 and 12 vials
• 100 mg in 50 mL vials also available in boxes of 1 vial
• 0.9% saline or 0.9% saline with 0.4% phenol or 0.9% saline with 0.9% benzyl alcohol as preservative must be used to reconstitute the powder
• Administered via inhalation using a nebulizer

REFERENCES

1. Morris JF, Koski WA, Johnson LC.
Spirometric standards for healthy non-smoking adults.
Am Rev Resp Dis 1971;103:57-67.

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