A Clinical and Structural Comparison of Industrial Methacholine and Provocholine*

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Methacholine, provided by industrial sources, has traditionally been used in studies of airways responsiveness. In 1986, a Food and Drug Administration approved formulation of methacholine (Provocholine) was released and replaced industrial methacholine in many pulmonary laboratories. To determine whether methacholine and Provocholine cause an equivalent degree of bronchoconstriction, a double blind, cross-over clinical trial was undertaken. After randomization, 19 medicine residents and respiratory therapists each performed methacholine challenge testing using either methacholine or Provocholine. Forty-eight hours later, each participant returned for repeat challenge testing with the alternate agent. The log of the dose-response slope (logslope) was calculated for each test. The mean logslope with methacholine (−0.15 ± 1.84) and with Provocholine (−0.26 ± 1.57) did not differ (paired Student's t test, p=0.84). Further, excellent agreement was found between each subject's logslope with methacholine and with Provocholine (intraclass correlation coefficient r1=0.82). Proton beam nuclear magnetic resonance revealed no structural differences between the two compounds. These findings suggest that methacholine from industrial sources and Provocholine are clinically and structurally similar and that the two agents may be used interchangeably in nonspecific bronchial provocation testing.

\[ \text{dose-response slope} = \text{logslope} \]

Since the early 1960s, bronchial provocation testing with histamine and methacholine has been used to measure airways responsiveness. For many years, methacholine was only available from industrial sources. In 1986, however, a Food and Drug Administration (FDA) approved formulation of methacholine, Provocholine (Roche, Nutley, NJ) was released and replaced industrial methacholine in many pulmonary laboratories and research investigations. Recently, Provocholine became unavailable, compelling clinicians and researchers to resume their use of industrial methacholine. To determine if methacholine from an industrial source and Provocholine cause an equivalent degree of bronchoconstriction, a double blind, cross-over clinical trial was undertaken.

METHODS

Nineteen medicine residents and respiratory therapists agreed to participate in the study. Standard questionnaires were used to obtain information on demographics, cigarette smoking, respiratory symptoms, and physician-diagnosed lung disease. After randomization, each subject performed methacholine challenge testing, following the protocol of O'Connor, with either methacholine (Spectrum Chemical Manufacturer, Gardenia, Calif) or Provocholine (Roche Laboratory, Nutley, NJ). Forty-eight hours later, each participant returned for repeat challenge testing with the alternate agent. Subjects were asked to refrain from cigarettes for at least 4 h, and chocolate and caffeinated beverages for at least 8 h or, if unable to follow these instructions, to behave similarly on both days of testing.

The dose-response slope (logslope), defined as the percent decline in FEV₁ from baseline divided by the cumulative dose of methacholine, was calculated for each methacholine challenge test. The natural log of the logslope was used in all analyses to normalize the distribution of the measurements.

The paired Student's t test and the intraclass correlation coefficient were used to compare logslopes obtained with methacholine and with Provocholine. The intraclass correlation, a measure of concordance for use with continuous variables, is mathematically equivalent to the kappa statistic and as such, accounts for chance agreement between two or more individual observations; it is derived by one-factor repeated-measures analysis of variance. Using suggested guidelines for interpreting kappa, values of the intracllass correlation coefficient from 0.41 to 0.60 were taken to indicate moderate agreement, 0.61 to 0.80 substantial agreement, and 0.81 to 1.0 almost perfect agreement.

Fitted multiple linear regression was used to determine if order of testing or intrindividual differences in prechallenge FEV₁ contributed to the prediction of logslope after Provocholine once logslope obtained with methacholine was considered. Proton beam nuclear magnetic resonance using 250 MHz and D2O solvent was used to analyze the structural composition of the two compounds.
RESULTS

The study group consisted of 8 women and 11 men, of whom 3(15.8 percent) were current smokers and 8(15.8 percent) were former smokers. The mean age for the group was 33.4 ± 6.8 years; 4 subjects were current asthmatics. The mean logslope with methacholine (−0.15) and with Provocholine (−0.25) did not differ (paired Student’s t test, p=0.64). Excellent agreement was found between each subject’s logslope with methacholine and with Provocholine (intraclass correlation coefficient r1=0.82, Fig 1). Once logslope with methacholine was considered, neither order of testing nor intra-individual differences in prechallenge FEV1 contributed to the prediction of logslope with Provocholine (fitted multiple linear regression, data not shown). Furthermore, an analysis using proton beam nuclear magnetic resonance revealed the two compounds to be structurally identical (Fig 2).

DISCUSSION

The results of this study suggest that methacholine from industrial sources and Provocholine might be used interchangeably for clinical and research purposes. If cost were the only issue, industrial methacholine clearly would be the preferred agent as it is 30 times less expensive than Provocholine. The former’s lack of FDA and United States Pharmacopedia approval, however, raises concerns about possible product impurities and lot variability. While we found no evidence of such problems, our study was limited in that we assessed only one lot of industrial methacholine produced by a specific manufacturer. It is reassuring, then, that others have been using methacholine from industrial sources for many years without apparent untoward events6,7 and that more than 70 percent of investigators in a recent survey reported their routine use of industrial methacholine in preference to Provocholine.8 As can be appreciated from the above discussion, deciding which agent to use is complicated. We urge further review of the issue and, perhaps, policy recommendations from bodies such as the American College of Chest Physicians. The latter will be all the more important if the current sole manufacturer of FDA-approved Provocholine (Roche Laboratories) permanently discontinues production of its product.
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