Limitations of the Anticholinergic Activity Assay and Assay-Based Anticholinergic Drug Scales

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Objective: *The anticholinergic activity (AA) assay is a common method to determine a patient's anticholinergic load. Several limitations, however, are expected when applying the AA assay to patients or using drug scales to estimate anticholinergic burden based on AA levels. This study aims to demonstrate common pitfalls in an experimental setting and outline their clinical consequences.* **Methods:** *The AA was analyzed for five drugs with reported interaction with muscarinic receptors. Concentration-response curves were constructed for furosemide (weak anticholinergic), diphenhydramine (moderate anticholinergic), the strong anticholinergic amitriptyline and its metabolite nortriptyline, and the cholinergic pilocarpine. The Combination Index (CI) was used to assess the interaction of three drug combinations with amitriptyline.* **Results:** *All compounds displaced the radioactive tracer from its receptor binding site in a concentration-dependent manner, and full displacement was reached for all compounds except furosemide (E_{max} 16%). The CI indicated that amitriptyline and thioridazine have antagonistic effects (CI = 1.46) at low and synergistic effects (CI = 0.88) at higher concentrations ($p < 0.0001$), whereas synergistic effects (CI = 0.47–0.48) were observed for amitriptyline in any concentration combined with pilocarpine ($p < 0.001$).* **Conclusion:** *When the patient's anticholinergic load is estimated using AA levels, the actual exposure, combination of anticholinergic drugs, their active metabolites, and also drugs with an opposite pharmacologic action will contribute to AA levels, whereas weak anticholinergic drugs in therapeutic concentrations are rather negligible.* (Am J Geriatr Psychiatry 2016; ■■:■■–■■)

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INTRODUCTION

The use of anticholinergic drugs is associated with physical and cognitive impairment, especially in vulnerable patients like the elderly.[1] As a proxy for anticholinergic adverse drug reactions, the anticholinergic load of a patient can be determined either by analyzing anticholinergic moieties in a patient's blood sample or by a theoretical appraisal of expected anticholinergic effects of a patient's medication. In the latter approach, each drug taken by the patient is scored according to a scale; summing up the scores yields the patient's anticholinergic load. In either approach, the drug's anticholinergic activity (AA) established by a radioreceptor assay plays a crucial role. This assay determines the drug's binding ability to muscarinic receptors of rat brain membranes and quantifies the displacement of a radioligand.[2] Hence, the assay can be used either for measuring the actual circulating AA of a patient's blood sample or for the development of a scale relating to known AA levels of anticholinergic drugs. In the scales, the drugs were scored according to AA levels published in the literature[3,4] or to the AA of pure compounds as tested in vitro.[5,6]

In theory, higher AA levels or drug scores indicate a higher anticholinergic load, conferring an increased risk of physical and cognitive impairment. A close linear relationship between peripheral and cerebral AA was detected,[7] and, thus, several studies reported an association between anticholinergic load and cognitive impairment or delirium,[8] whereas others did not confirm AA as a marker of cognitive dysfunction.[9] The results were not consistent throughout different study populations and diverse cognitive tests,[10] potentially because of major limitations of the AA assay and/or the methodology of the scales based on AA levels.

The first (expected) limitation relates to the rating of the drugs listed in the scales because the scores were assigned to individual drugs regardless of the administered dose. However, as known for all receptor-mediated effects, the exposure will be a decisive factor of the effect and, thus, the anticholinergic load. Indeed, the occurrence of dementia was associated with the cumulative dose of anticholinergic drugs,[11] and also the anticholinergic adverse drug reaction rate of solifenacin increases with higher doses.[12] Second, the scales generally do not consider metabolites, even though the binding of metabolites may potentially strengthen or weaken the anticholinergic effect of their parent drug. For instance, although clozapine mainly acts as a cholinergic antagonist, its metabolite *N*-desmethylclozapine is a partial agonist.[13] Third, in the AA assay only the drug's affinity to muscarinic receptors is measured but not its intrinsic pharmacologic activity. It thus appears to be intuitive that agonists and antagonists will cause similar AA levels because both are able to bind to muscarinic receptors, despite their opposite pharmacologic effects. A fourth limitation refers to the calculation of the patient's anticholinergic load. The summation of scores assumes that the pharmacologic effect is not influenced by the applied drug concentration and therefore increases in a linear way when other drugs are added to the medication regime. However, such a relationship will likely not infinitely increase but rather reach a plateau at higher concentrations because of the finite number of muscarinic receptors.[10] In this study we aimed to quantify the (expected) limitations of the AA assay and therefore also AA-based drug scales in an experimental setting and to discuss the resulting consequences for their clinical use.

METHODS

Determination of AA

The concentration-dependent displacement of muscarinic receptor binding was measured in a radioreceptor binding assay as described by Tune and Coyle.[2] Instead of patient blood samples, pure compounds (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) were used. The compounds were dissolved in 200 µL 0.1 N-hydrochloric acid and 50 mM potassium biphosphonate buffer and added to 200 µL human male plasma (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). In this assay, the drugs compete with 200 µL of the 56,000 dpm radioactive labeled cholinergic antagonist L-quinuclidinyl benzilate (^{3}H-QNB; Hartmann Analytic, Braunschweig, Germany) for muscarinic receptors obtained from 50 µL homogenized brain of male Wistar rats (Analytical Biological Services, Wilmington, DE). The AA is derived from the quantitative displacement of ^{3}H-QNB by the drug (=response) and is expressed as a percentage of the total inhibition of ^{3}H-QNB binding. For each drug and drug combination, the indicated concentrations were measured in triplicate followed by a calculation of a sigmoid