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LETTER TO THE EDITOR

The frequent deficiency of lack of assay sensitivity

Dear Editor,

I read with interest the article by Jantikar et al.[1] that was published in the April 2007 edition of Respiratory Medicine, which reported similar bronchodilator response between 200 μg of salbutamol and 100 μg of levosalbutamol. A sensitive comparison on the bronchodilator effect of salbutamol and levosalbutamol ((*R*)-salbutamol) would have been useful to clarify the controversy regarding the clinical relevance of the preclinical and *in vitro* negative effects of dextrosalbutamol ((*S*)-salbutamol).[2,3] However, in order to avoid the constant repetition of the same methodological deficiencies, I believe that it is important to highlight these flaws that invalidate the study conclusions and, therefore, do not provide relevant information to the controversy, like many other manuscripts before, as it was already highlighted by Ahrens and Weinberger.[4] Even the paper by Lotvall et al.[5] that seemed to be sensitive according to Ahrens and Weinberger, has to be questioned according to Fishwick et al.[6]

In the paper by Jantikar et al. we can find three major deficiencies.

Firstly, it is well known that the comparison of the bronchodilating or bronchoprotective effect of β_2-agonists has to be performed with at least two doses of each product to calculate a simple dose–response curve for each product,[7] which indicates if the study presents assay sensitivity to detect differences. In addition, the comparison is not based on the response obtained with equivalent doses of each product but on the shift of the dose–response curve of both products, which is called the relative potency.[8] This concept was applied to the comparison of inhalation products more than 30 years ago[9–11] and has been incorporated into the requirements of the Regulatory Agencies to avoid false conclusions of equivalence due to the flatness of the dose–response curve of β_2-agonists.[12–14] Therefore, it is surprising that insensitive studies are still being designed by scientists that should be updated, approved by ethical committees that should avoid human experimentation without any usefulness and publication in peer-reviewed journal that give credibility to insensitive designs and their conclusions.

The sensitivity of this trial could have been investigated without an increase of complexity. The inclusion of a placebo treatment in the cross-over design is unnecessary (nice to see but not needed), because there is no doubt that any dose of salbutamol is going to be superior to placebo. Placebo is usually required to give internal validity in studies that investigate drugs that have not shown superiority to placebo consistently (e.g. topical NSAIDs). The use of another dose of any active treatment (e.g. 100 μg of salbutamol or 50 μg of the eutomer) would have been preferable to provide assay sensitivity since it allows the estimation of the relative potency (even with methods different to the Finney bioassay) and demonstrates whether different doses provide similar responses and, therefore, the comparison of the responses is insensitive to detect any difference.

Secondly, this study has compared 200 μg of salbutamol versus 100 μg of levosalbutamol. It is also known that a single actuation of 100 μg of salbutamol produces and increment in FEV_1 that is close to peak response in mild-to-moderate asthmatics.[15] Similarly, it has been described that 40 μg of salbutamol provides 80% of the personal best value of FEV_1.[16] Therefore, the comparison at a dose of 200 μg has been performed at the upper plateau of the dose–response curve of salbutamol. Then, the study is insensitive to detect any difference if such a difference exists.

Thirdly, the authors have always considered that the lack of statistical significant differences is indicative of similarity. It has to be remembered that absence of evidence (of a difference) is not evidence of absence (of a difference). In other words, the inability to reject the null hypothesis does not support the validity of the null hypothesis. Only the alternative hypothesis can be proved in a test of hypothesis. In conclusion, the authors should have employed the hypothesis of equivalence in the alternative hypothesis. The authors' confusion is shown in the wording employed to justify the sample size calculation. This wording is confusing because at the same time it claims that 24 subjects were required to detect differences between study medication with 80% power (which shows that it is a superiority test) and to show equivalence at a significance level of 5% (which seems to be an equivalence test, but no equivalence limits have been defined). In order to calculate the sample size for an equivalence test it is necessary to define the acceptance range (δ), the difference expected between treatments (Δ), the variability of the response (σ), the consumer risk ($\alpha = 5\%$) and the produce risk ($\beta = 20\%$ to have a power of 80%). The authors only mention that the size estimation was

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done considering the mean maximum change in FEV_1 following salbutamol administration in a previous study, which is none of the parameters needed and apparently irrelevant. This confusion is confirmed with the use of Student's *t*-test for paired variables employed to detect differences (superiority test) and the Schuirmann's two one-sided test of equivalence, which are equivalent to confidence intervals for the ratio or difference. Unfortunately, no confidence interval is provided and the similarity is concluded when statistically significant differences are not detected ($P > 0.05$).

Besides these three major deficiencies, other interesting issues can be identified.

The authors have compared two identical formulations and devices manufactured by Cipla Ltd. that apparently only differ in the amount of active substance. This has been necessary in order to assure that the difference if detected is not due to the different formulation or device. However, although unlikely, it should be confirmed that both formulations show a similar lung deposition by means of, for example, a pharmacokinetic lung deposition study, which can be easily performed by a generic company like Cipla Ltd.

From a purely scientific point of view, the selection of the reference product, which is identical in formulation and device, is the correct one. However, it is difficult to understand why a CFC formulation is developed presently when the CFC are almost completely replaced by HFA.

Finally, although a bronchodilator or bronchoprotection model can be used to demonstrate therapeutic equivalence of generic products of short-acting β_2 agonists[12,14] because they contain the same active substance, some might think that in order to conclude similarity between salbutamol and levosalbutamol it would be necessary to investigate not only the bronchodilating effects but also the bronchoprotecting effects, since they are not the same chemical entity.

This represents the personal opinion of the author and does not necessarily represent the views or policy of the Spanish Agency for Medicines and Health Care Products.

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Alfredo García Arieta
Division of Pharmacology and Clinical Evaluation, Spanish Agency for Medicines and Health Care Products, Campezo 1, Edificio 8 Planta 2 Oeste, E-28022 Madrid, Spain
E-mail address: agarciaa@agemed.es