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EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Nonirritant intradermal skin test concentrations of ciprofloxacin, clarithromycin, and rifampicin

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Keywords

antibiotics allergy; ciprofloxacin; clarithromycin; digital image analysis; intradermal testing; laser Doppler flowmetry; rifampicin.

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Abstract

Background: Intradermal skin testing of the clinically important antibiotics ciprofloxacin, clarithromycin, and rifampicin in the case of suspected allergies to antibiotics is poorly standardized. For clinical practice, standardized procedures and protocols are desired.

Methods: Fifteen healthy volunteers were tested with different concentrations of the antibiotics as well as with appropriate controls. Test readings included wheal area measured by digital image analysis and blood flow increase measured by laser Doppler flowmetry (LDF). To reduce interpersonal variability, test results were normalized with the individual controls using a novel protocol.

Results: Nonirritating concentrations of the three antibiotics (ciprofloxacin ~0.0067 mg/ml, clarithromycin ~0.05 mg/ml, rifampicin ~0.002 mg/ml) could be defined for healthy volunteers. Laser Doppler flowmetry generates comparable results to wheal area measurement. Normalization of the test results is necessary and can be applied in a practical algorithm.

Conclusions: Standardized skin testing to detect sensitization to broadly used nonbetalactam antibiotics was presented and should be applied in truly sensitized patients. This approach should help to minimize the inter- and intraindividual differences in reactivity.

Adverse reactions to antibiotics are common and are associated with morbidity and rarely mortality. Convenient and reliable testing for sensitization to antibiotics is desired and might reduce unjustified omissions owing to the concerns regarding allergy.

Up to 15% of all adverse drug reactions are considered to be of allergic or pseudoallergic nature. Most allergic reactions are either immediate-type immunoglobulin E-mediated reactions that cause anaphylactic reactions or delayed-type reactions that cause anaphylactoid reactions or delayed-type reactions mostly T-cell-mediated reactions manifesting as various exanthems (1, 2). Pseudoallergic reactions are thought to be mediated by the direct release of histamine and other inflammatory mediators by mast cells and basophils (3, 4). Diagnosis of antibiotic allergy is based on a thorough patient's history and clinical manifestation as well as on the diagnostic tests. The diagnostic gold standard is controlled reexposure with the suspected drug, but application is often limited by fear of severe reactions. Skin testing can be used

to detect immediate-type IgE-mediated skin reactions, in which intradermal is more sensitive than prick testing, but it is more difficult to interpret because of potential irritative test reactions.

While skin testing for penicillin and cephalosporin allergy is well established (1), there are no accepted standard protocols for most nonbetalactam antibiotics (5). In the case of commonly used antibiotics such as fluoroquinolones, macrolides, or rifampicin, literature data are limited even though hypersensitivity reactions are also known for these antibiotics (6). The allergenic determinants of these antibiotics are unidentified, and some of them have a high irritant potential. Therefore, the sensitivity and the specificity of intradermal skin tests are limited, mostly owing to the irritant reactions to higher concentrations of the test drug or to excipients that are present in commercial preparations (7). Prior to widespread use of skin testing with these antibiotics, nonirritating concentrations for individual antibiotics have to be defined

for nonallergic subjects, the readings have to be standardized, and the protocols have to be defined and evaluated.

The classical readings in skin testing are areas of wheal and erythema (8). Laser Doppler flowmetry (LDF) can be used as an objective and semi-quantitative tool to assess the dynamics of the immediate-type hypersensitivity reaction and may be helpful in reducing false-negative or false-positive test results (9, 10). Laser Doppler flowmetry quantifies microcirculatory skin blood flow in real time based on the Doppler effect. A monochromatic laser beam is directed onto the skin, and a photodetector measures the reflected light and calculates the average velocity of blood cells within the tissue.

In this work, we evaluated clinical readings and LDF to discriminate between irritating and nonirritating skin test concentrations of ciprofloxacin, clarithromycin, and rifampicin in nonallergic subjects and assessed the possibility to reduce their irritant potential by using different vehicles and concentrations.

Material and methods

Subjects and test substances

The local ethics committee approved the study. After obtaining written consent, we enrolled 16 healthy nonallergic or nonexposed volunteers, one had to be excluded from the analysis because of urticarial dermatographism. Ciprofloxacin (Ciproxin®; Bayer, Zuerich, Switzerland), clarithromycin (Klacid®; Abbott, Baar, Switzerland), and rifampicin (Rimactan®; Sandoz, Steinhausen, Switzerland) for intravenous use were reconstituted according to the instructions of the manufacturer and diluted either in physiological saline (NaCl, 0.9%) or in 0.3 mg/ml human serum albumin (ALK Abello, Horsholm, Denmark). It was hypothesized that albumin might influence the irritant potential of the injected antibiotics by alterations in pH, the physicochemical or immunological properties. Full-strength concentrations were 2 mg/ml for ciprofloxacin, 50 mg/ml for clarithromycin, and 60 mg/ml for rifampicin. Different dilutions of ciprofloxacin (1 : 300, 1 : 1000, and 1 : 3000, resulting in estimated concentrations of ~0.0067, ~0.002, and ~0.00067 mg/ml, respectively), clarithromycin (1 : 300, 1 : 1000, 1 : 3000, 1 : 10 000 and 1 : 30 000; ~0.167, ~0.05, ~0.017, ~0.005 and ~0.0017 mg/ml, respectively), and rifampicin (1 : 10 000, 1 : 30 000 and 1 : 100 000; ~0.006, ~0.002 and ~0.0006 mg/ml, respectively) were administered intradermally on the upper back (large even area necessary for scanning LDF) by one investigator (T.H.) with a 1-ml syringe (CODAN Medical, Rodby, Denmark) and 0.4 × 20 mm needle (Terumo Europe, Leuven, Belgium). The volume was 0.03–0.04 ml, resulting in a bleb of approximately 5–6 mm. The drug concentrations were chosen based on available data for nonirritating concentrations and prepared according to published recommendations (3, 6, 11). To rule out the influence of pH of the solutions, it was measured in all drug concentrations. As positive controls, different histamine (Allergopharma, Therwil, Switzerland) concentrations (dilution 1 : 1, 1 : 10, and 1 : 100; 0.1 mg/ml, ~0.01 mg/ml, and ~0.001 mg/ml, respectively) were chosen and as negative controls physiological

saline and human serum albumin. This resulted in 24 skin tests per subject. The area of the wheal was measured after 20 min, the flow measurement by LDF was performed at baseline, and then every 4 min for 20 min.

Clinical test evaluation

The area of the wheal was measured by photography as published previously (12, 13). Twenty minutes after injection, digital images of the resulting wheals were taken using a Coolpix 5000 camera (Nikon Corporation, Egg, Switzerland) with an optical device with integrated scale to allow skin contact photography (Optical Service Neuhaus, Gams, Switzerland). The images were analyzed using ImageJ software (U.S.

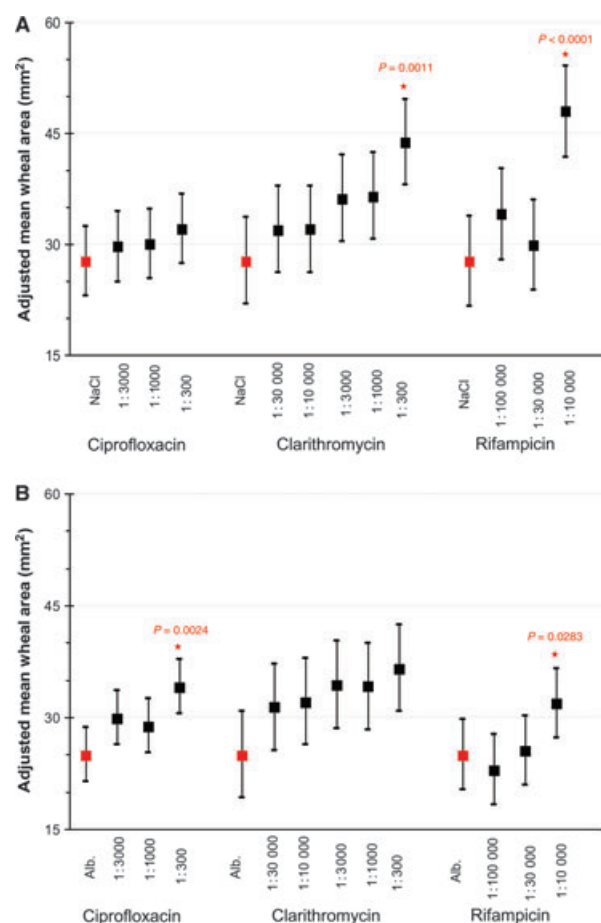


Figure 1 Mean (+ 95% confidence interval) wheal area in mm² measured optically on the back of the subjects 20 min after intradermal injection of different concentrations of the test substances or controls. The vehicle for dilution was physiological saline (A) or human serum albumin (B). The wheal area was adjusted by the effect of the individuals, taking also into account that each subject received all the tests. *P*-values are given if the difference is significant compared to the negative control in group comparisons. The *P*-values were adjusted for multiple comparisons, using the Dunnett-Hsu correction.

National Institutes of Health, Bethesda, MD, USA). The perimeter of the wheal was drawn in triplicate, and the corresponding area of the wheal was calculated.

Additionally, the wheal area data were analyzed according to the standard methods used for betalactam antibiotics testing (1). In short, an increase in wheal diameter of more than 3 mm 20 min after the injection of an antibiotic compared to the reaction to the negative control was considered to be positive.

$$\text{Cutoff factor} = \frac{\text{wheal area of test concentration} - \text{wheal area of negative control}}{\text{wheal area of positive control} - \text{wheal area of negative control}}$$

Laser Doppler flowmetry

Laser Doppler flowmetry measurements were taken in a darkened room with the PeriScan PIM II Imager with LPDIwin-Software (Perimed AB, Stockholm, Sweden), equipped with a low-power 670-nm laser beam that scans the tissue surface sequentially and stepwise. The PeriScan system was used according to the standards of the manufacturer. Scans were performed at a distance of 20 cm ($\pm$ 2 cm) and in a low-resolution mode, which scans an area of 18 $\times$ 18 cm to cover all 24 skin tests (64 $\times$ 64 sampling points). By linear interpolation, this area was converted to an image containing 256 $\times$ 256 pixels (pixel size 0.75 $\times$ 0.75 mm). Scan time was 4 min, images were coded based on a defined color scale between 0 and 3, and a region of interest (ROI) was defined manually. The ROI calculation report compares the minimal, maximal, and mean perfusion intensity, including the standard deviation based on the baseline measurement as a reference.

Statistical analysis

The wheal area for each test was calculated by averaging the three performed readings. Categorical variables were summarized as proportions and continuous variables as means and standard deviation. Mixed regression models adjusted for the

effect of the individuals were used to compare the wheal area size produced by different concentrations of an antibiotic or to compare blood flows at different times. Mixed models provide an allowance for the effect of repeated measurements as the same individual received all tested antibiotics and respective concentrations. The effects were adjusted for multiple comparisons, using the Dunnett-Hsu adjustment.

To determine whether a reaction was irritating, the following factor for the wheal area was defined for each antibiotic:

Negative values for the factor were set to zero (i.e., area of test was smaller than the negative test). This factor takes into consideration the reaction of each individual to the different test agents. A concentration was considered irritating if its test yielded a reaction with a defined cutoff point of a factor higher than 0.4. The LDF data were treated in the same way.

Results

Subject and test solution characteristics

The mean age of the 15 tested subjects (seven men, eight women) was 34.2 years (range, 24–60 years); 40% reported a personal history of allergies, mostly rhinoconjunctivitis and atopic dermatitis. Four subjects had been previously exposed to the tested antibiotics, but none of the subjects had a known allergy to any antibiotic.

Measurement of pH showed no relevant difference among the different drug concentrations in the two vehicles. pH for albumin was 5.65, for the drug dilutions in albumin was 5.61–5.77, for NaCl 0.9% was 5.81, and for the drugs in NaCl between 5.71 and 6.00, independent of dilution. Therefore, it was estimated that pH was not relevant to the irritant potential of higher drug concentrations. Also, no

Table 1 Statistically derived threshold factor of 0.4 (confer to main text for calculation details) applied to the test data

Test substance	Dilution	Number of subjects with a factor $\geq$ 0.4	Percentage of subjects with factor $\geq$ 0.4	Number of subjects with a diameter $>$ 3 mm than control	Percentage of subjects with a diameter $>$ 3 mm than control
Ciprofloxacin (2 mg/ml)	1 : 3000	1	6.67	5	33
	1 : 1000	2	13.33	5	33
	1 : 300	1	6.67	6	40
Clarithromycin (50 mg/ml)	1 : 30 000	3	20.00	5	33
	1 : 10 000	4	26.67	5	33
	1 : 3000	1	6.67	7	47
	1 : 1000	3	20.00	7	47
	1 : 300	8	53.33	9	60
Rifampicin (60 mg/ml)	1 : 100 000	5	33.33	2	13
	1 : 30 000	5	33.33	5	33
	1 : 10 000	10	66.67	7	47

irritant additives were present in the freshly reconstituted antibiotic solutions.

Wheal area measurements

Subjects showed a high grade of interpersonal variability in terms of wheal area, independent of the applied substance or concentration. The mean wheal area for 0.1 mg/ml histamine was 197.0 mm², whereas the wheal areas for the test substances were in the range of 30–48 mm².

For ciprofloxacin in physiological saline, all concentrations were nonirritating. In albumin, the adjusted mean wheal area for ciprofloxacin 1 : 300 was significantly larger than that of the negative control, indicating a nonallergic, irritative reaction. In saline, rifampicin and clarithromycin both showed a trend to a stronger irritant potential than ciprofloxacin. Rifampicin 1 : 10 000 and clarithromycin 1 : 300 showed a significant irritating reaction. In albumin, the irritant potential for clarithromycin, but not for rifampicin, was decreased (Fig. 1A,B).

To generate a simple calculation model, we propose the use of an individualized cutoff point as described earlier. As shown in Table 1, some of our nonallergic subjects developed reactions that were classified above the proposed cutoff point. For clarithromycin 1 : 300 and rifampicin 1 : 10 000, more than half of our subjects had irritative reactions and surpassed the cutoff point, being in parallel to the adjusted mean wheal area measurements shown in Fig. 1.

Compared to our proposed calculation model that takes into account the reaction area of the negative as well as that of the positive control, analysis by the standard methodology for betalactam antibiotics testing leads to a less clear-cut image. For most of the antibiotic concentrations, at least one-third of our subjects would be classified as positive, whereas the highest concentration of clarithromycin (1 : 300) leads to a positive test in more than half of the subjects.

Flow measurements

The mean flow increase after 20 min for 0.1 mg/ml histamine was 284.3% of baseline, whereas the flow increase for the test substances was mostly in the range of 28–55%. Mean flow increase compared to baseline after 20 min was 21.7% for saline and 25.6% for albumin, reflecting the physiological blood flow increase caused by the intradermal injection. The test data were also adjusted with the individual negative controls because of the high interpersonal variability.

Ciprofloxacin showed a significantly higher increase in blood flow in both saline and albumin compared to the negative controls. In saline, ciprofloxacin 1 : 300 and 1 : 1000 caused a statistically significant increase in blood flow.

Clarithromycin clearly showed the highest increase in blood flow, especially in albumin. In saline, dilutions 1 : 300 and 1 : 1000 reached significance compared to the negative control. In albumin, all dilutions between 1 : 300 and 1 : 10 000 showed a significant increase in blood flow.

For rifampicin in saline, dilution 1 : 10 000 showed an increased blood flow compared to the negative control, the

lower concentrations showed no significant difference. The same results were obtained in albumin (Fig. 2A,B).

Figure 3 illustrates blood flow changes in the most irritant concentration for every substance after intradermal injection. With all three substances, maximal blood flow change was seen 4 min after injection, with a decline afterward. Ciprofloxacin showed the lowest, while clarithromycin showed the highest irritant potential in LDF. Blood flow increase was lower and decreased faster at lower concentrations (data not shown).

Discussion

Clinical use of intradermal skin testing with nonbetalactam antibiotics is still limited by technical problems. Standardiza-

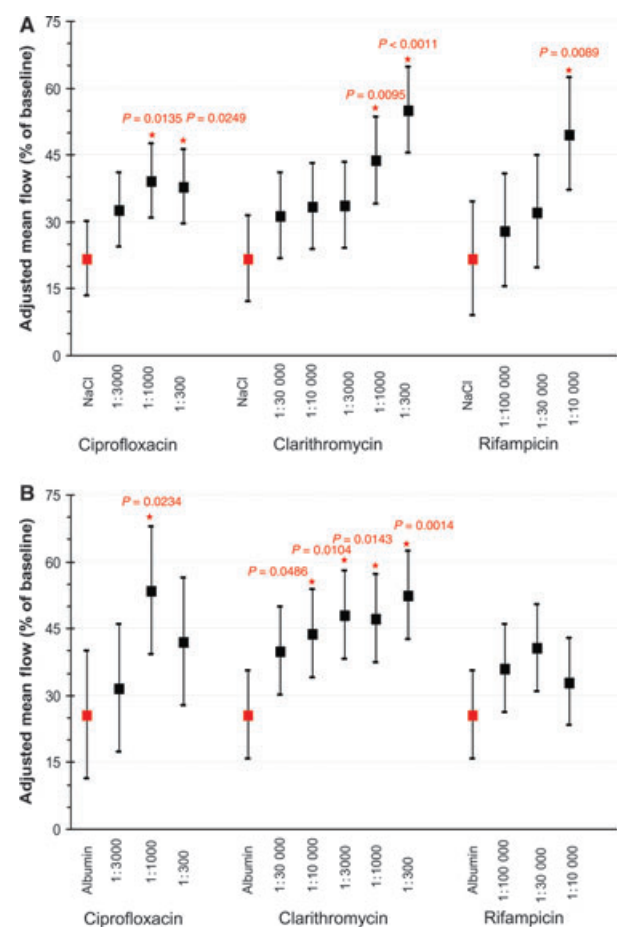


Figure 2 Mean (+ 95% confidence interval) increase in blood flow in percentage measured by laser Doppler flowmetry on the back of the subjects 20 min after intradermal injection of different concentrations of the test substances or controls. Vehicle for dilution was physiological saline (A) or human serum albumin (B). The mean flow adjusted by the effect of the individuals, taking also into account that each subject received all the tests. *P*-values are given if the difference is significant compared to the negative control in group comparisons. The *P*-values were adjusted for multiple comparisons using the Dunnett-Hsu correction.

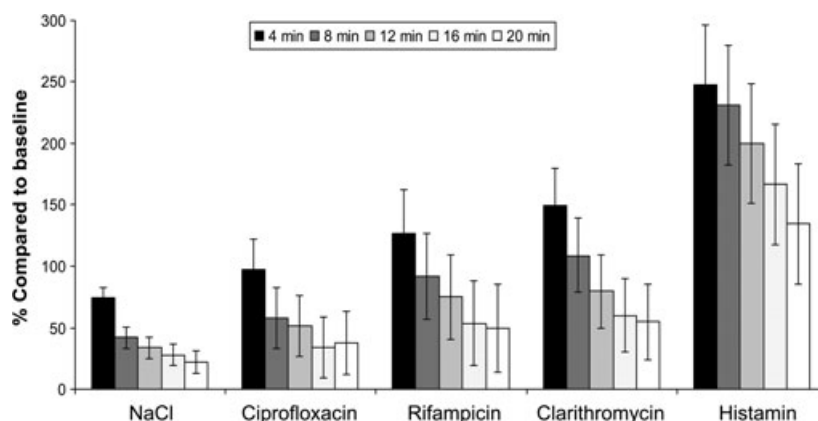


Figure 3 Mean (+ 95% confidence interval) changes in blood flow compared to baseline over time after intradermal skin tests is given for the most irritant concentration of the test substances. The vehicle used was physiological saline. Means were adjusted by the effect of the individuals, taking into account repeated measurements.

tion is lacking, and interpretation of the test results is difficult owing to a high irritant potential of some of the antibiotics. Standard evaluations for the intradermal testing of betalactams such as increase in the initial bleb by 3 mm or doubling of the bleb diameter tend to generate a number of positive test results that may reflect irritative, nonallergic reactions.

Here, intradermal skin testing was performed according to the accepted standards, which were mostly evaluated for betalactams. Readouts were the area of the wheal measured photo-optically and the dermal blood flow increase measured by LDF. For the interpretation of the data, we propose a novel normalization protocol based on the data of nonallergic test subjects. This step is mandatory because of the high interpersonal variability of the wheal areas when injecting test substances.

We also tested the value of an alternative vehicle, namely human serum albumin instead of physiological saline. There, we did not observe any relevant differences. Furthermore, we propose the use of smaller intervals in dilution steps, using in-between concentrations in the region of nonirritating concentrations.

Our results show that it may be possible to define nonirritating concentrations for nonallergic subjects using the proposed factor which takes into consideration the high inter-individual variation. In our test series, albumin did not relevantly decrease the irritant potential of the drugs, but a tendency toward a lower irritant potential was observed for rifampicin. In saline and wheal area as a readout, the highest

nonirritating concentrations were 1 : 300 for ciprofloxacin, 1 : 1000 for clarithromycin, and 1 : 30 000 for rifampicin. In albumin, the highest nonirritating concentrations were 1 : 1000 for ciprofloxacin, 1 : 300 for clarithromycin, and 1 : 30 000 for rifampicin. Similar nonirritating concentrations can be found in the literature for rifampicin (12), ciprofloxacin (7, 14), and clarithromycin (15). For ciprofloxacin, one study stated a high irritant potential, but it remains unclear whether the reaction at low concentrations in this study was caused by additives instead of the drug itself (11).

Compared with the standard methodology for betalactam antibiotics, the proposed calculation model leads to a more clear-cut distinction between negative and positive reactions, which might reflect true sensitization. Further tests in truly allergic patients will generate a better basis for clinical application by delivering more precise data regarding sensitivity, specificity, and negative predictive value.

For daily practice, we propose to use at least two concentrations of the test substances as well as appropriate intradermally injected negative and positive controls (see Table 2). The resulting values of the wheal area measurements should then be normalized using the presented formula. Test values surpassing the 0.4 cutoff point should be considered as suspicious, warranting additional testing or higher dilutions to rule out an irritative reaction. Reactions with a value below the cutoff point and below the cutoff concentrations of the test substance found in literature can be considered as negative, even when the wheal diameter is >3 mm larger than the negative control.

Table 2 Recommended standard procedure for intradermal drug testing of three nonbetalactam antibiotics (confer to main text for details)

Test substance	Concentration of stock solution (mg/ml)	Recommended vehicle	Recommended test dilutions
Ciprofloxacin	2	Physiological saline	1 : 300/1 : 1000
Clarithromycin	50	Physiological saline	1 : 1000/1 : 3000
Rifampicin	60	Physiological saline	1 : 30 000/1 : 100 000

Laser Doppler flowmetry measurement of the test areas reveals irritating reactions already at lower concentrations not yet visible by the naked eye. The reason for this difference remains unclear; especially, it is unclear whether it reflects a higher sensitivity in measuring a vascular reaction to the test substance. Future experiments with truly sensitized patients might show whether LDF is able to indicate significant reactions already at lower concentrations of the tested drugs.

Our work has some limitations that have to be considered. We have chosen healthy subjects without known allergies to antibiotics to establish nonirritant cutoff concentrations. However, patients with a suspected allergic reaction may have a stronger baseline reactivity to injected allergenic substances. On the other hand, the proposed approach with normalization of the test results might be able to diminish the interpersonal differences in skin reaction capacity.

Secondly, the evaluation is based on the data of 15 persons only; a larger cohort may result in more homogenous results with a lower degree of variability. The defined cutoff factor of 0.4 was chosen arbitrarily; lower and higher values were tested but were considered to be less cohesive with clinical readouts. When testing the truly sensitized patients, the choice of the level of the cutoff factor will clearly influence sensitivity and specificity as well as negative and positive predictive values.

We compare wheal area measurement and LDF for intradermal skin testing of three nonbetalactam antibiotics and

present a novel method for the interpretation of the test results by normalizing the results based on individual negative and positive controls. We established nonirritating concentrations of the three tested antibiotics and defined cutoff values for possibly true-positive skin test reactions. Further studies in patients sensitized to these three evaluated antibiotics are needed to evaluate whether the proposed test procedure is valid for the correct interpretation of intradermal skin tests.

Authors contributions

P. Brož has drafted, designed, and written the complete manuscript. Th. Harr and C. Hecking have recruited the volunteers and taken all the measurements of digital analyses and laser Doppler flowmetry, data acquisition and analyses. L. Grize has performed data and statistical analyses. K. Scherer has established the concept, test concentrations, digital imaging, and performed analyses in previous studies. K. A. Jaeger and A. J. Bircher have established the concept, and designed and supervised the study. All authors have revised and approved the final article.

Conflict of interests

All authors declared that they have no conflict of interest with regard to the study.

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