Repeatability of transcranial Doppler measurements of arterial blood flow velocities in healthy subjects

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(Received 4 January/1 March 1993; accepted 11 March 1993)

1. Transcranial Doppler measurements of the mean blood flow velocity and the resistance index in the middle cerebral artery are used to assess cerebral haemodynamics. The prerequisite for the use of these parameters in clinical pharmacology studies is an evaluation of their repeatability and spontaneous variation with time.

2. Repeatability of transcranial Doppler measurements of mean blood flow velocity and resistance index was investigated in healthy subjects by calculation of the repeatability coefficient as defined by the British Standards Institution. Intra-observer repeatability (comparison of two successive determinations by one observer, n=30 subjects), between-observer repeatability (comparison of two successive measurements each performed by a different observer, n=30) and long-term repeatability (comparison of two determinations performed at the same hour with a 1 week interval by one observer, n=14) were assessed. In addition, the spontaneous evolution with time of mean blood flow velocity and resistance index was determined over an 8 h period (n=14).

3. Repeatability coefficients for intra-observer repeatability, between-observer repeatability and long-term repeatability were 4.83, 4.59 and 3.32 cm/s for mean blood flow velocity (normal value 61.2±10.2 cm/s) and 2.62, 3.12 and 3.49% for resistance index, respectively (normal value 53.6±5.9%), indicating that transcranial Doppler measurements are repeatable enough to be used over periods of time of up to 1 week in clinical pharmacology studies.

4. Finally, a time effect was detected for mean blood flow velocity, indicating that this parameter undergoes diurnal variation.

INTRODUCTION

The transcranial Doppler technique allows instantaneous measurements of blood velocity in intracranial arteries. It has been used for a decade to investigate cerebral haemodynamics under pathological conditions such as atherosclerosis of intracranial vessels [1] or cerebral vasospasm [2]. More recently, the transcranial Doppler technique has also been used to investigate the effects of drugs on cerebral circulation in clinical trials [3, 4]. Other methods used in previous studies were either invasive (oxygen arteriovenous difference method) [5] or involved the use of radioactivity (133Xe dynamic single-photon-emission computerized tomography) [6]. In contrast, the transcranial Doppler technique is non-invasive and can be repeated, giving access to the kinetics of a drug's effect on blood velocity in large intracerebral arteries, e.g. the middle cerebral artery.

The prerequisite for the use of the transcranial Doppler technique in clinical pharmacology studies is the demonstration of its repeatability. However, as this has not yet been adequately established under standardized conditions [7], the main goal of the present study was to investigate, in healthy subjects, the repeatability of middle cerebral artery blood velocity measurements using the transcranial Doppler technique. In this study, repeatability was assessed under three different aspects: (a) intra-observer repeatability (IOR), i.e. the comparison of two determinations obtained at a 5 min interval by the same observer; (b) between-observer repeatability (BOR), i.e. the comparison of two measurements each performed by a different observer at a 10 min interval; (c) long-term repeatability (LTR), i.e. the comparison of two determinations performed at the same hour but with a 1 week interval by the same observer. In addition, we also investigated the spontaneous evolution with time of middle cerebral artery blood flow velocity, a single observer performing at regular intervals and over an 8 h period successive transcranial Doppler measurements in healthy subjects at rest.

METHODS

Subjects

The studies were conducted in healthy subjects. These were deemed healthy on the basis of a complete medical examination and were free of any medication. All subjects gave their written informed consent for the study, which was approved by the
Hospital Ethical Committee. The characteristics of the subjects are listed in Table I.

IOR and BOR were evaluated together during the first study (study 1, n=30). LTR and spontaneous evolution with time were assessed in another group of subjects (n=14) during studies 2 and 3, respectively.

Parameters investigated

Middle cerebral artery mean blood flow velocity (MV) was measured using a transcranial pulsed Doppler (Medasonics Transpect; 2 MHz) device. The probe was fixed over the right temple using a special headset to ensure the stability of the device during the entire experimental period.

Spectral analysis was accomplished with fast Fourier transformation. Spectral information was displayed as velocity, assuming an angle between the ultrasonic beam and the blood column of 0 degree.

The depth of focus was increased from 45 mm by 2 mm steps until bidirectional flow appeared from the bifurcation of the carotid artery. The depth was increased until a negative signal, characteristic of the anterior cerebral artery, was observed. Then, the depth was decreased until a positive signal, characteristic of the middle cerebral artery, was obtained. MV was recorded over an 8 s period, at rest, in the supine position, with no acoustic or visual distraction. MV is expressed in cm/s.

Middle cerebral artery resistance index (RI) was calculated from the following formula:

\[ \text{RI} = \frac{\text{systolic velocity} - \text{diastolic velocity}}{\text{systolic velocity}} \]

Systolic and diastolic velocities were measured on the same recordings as MV and were the maximal and minimal instantaneous velocities measured on each systolo–diastolic complex. Mean RI values were calculated over an 8 s period. RI is expressed as a percentage.

Systolic and diastolic blood pressures were also measured in study 3 using a Roche Sentron automatic monitor [8] positioned around the right arm of the recumbent subjects and programmed to record these parameters every 10 min. One given value corresponds to the mean of three consecutive measurements. Mean arterial pressure was calculated as (systolic blood pressure + 2 × diastolic blood pressure)/3.

Experimental protocols

After a light breakfast at 06.00 hours, each subject arrived at the laboratory at 07.00 hours. The subject rested in the supine position for an hour. In all three studies, the first transcranial Doppler measurement of middle cerebral artery MV and RI started at 08.00 hours.

**Study 1.** Subjects were investigated by two different observers (A and B). Observer A performed two successive measurements (A1, and 5 min later A2) without changing the position of the probe and the conditions of measurement. The Doppler probe and the headset were then removed. Thereafter, observer B also performed two successive measurements (B1 and B2) without having any knowledge of the first observer’s results. The order of observers A and B was randomized. The subjects remained resting in the supine position during the whole measurement period and were asked not to help the second observer in any way. A1 and A2 were used to assess IOR, whereas A1 and B1 were used to assess BOR.

**Study 2.** Two measurements were performed by the same observer with a 1 week interval (C1 and C2).

**Study 3.** After the initial determination performed at 08.00 hours, the subjects underwent six further middle cerebral artery MV and RI measurements at 09.00, 10.00, 11.00 and 12.00 hours, and at 14.00, and at 16.00, respectively. The subjects were asked to remain at rest in the supine position during the whole duration of the experiments, except for half an hour (between 13.00 and 13.30 hours) when a standardized meal was served. No tea, coffee, alcohol or smoking was allowed on the day before and during the whole measurement period.

Data and statistical analysis

Data are reported as means ± SD. When two series of paired measurements were compared (studies 1 and 2), the results were analysed in four different steps, according to the recommendations of Bland and Altman [9].

(a) The correlation between the values of measurements (equation of the linear relationship, correlation coefficient \( r \), and \( P \) value) (A1 versus A2 for IOR, A1 versus B1 for BOR, C1 versus C2 for LTR) was investigated. This first step was used to gauge the degree of agreement between the two series of measurements.

(b) Relative (positive or negative) observed differences (\( D_1 = A1 - A2 \), or \( A1 - B1 \), or \( C1 - C2 \) for IOR, BOR and LTR, respectively) between values within each pair were averaged. The mean relative observed difference (\( D_{\text{mean}} \)) was compared with zero to detect if the nature of the series influenced the value of the measurement (e.g. if values obtained by one
Table 2. Equations of regression lines, correlation coefficients and corresponding \( P \) values for IOR, BOR and LTR and values of differences \( (D_m) \) with their 95% CIs

<table>
<thead>
<tr>
<th>Equation</th>
<th>( r )</th>
<th>( P )</th>
<th>( D_m \pm 95% \text{ CI} )</th>
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<tbody>
<tr>
<td>IOR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MV ( y = -1.25 + 1.02x )</td>
<td>0.902</td>
<td>&lt; 0.001</td>
<td>+0.067 \pm 1.76</td>
</tr>
<tr>
<td>RI ( y = +3.99 - 0.91x )</td>
<td>0.926</td>
<td>&lt; 0.001</td>
<td>+0.630 \pm 0.90</td>
</tr>
<tr>
<td>BOR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MV ( y = +1.37 + 0.98x )</td>
<td>0.908</td>
<td>&lt; 0.001</td>
<td>+0.470 \pm 1.67</td>
</tr>
<tr>
<td>RI ( y = +0.90 + 0.98x )</td>
<td>0.898</td>
<td>&lt; 0.001</td>
<td>+0.100 \pm 1.14</td>
</tr>
<tr>
<td>LTR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MV ( y = +11.1 - 0.81x )</td>
<td>0.951</td>
<td>&lt; 0.001</td>
<td>+0.793 \pm 3.34</td>
</tr>
<tr>
<td>RI ( y = +5.29 + 0.92x )</td>
<td>0.762</td>
<td>&lt; 0.01</td>
<td>+0.657 \pm 3.56</td>
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</table>

of the observers were significantly different from the values obtained by the other one.

(c) The relative differences within each pair of measures \( (D_i) \) were plotted against the mean of the pair [i.e. \((A_1 + A_2)/2, (A_1 + B_1)/2, (C_1 + C_2)/2\) for IOR, BOR and LTR, respectively] to make sure that no obvious relation appeared between the estimated value (mean) and \( D_i \).

(d) Finally, the repeatability coefficient (RC) was calculated as defined by the British Standards Institution [10], i.e. according to the formula:

\[
RC = \sqrt{\left(\frac{\sum D_i^2}{n}\right)}
\]

where \( n \) is the sample size. This coefficient is the SD of the estimated difference between two repeated measurements. The 95% confidence interval (CI) of the expected difference was calculated as \( \pm 1.96 \) RC. In other terms, repeated measurements are expected to differ by more than RC with a probability of only 5%.

In study 3, repeated measures analysis of variance was performed to investigate a time effect on parameters assessed by the transcranial Doppler technique.

RESULTS

Basal values of MV and RI in our population of healthy subjects \((n=44)\) at 08.00 hours were 61.2 \( \pm \) 10.2 cm/s and 53.6 \( \pm \) 5.9%, respectively.

Repeatability of transcranial Doppler technique measurements (studies 1 and 2)

Table 2 summarizes for MV and RI the equations of the regression lines of \( A_2 \) versus \( A_1 \) (IOR), \( B_1 \) versus \( A_1 \) (BOR) and \( C_2 \) versus \( C_1 \) (LTR) as well as the corresponding correlation coefficients and the mean values of the \( D_m \) differences with their 95% CIs. As can be seen, all correlation coefficients were significant.

Figs. 1 and 2 show for MV and RI, respectively, the plots of the \( D_i \) differences within pairs against means of pairs under the same conditions. As can be seen, none of the mean relative differences \( D_m \) was significantly different from zero, and there was never any obvious relationship between the \( D_i \) differences and the measurement's level. Thus, these results allowed us to calculate the RCs and the 95% CI of the expected difference which are listed in Table 3.

Spontaneous evolution with time of parameters assessed by the transcranial Doppler technique (study 3)

Analysis of variance demonstrated a significant time effect for MV (Fig. 3), which exhibited a maximal decrease at 11.00 hours. No time effect was detected for RI. Regarding MAP, the evolution with time was similar to that observed for MV, but statistical analysis did not demonstrate any significant time effect.

DISCUSSION

The available techniques for cerebral haemodynamic measurements such as oxygen arterio-
Table 3. RCs and 95% CIs of expected differences for both MV and RI. Values at 08.00 hours in our population of 44 subjects were 61 ± 10.2 cm/s and 53.6 ± 5.9% (means ± SD) for MV and RI, respectively.

<table>
<thead>
<tr>
<th></th>
<th>RC</th>
<th>95% CI of expected error</th>
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<tbody>
<tr>
<td>IOR</td>
<td>MV</td>
<td>4.83 ± 9.66 cm/s</td>
</tr>
<tr>
<td></td>
<td>RI</td>
<td>2.62 ± 5.24%</td>
</tr>
<tr>
<td>BOR</td>
<td>MV</td>
<td>4.59 ± 9.18 cm/s</td>
</tr>
<tr>
<td></td>
<td>RI</td>
<td>3.12 ± 6.25%</td>
</tr>
<tr>
<td>LTR</td>
<td>MV</td>
<td>3.32 ± 6.64 cm/s</td>
</tr>
<tr>
<td></td>
<td>RI</td>
<td>3.49 ± 6.98%</td>
</tr>
</tbody>
</table>

Fig. 2. RI plots of differences within a pair (D) against means of the pairs for (a) IOR, (b) BOR and (c) LTR. Broken lines and stippled areas symbolize the mean differences (D̄) and their 95% CIs, respectively.

Fig. 3. Spontaneous evolution with time of middle cerebral artery, RI and mean arterial pressure. Time effect: for MV, P < 0.001, for RI and MAP, not significant.

Repeatability of transcranial Doppler measurements (studies 1 and 2)

One theoretical disadvantage of the transcranial Doppler technique is that a possible change in the θ angle during repeated measurements may be prejudicial to the repeatability. However, the use of a part directly directed to the temporal bone, it is easy to bring the ultrasound beam into alignment with the middle cerebral artery axis. Hence, given the Doppler equation, one can accurately determine the actual blood flow velocity inasmuch as the incidence angle θ between the ultrasound beam and the middle cerebral artery axis being nil, cosine θ equals 1.

It is beyond the scope of this work to justify the use of MV and RI in investigations of cerebral haemodynamics as this issue has already been extensively discussed in previous reports [10, 11]. Hereafter, we will rather focus the discussion on the repeatability of transcranial Doppler measurements and on the spontaneous evolution with time of middle cerebral artery MV and RI.

venous difference [5] and 133Xe clearance (single-photo-emission computerized tomography) [6], have been applied to clinical pharmacology studies, but they are either invasive or involve the use of radioactivity, and hence are not suitable for multiple measurements. In contrast, the transcranial Doppler technique is a newly developed method that allows instantaneous measurements of blood flow velocity in large intracranial arteries, and especially in the middle cerebral artery. The latter being in its initial
Transcranial Doppler repeatability

specially designed headset and of a standardized methodology should provide a better agreement between repeated measurements.

In this study, our scope was to investigate in healthy subjects the repeatability of middle cerebral artery MV measurements performed using the transcranial Doppler technique. Healthy subjects frequently participate in clinical pharmacology studies in which measurements are usually performed at different time intervals over a 24-hour period after a single administration of a drug and, in controlled studies, after a wash-out period not usually exceeding 1 week. This was the reason why our LTR measurements were performed at a 1-week interval.

In the literature [7], there has been one attempt to evaluate the reproducibility of transcranial Doppler measurements in 15 healthy subjects. However, the tools used (correlation studies, calculation of coefficients of variation) may be questioned inasmuch as (a) correlation coefficients test whether there is a linear relationship between two variables and measure the strength of this relationship, but do not give any quantitative information on the error; and (b) coefficients of variation (CV, in per cent) calculated as

\[ CV = \frac{SD}{M} \times 100 \]

(where SD is the standard deviation of the measurements and M is the mean in one series) depend on the value of M and can be applied only to studies in which errors depend upon the measurements’ level, whereas there is usually no relationship between error and measured value.

As a consequence, in our study we have used another approach to investigate the repeatability of transcranial Doppler measurements, i.e. the calculation of RCs [9]. The difference within a pair of measurements can be considered as depending on a probability law where random and experimental conditions are the only causes of variability. If the two series of measurements can be assumed as equivalent (i.e. the two observers, the two different times of measurement), the mean error will be nil. If the differences are normally distributed, 95% of the differences will lie between \(-1.96\)RC and \(+1.96\)RC. This RC gives an estimation of the theoretically expected difference between two measurements. It is expressed in the same units as the investigated parameter and can be compared with both normal values and expected variations.

Our results indicate that, for both MV and RI, the repeatability of transcranial Doppler measurements allows repeated measurements in clinical studies. For example, there is a probability of only 5% for two measurements of MV performed by the same observer, using the previously described methodology, to differ by more than 9.66 cm/s (Table 3). In other terms, if such a difference or a greater one occurs, it is unlikely that it is due to the intra-observer variability and it can reasonably be imputed to other factors (e.g. a drug or a physiological stimulus). The same 5% probability for the mean difference in a sample of \(n\) differences obtained after \(n\) paired measurements corresponds to a reduced CI obtained by dividing the first CI (calculated for one difference) by \(\sqrt{n}\).

The knowledge of a technique’s repeatability allows us to interpret experimental results. Recent reports have investigated the influence of several stimuli on MV or RI. During cognitive tasks right middle cerebral artery MV was reported to increase significantly by \(7.3 \pm 1.1\) cm/s in 19 subjects [12].

Given our data (as \(9.66/\sqrt{19} = 2.22\)), this increase is clearly not related to intra-observer variability.

During a tilt-test in 30 patients with recurrent unexplained syncope [13], a fall of \(26 \pm 9.5\) cm/s in MV and a rise of \(34.8 \pm 11.3\%\) (absolute values) in RI were observed. Such wide changes are far beyond the limits of intra-observer repeatability as assessed in our experiment (9.66/\(\sqrt{30} = 1.76\) for MV and 5.24/\(\sqrt{30} = 0.96\) for RI).

We expected IOR to be better than both BOR and LTR. And indeed, the fact that for the determination of BOR and LTR the headset had been removed between the two measurements, could have led to variations of the site of observation (i.e. of the \(\theta\) angle). But surprisingly, in our study, the fixity of the probe during IOR determination did not produce a better repeatability. This is probably due to the fact that middle cerebral artery blood flow measurement was performed only when the middle cerebral artery, internal carotid artery and anterior cerebral artery could be detected at different depths on the same axis, i.e. when the variations of the \(\theta\) observation angle were reduced to the minimum.

**Spontaneous evolution of parameters assessed by the transcranial Doppler technique (study 3)**

As can be seen from our data, MV values varied with time. These changes (a) occurred even though the measurements were always performed by the same investigator and under the same experimental conditions, (b) were observed in all subjects with the same kinetics, and (c) were large enough to exceed the variability of the measurement technique. Thus, in our group of 14 subjects, the mean variations in MV due to intra-observer repeatability are expected to be less than \(9.66/\sqrt{14} = 2.6\) cm/s. Hence, the maximal observed mean difference which occurred at 11.00 hours and amounted to 6.5 cm/s (95% CI = 3.5 to 8.6 cm/s) was most likely not due to intra-observer variations.

It should be noted that the changes observed in MV occurred together with variations in arterial blood pressure, but there is probably no link between these two phenomena as cerebral blood flow remains constant within a wide range of blood pressure values (autoregulation). These findings could indicate diurnal variation in MV, but further experiments are necessary to confirm or invalidate this hypothesis. However, these variations must be borne in mind when organizing an experiment and
choosing the chronology of transcranial Doppler measurements.

In conclusion, the knowledge of RCs allows the investigator to calculate the number of subjects or patients required, and to compare an observed difference (if statistically significant) with the repeatability of the method. It appears from our data that transcranial Doppler measurements of both MV and RI of the middle cerebral artery are sufficiently repeatable to allow comparison of results obtained from repeated investigations over a period of up to 1 week in healthy subjects. In addition, MV measurements can be compared only if they are performed at the same hour or compared with a control evolution because of spontaneous diurnal variations.

REFERENCES