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On the number of measurement sites required to assess regional cerebral blood flow by laser-Doppler scanning during cerebral ischemia and reperfusion

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Abstract

The aim of this study was to determine whether the number of measurement sites affected the precision of regional cerebral blood flow (CBF) assessment by Laser-Doppler (LD). A simulation study was applied based on data obtained by scanning the cortex in 25 rats during baseline conditions, 15 min global cerebral ischemia and reperfusion. Random samples were repeatedly collected from 1 to 100 locations and deviations from the median of the entire CBF data pool (800 locations) were determined. Single location CBF measurements missed the true median by 24.8 ± 2.2 LD-units (baseline conditions, $n = 100$ simulations, mean $\pm$ SEM), 2.7 ± 0.6 LD-units (ischemia), and 31.9 ± 2.4 LD-units (30th min reperfusion), which can be reduced to 10.9 ± 1.0 LD-units (baseline), 0.9 ± 0.1 LD-units (ischemia), and 15.5 ± 1.3 LD-units (30th min reperfusion) by scanning ten locations. Reliability is further improved by scanning 30 sites with deviations of 6.1 ± 0.6 LD-units (baseline), 0.4 ± 0.0 LD-units (ischemia), and 8.9 ± 0.7 LD-units (30th min reperfusion). Single location CBF assessment was sufficient during global ischemia only. In order to keep the deviation from the true flow below 10 LD-units, at least 15 locations are recommended during baseline conditions and 25 during reperfusion. Laser-Doppler scanning improves the reliability and reduces the variability of CBF measurements.

Keywords: Cerebral blood flow; Global cerebral ischemia; Laser-Doppler scanning; Rats; Simulation study

1. Introduction

Since Laser-Doppler flowmetry (LDF) provides non-invasive, instantaneous and continuous measurement of microcirculatory blood flow (Dirnagl et al., 1989; Öberg, 1990; Kempski et al., 1995), it has become a widely used technique. When applied to the cerebral cortex, it assesses local rather than regional blood flow, due to its high spatial resolution of approximately 1 mm^3. Such LDF measurements vary significantly even at adjacent sites (Dirnagl et al., 1989), since the cerebral cortex shows substantial spatial heterogeneity of perfusion. Scanning techniques have been introduced considering that multiple location measurements would reflect regional tissue perfusion more precisely than single point assessment. Indeed, Laser-Doppler scanning permits estimation of *regional* cerebral blood flow (CBF) from *local* measurements (Ungersböck et al., 1993a,b; Heimann et al., 1994).

Up to now, it remains uncertain as to how many locations are required to obtain a certain precision standard. Previous studies have shown that a minimum of four to six measurements are required for human skin, gastric mucosa and pig kidney for an acceptable precision estimate (Line et al., 1992), whereas at least 15 sites should be scanned in rat diaphragm, even when locations over larger vessels were excluded (Chang et al., 1997). Sample sizes above 25 have been recommended in rabbit cerebral microcirculation (Kempski et al., 1995). Those studies were conducted under physiological conditions, whereas only little is known about the precision of LDF under pathological circumstances, such as ischemia.

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Therefore, the current study was performed to determine the number of measurements necessary to achieve sufficient reliability during baseline conditions, global cerebral ischemia and reperfusion. To do so, a simulation technique (Kempski et al., 1995) was applied based on raw data from 25 animals: regional CBF was calculated as the median of a given number of 1–100 measurements and compared with its deviation from the 'true' regional CBF, i.e. the median of the total data pool.

2. Methods

The study was conducted according to the current animal protection legislation and was reviewed by the regional ethics committee (AZ 177-07/931-20). Twenty-five male Wistar rats (250–370 g) were anaesthetized by intraperitoneal injection of chloral hydrate (360 mg/kg bw), orally intubated and ventilated throughout the entire experiment. Body temperature was maintained at 37 °C. Both common carotid arteries were exposed, the left was looped with a loose thread for later ligation during ischemia, whereas the right was catheterised for continuous arterial blood pressure monitoring and blood gas analysis (Soehle et al., 1998). The head was fixed in a stereotactic frame and a 5×3 mm large cranial window was drilled, centered 4 mm lateral and 4 mm caudal to the bregma. The dura was left intact. The lower body portion of the animals was placed in a sealable chamber, connected to an electronically controlled vacuum pump for later induction of hypobaric hypotension (Dirnagl et al., 1993; Heimann et al., 1994; Soehle et al., 1998). To do so, the barometric pressure within the chamber could be reduced to -30 cm H_2O (-2.9 kPa), thereby causing a pooling of venous blood in the lower body portion of the rat.

Local cortical blood flow (lCBF) was measured using a laser flow blood perfusion monitor (model BPM 403a, TSI, St. Paul, MN) with a 0.8 mm needle probe. lCBF is expressed in 'LD units' since the calibration of Laser-Doppler's (LD) to absolute flow units remains controversial. A 15 min baseline period was followed by 15 min of global cerebral ischemia, as performed by temporary bilateral occlusion of the common carotid arteries and MABP-reduction below the lower autoregulation threshold to 42 mmHg using hypobaric hypotension. lCBF was sequentially measured at 32 (8×4) parietal cortical locations 300 μm apart using a computer-controlled micromanipulator scanning technique. Data were sampled with a running average for 8 s (i.e. approximately ten breathing cycles) and transferred to a PC for storage and analysis. Scans were performed at baseline conditions, ischemia and reperfusion (1st, 10th, 20th, 30th, 45th, 60th, 75th min). One scan took 4 min.

Data from the 32 individual sites in 25 rats were pooled and considered as independent samples representing the possible range of data to be encountered under physiological baseline conditions in the parietal cortex of rats in chloral hydrate anaesthesia. The CBF data from individual sites are *local* measurements, whereas the median CBF characterizes *regional* blood flow. Those medians of the entire data pool are termed 'true medians' in the following. In order to answer the question whether N measurements would have been sufficient to reliably assess regional CBF, a simulation study was applied (modified from Kempski et al., 1995). The deviation between the median of N data and the median of the entire data pool (true median) was taken as a measure of sufficiency. It was calculated by a computer program written in Visual Basic (Microsoft) as follows: N samples were randomly drawn from the entire data pool, simulating N CBF measurements in one animal. Repetitive drawings from the same location were prohibited. The median of the N data, as well as its deviation from the true median were calculated. To achieve more reliable data, the random drawing of N data was repeated 100 times, simulating the scanning of N locations in 100 animals. The mean deviation of those 100 simulated medians from the true median gives the required measure and is displayed in Figs. 1 and 2, as well as in the results. The described algorithm was used for sample sizes between $N = 1$ and $N = 100$ and for different times (baseline, ischemia and reperfusion).

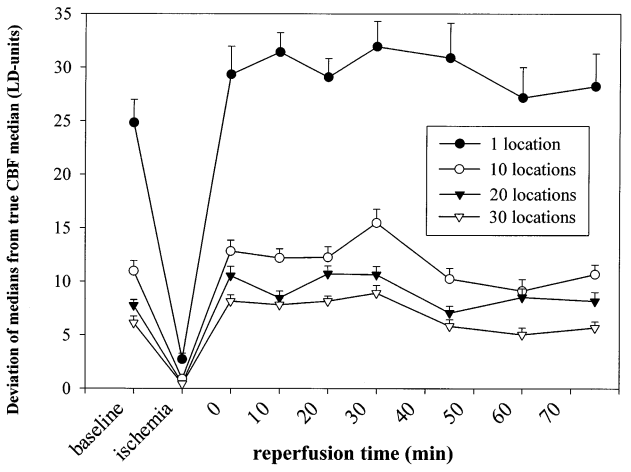

Fig. 1. Deviation of simulated medians ($n = 100$ simulations, data = mean ± SEM) from the 'true' regional CBF median during baseline conditions, 15 min global cerebral ischemia and reperfusion. The simulation was performed for scanning 1, 10, 20 or 30 locations, based on 800 CBF data from 25 animals.

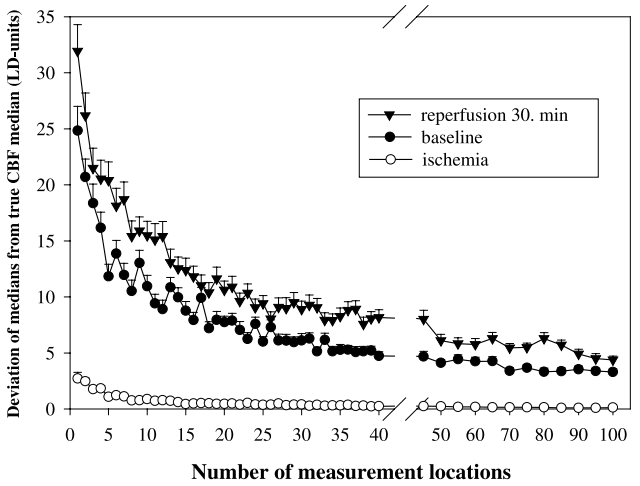

Fig. 2. Simulation with CBF data from 800 measuring sites in 25 rats during baseline conditions (black filled circles), 15 min global cerebral ischemia (non-filled circles) and 30 min reperfusion (black filled triangles). The plots illustrate the deviation of the simulated medians ($n = 100$ simulation, data = mean ± SEM) from the 'true' CBF median with given number of measurement locations, i.e. number of scanning points.

3. Results

The physiological variables of the 25 animals, such as arterial pH, pCO_2 and pO_2 were within the physiological range throughout the experiment. The mean deviation of the simulated medians from the CBF median of the entire data pool (35.6 LD-units, true median) changed during baseline conditions, global cerebral ischemia and reperfusion, as shown in Fig. 1: during baseline conditions, the deviation of CBF medians was 24.8 ± 2.2 LD units (1 location measurement, $n = 100$ simulations, mean ± SEM), 10.9 ± 1.0 LD units (ten locations) and 6.1 ± 0.6 LD units (30 locations). It reached its minimum during ischemia with 2.7 ± 0.6 LD units (one location), 0.9 ± 0.1 LD units (ten locations) and 0.4 ± 0.04 LD units (30 locations). During the first 20 min of reperfusion, the mean deviation was 2–5 LD units elevated as compared to baseline conditions. The maximum mean deviation was observed at 30 min of reperfusion, where values of 31.9 ± 2.4 LD units (one location), 15.5 ± 1.3 LD units (ten locations) and 8.9 ± 0.7 LD units (30 locations) were determined. Therefore the deviation at this time point was approximately one third larger than during baseline conditions. The mean deviation of CBF medians during late reperfusion (60th and 75th min) was almost equal to pre-ischemic values.

The deviation of the simulated from the true medians decreased exponentially with a rising number of measurement locations, as shown in Fig. 2. Deviation from true CBF median during ischemia is small: it is less than 3 LD units no matter how many locations are scanned and can be reduced to less than 1 LD unit by scanning ten locations or more. To achieve a deviation of less than 10 LD units from the true median, at least 15 locations have to be scanned during baseline conditions, and 25 locations during reperfusion, whereas single point measurement is sufficient during global cerebral ischemia only.

Increasing the number of measurement locations from 50 to 100 goes along with a minor decrease of the deviation from true CBF medians, but requires long measurement times and sophisticated technology to do so properly. The steepest section of the deviation curve (Fig. 2) is between 1 and 25 measurement locations. In those ranges, an increasing number of scanning locations results in a marked decrease of the error probability whereas it requires only a moderately longer measuring time.

4. Discussion

In a normally distributed population, the error probability of a given sample size can be calculated using standard statistic procedures. In a non-normal distribution, however, simulation studies have to be applied, especially if the mathematical transformation of the non-normal into a normal distribution is difficult or impossible. Previous studies recommended sample sizes of five–six using statistical methods assuming a Gaussian distribution of LD readings (Line et al., 1992). In contrast, Kempski et al. (1995) in rabbits found, in accordance with our current study, a non-Gaussian distribution and quite remarkable error probabilities of observed median flows as compared to a 'true' median flow. The error probability might be underestimated when assuming a Gaussian distribution.

Therefore we decided to apply a simulation technique similar to that previously described by Kempski et al. (1995). It could be argued that by pooling data sets with varying distributions from 25 animals into one large data pool, the nonhomogeneity of the data pool might be increased, resulting in an overestimation of the acceptable sampling numbers. Chang et al. (1997) applied the same sampling method as used here as well as statistics based on ANOVA. They demonstrated a linear relationship between the results of both methods and concluded that the values of the sampling method were on average minimally larger than those of the ANOVA method. Therefore these authors excluded a vast overestimation of the required sample sizes when using the sampling technique.

As described before in rabbits (Kempski et al., 1995), we also found a wide range of local CBF readings during baseline conditions, and the deviation of simulated medians from the true CBF median decreased with an increasing number of measurement locations. During global cerebral ischemia, CBF was found to be close to zero with only a small range of individual CBF

readings. Therefore, even a single point measurement is sufficient in ischemia and scanning of more locations increases measurement time rather than reliability of scanning. However, during the first 30 min of reperfusion, the deviation of simulated medians was elevated as compared to baseline conditions. This is presumably caused by a much more heterogeneous and on average larger microcirculatory blood flow during reperfusion than under resting conditions.

The number of measurement sites required in LD-scanning depends on the aim of a study. If it is used to confirm ischemic CBF in a global ischemia model, even single point measurements are sufficient. Considering a baseline CBF of 35 LD-units as found with the current experimental setup, a deviation from the true CBF of less than 10 LD-units is acceptable for many applications. This requires scanning of 15 locations during baseline conditions, one during ischemia and 25 during reperfusion (30th min). With less locations and hence higher deviations, phenomena such as postischemic hyper- and hypoperfusion can hardly be assessed reliably. However, if a study is focussed to assess effects of therapeutical interventions on CBF during reperfusion, we recommend the study of 30 to 40 scanning locations. By doing so, even subtle rCBF differences between individual animals can be found with predictable error.

The use of a computer controlled stepping motor-driven micromanipulator permits location of the probe of a Laser-Doppler above the same cortical location as in previous scans with a precision of a few micrometers. Hence the reproducibility of repeated scans using the same technique has been found to be excellent (Ungersböck et al., 1993a; Heimann et al., 1994; Kempski et al., 1995). With the given high spatial resolution of Laser-Doppler probes, reliable and sufficient information on tissue perfusion can be obtained, even in tissues with rather heterogeneous perfusion such as the cerebral cortex. Other techniques with a good spatial resolution, such as the iodoantipyrine, the H_2 clearance or the microsphere technique, usually do not permit repeated measurements at a sufficient frequency.

Many authors express locally sampled LD data as a percentage of an initially determined baseline value. Considering the enormous variability of lCBF values encountered in rat cerebral cortex, flow values, expressed in percent, can only be compared if locations with comparable baseline flow are selected. Using the scanning technique, on the other hand, it is just necessary to include enough locations into the scan. Then, the median value of such a scan can be used as a reliable baseline value which will allow for comparisons not only with successive measurements in the same animal but also — more importantly — with data from other animals.

In summary, this is the first study to examine the number of measurements necessary to obtain regional cerebral blood flow under pathophysiological conditions such as cerebral ischemia and reperfusion. We conclude, that the main disadvantage of Laser-Doppler flowmetry, the rather local than regional measurement resulting in unrepresentative data, can be overcome by using a scanning technique. Especially in tissues with a heterogeneous microvasculature, such as the brain, scanning is recommended.

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