Effect of captopril on skin blood flow following intradermal bradykinin measured by laser Doppler flowmetry

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Summary. The effect of captopril on skin response to intradermal injection of bradykinin was investigated by laser Doppler flowmetry (LDF) and weal and flare measurements in this randomised, double-blind, placebo-controlled, cross-over balanced study. Intradermal injections of 1 and 2.5 μg of bradykinin and normal saline were made into the forearm skin of six healthy volunteers between 1 and 2 h (t1) and between 3 and 4 h (t2) after either 25 mg captopril or placebo. Skin blood flow outside the induced weal was monitored continuously by LDF for 15 min and the mean LDF values over the last 15 s were used for analysis. Weal and flare sizes were measured at 15 min.

On the placebo days, the mean LDF output, weal volume and flare area increased with incremental bradykinin dose. Pre-treatment with captopril significantly increased LDF output following intradermal bradykinin at t1 but not at t2. At both t1 and t2, captopril significantly increased weal volume. There was no significant difference between treatments in flare areas. Skin response following intradermal normal saline, measured by the above parameters, was not affected by captopril.

This study showed that captopril potentiated the effects of intradermal bradykinin both with respect to blood flow changes and weal formation. The non-invasive technique of laser Doppler flowmetry (LDF) enables changes in cutaneous blood flow to be measured and has been shown to be a useful and sensitive method for quantifying changes in cutaneous blood flow after intradermal injections of leukotrienes [6] and histamine [7, 8].

This study reports on the use of LDF to quantify changes in cutaneous blood flow following intradermal bradykinin and investigates the effect of captopril on this response and on weal and flare sizes.

Materials and methods

Materials

Captopril 25 mg and matched lactose placebo were prepared in capsules by the Pharmacy Department. Bradykinin (Sigma Chemical Co. Ltd., Poole, UK) was dissolved in 0.9% normal saline to give concentrations of 0.01, 0.025, 0.05 and 0.1 and 0.2 mg/ml. Normal saline acted as control. Intradermal injections of 0.1 ml of the solutions were made using a 1-ml Sibre syringe and a 25 g × 5/8 sterile needle.

Laser Doppler flowmetry

Skin blood flow was recorded with a laser Doppler flowmeter [9]. The essentials of this system are as follows: monochromatic light with a wavelength of 632.8 nm is transmitted from a 2 mW helium-neon laser by an optical fibre to illuminate the skin where the light is scattered and absorbed. Light beams scattered by moving red
cells undergo a frequency shift according to the Doppler effect, while those scattered by static tissues remain unshifted. A portion of the backscattered light is conveyed by a second optical fibre, separated by 1 mm from the transmitting fibre, to a photodetector. A processing circuit then attenuates this input signal to generate an output signal which is proportional to the flow of blood cells (velocity x concentration) in the microvasculature of the superficial skin. The bandwidth of the instrument is 50 Hz to 7 kHz and the output time constant is 0.5 s.

The LDF signal was digitalised at 8 Hz using an IBM PCAT and CED1401 intelligent interface. Mean values for each 15 s period were computed on-line and the data were stored on a floppy disk.

The LDF output is expressed in arbitrary units (a.u.) since there is no standard technique against which the signal may be calibrated and the complexity of the microvasculature precludes a theoretical calculation.

Protocol

In order to select the bradykinin doses for the definitive study, a dose-response curve was first established in 5 normal volunteers, 3 males and 2 females, aged 23 to 33 years (mean 29 years). Solutions of 0.1 ml of 1, 2.5, 5, 10 and 20 μg of bradykinin and normal saline alone were injected intradermally at 20-min intervals into either forearm. Measurement bias was avoided by coding the solutions which were then assigned randomly to marked sites on the flexor surface of the forearms. The injection sites were separated by at least 6 cm and any visible cutaneous veins avoided. For 10 min prior to the injections and for the duration of the blood flow measurements, the subjects were seated with the forearms supported at an angle of 30° so that the hands were at heart level.

Laboratory temperature was maintained at 24 ± 1°C and relative humidity at 30–40%. LDF output was recorded at a fixed distance of 5 mm from each injection site for 2 min prior to and for 15 min after each injection. Dose–response curves were constructed using both the absolute LDF values and the percentage increase relative to baseline for the last 15 s of the recording period.

From the results of this preliminary study, doses of 1 and 2.5 μg of bradykinin were selected to investigate the effect of captopril on bradykinin-induced blood flow changes and weal response. Six healthy volunteers, 3 males and 3 females, aged 22 to 34 years (mean 29 years) were studied. Each received either 25 mg captopril or placebo after a light breakfast on two separate occasions, at least 1 week apart, in a randomized, double-blind, placebo-controlled, cross-over balanced study which was approved by the local hospital Ethics Committee. Solutions of 0.1 ml of 1 and 2.5 μg bradykinin and normal saline were injected intradermally at 20-min intervals into either forearm between 1 and 2 h (t1) and between 3 and 4 h (t2) after the medication. The same method described above was used to measure the skin blood flow changes following intradermal bradykinin. In addition weal and flare outlines were traced onto transparent sheets at 15 min and areas were then measured from the tracings by computerized planimetry (Hewlett Packard). Skin thickness was measured with modified skin calipers before and 15 min after each injection and weal thickness was calculated from these measurements by a method described by Cook and Shuster [10]. Weal volume was obtained by multiplying weal thickness by weal area.

Using these techniques, the within-day coefficients of variation of the absolute LDF output, flare area, and weal volume following intradermal injection of 2.5 μg bradykinin in one subject were previously found to be 23%, 43% and 54% respectively. The between-day coefficients of variation were 19%, 25% and 51% respectively.

Statistical evaluation

Multiple linear regression analysis with subjects, concentrations of bradykinin and treatments as independent variables employing the dummy variable technique, was used to assess the effect of treatment on LDF output, weal and flare sizes at t1 and t2. Two-way ANOVA was used to identify any significant difference between treatments following intradermal normal saline which acted as control.

Results

Bradykinin dose-response study

The skin blood flow measured by LDF increased rapidly during the first two minutes following intradermal bradykinin and peaked between 1.5 and 3 min (Fig. 1). The peak LDF values did not allow clear differentiation between the bradykinin doses. However there was a bradykinin dose-related increase in LDF output when the data at 15 min were used. The LDF output fell with the 20-μg dose. Dose–response curves were similar irrespective of whether the values were expressed in absolute terms or as a percentage increase (Fig. 2). Consequently, only the absolute LDF values are presented in the subsequent study.