Brief Communication

Free radical injury for *in vivo* induction of platelet aggregation. *A model in microcirculatory studies of the brain*

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Blood supply to tissues is very important, as it carries oxygen and nutrients needed for normal biological processes in cells. Exchange between the blood and tissue takes place at the microcirculatory level. The brain is very sensitive to low oxygen and glucose, and therefore requires sufficient blood flow to its cells. Formation of clots (thrombi) in brain vessels reduces or blocks blood flow with serious adverse effects. Circulating platelets do not normally adhere to the endothelial cells of the vascular wall, or aggregate, except when activated or when damage to the endothelium is attained. Inducing platelet aggregation in an experimental model would allow the study of blood flow and for the study of factors that influence tissue perfusion. The objectives of this work were to devise a method in which blood clots are experimentally-formed by a free radical injury, to apply this method in experiments on brain circulation in live animals, and to explore other possible applications in different tissues and settings.

Animals and methods. This method used laboratory animals such as mice, rats, and cats. The free radical is generated by direct exposure of vessels, in which a dye (for example, sodium fluorescein) is injected and circulates in the blood, to intense mercury light. This produces a singlet oxygen free radical, which causes injury to the endothelial lining of the blood vessel in live laboratory animals. In response, platelets begin to adhere to the site(s) of endothelial injury and then aggregate. The times for both the first observed platelet aggregate and for platelets to grow and block the vessel are measured in seconds by several stop watches and all observations are also recorded on video tapes, for further viewing and analysis.

Experimental protocol. Mice, for example, are anesthetized with urethane (1-2 mg/g body weight, intraperitoneally) and the trachea is intubated. A craniotomy (3 x 3 mm) is performed on the left side, using a micro-drill, and the dura mater is stripped open. Only untraumatized preparations are used and those showing trauma to either microvessels or underlying brain tissue are discarded. The mouse is then placed on the stage of a microscope (Olympus-BH2, fluorescence) to which a video camera and a recorder are attached (**Figure 1**). A heating mat is placed under the mouse and body temperature is raised to 37°C (within 30 minutes), as monitored by a rectal thermoprobe (Physitemp, model RET-3, Clifton, NJ, USA) connected to a temperature reader (Physitemp, Thermalert model TH-5, Clifton, NJ, USA). The cranial preparation is moistened continuously with artificial cerebrospinal fluid (ACSF) of the following composition (mM): sodium chloride 124, potassium chloride 5, sodium phosphate 3, calcium chloride 2.5, magnesium sulphate 2.4, sodium bicarbonate 23, and glucose 10; pH 7.3. A sub-field containing arterioles and venules of 25-50 microns in diameter is chosen. The entire intravital microscope-TV set up and the ACSF delivery and warming and body temperature controlling are shown in detail in **Figure 1**. Microvascular sub-fields are videotaped prior to

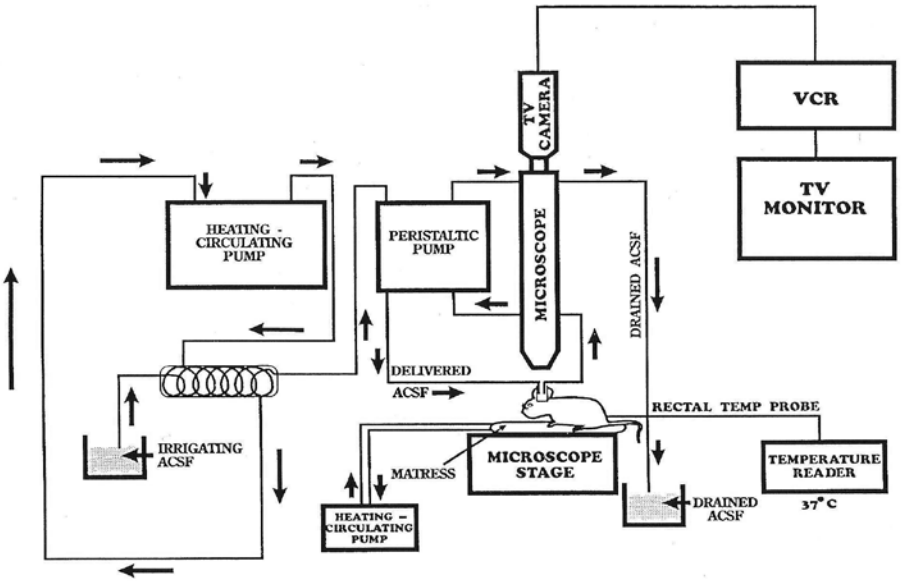

Figure 1 - Schematic presentation of the intravital microscope-TV set-up, the artificial cerebrospinal fluid and body temperature-controlling systems. VCR - video cassette recorder, ACSF - artificial cerebrospinal fluid

and during the photochemical insult. All microscopic observations were made using a 4x objective lens and a 10x eyepiece.

Treatment and vehicle solutions. Treatment and vehicle solutions are prepared fresh daily. All solutions have a pH of approximately 7.3. The vehicle solution is that of sodium bicarbonate in distilled water (or normal saline). Injection of solutions is intraperitoneally 60 minutes before the intended *in vivo* induction of platelet aggregation procedure (exposure to light).

Induction of photothrombosis in vivo. The photochemical insult, photothrombosis, is carried out by injecting sodium fluorescein (0.1 ml/25 g body weight of a 5% solution) via the tail vein, which is allowed to circulate for 30-40 seconds. The cranial preparation is then exposed to a stabilized and intense (44000 lux candle/cm^2) mercury light. The combination of light and dye produces a free radical, which injures the endothelium of the microvascular lumen. This, in turn, causes platelets to adhere at the site(s) of endothelial damage and then aggregate. Platelet aggregates grow in size until complete vascular occlusion occurs. The time when the first observable aggregate appeared (time to first aggregate) and until full vascular occlusion (time to flow stop) in the chosen arteriole and venule are all measured in seconds with 4 stop watches. The time of aggregate growth was calculated, as the difference between the time to flow stop and the time to first aggregate. Vessel diameters before insult and immediately after full vascular occlusion were measured from video recordings and changes were calculated.

Applications of the method. This model involves the induction of *in vivo* platelet aggregation (thrombus formation) in brain microvessels of laboratory animals. This *in vivo* animal model has the advantage over the *in vitro* method, as all biological interacting factors are present. Thus, influences on platelet aggregation (whether anti- or pro-aggregatory) can be studied under well-controlled experimental conditions, which may involve: medications, natural products, medicinal plants, and environmental and nutritional factors. This method has been utilized as an *in vivo* model for stroke,[1-4] and to induce synaptic dysfunction in neuronal tissue, *in vitro*.[5] Other studies involving blood flow in the microcirculation, such as microvascular permeability, and vasoactivity, can utilize this method.

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