Cutaneous Blood Flow Measurements: A Standardization of the Microsphere Assay for Vasoactive Agents¹)

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Abstract

Other studies have shown that the number of isotopically labelled microspheres localized in a region, following injection into the left heart, is a function of the relative blood flow to that region. The present studies show that the number of 10 μ size 113Sn-labelled microspheres impacted in skin and various organs of rabbits under urethane anesthesia is directly proportional to the number of injected, over a wide range, with no evidence of saturation of the microcirculatory bed. At the lowest dose tested, there were 300 microspheres per skin site and at the highest dose an equal-sized skin site contained 15,000 microspheres. Saline-injected test sites assayed 45 min after injection were not significantly different from uninjected sites. It was found that a standardized 177 mm² area of skin received 0.023 ± 0.001% of the cardiac output. To assay various agents for their effect on dermal blood flow and to determine the time course of the effects, it was convenient to express the radioactivity of the test site as a ratio relative to the average radioactivity of uninjected skin sites. Standard errors less than 10% of the mean could be obtained when 13 × 10⁶ microspheres were injected into the left heart via a catheter in the carotid artery, providing 4 to 6 replicate test sites were averaged. It was possible to directly compare lesions from groups of animals. Enhanced blood flow was produced by the injection of histamine and bradykinin. The effect was transient and subsided by 20 min. Prostaglandin E₂ was the most potent mediator tested and its action lasted between 1-2 h. Casein, calcium pyrophosphate, carrageenan, endotoxin and glycogen were injected and their effect assayed at the arbitrary time of 100 min. Casein produced a 3-fold enhancement of blood flow. Calcium pyrophosphate gave a positive but mild (less than 2-fold) effect. The other agents had no effect. The intradermal injection of adrenaline significantly reduced the blood flow to normal skin, but this was overcome by the intradermal injection of casein, bradykinin and PGE₂. This assay has potential application to study the kinetics and the inhibition of this fundamental component of the inflammatory response.

Introduction

Conventional techniques of blood flow measurements include plethysmography, dye dilution, xenon clearance, and the use of radioactive microspheres. The microsphere technique has been used for organ studies and also for the fractionation of the cardiac output [1]. Other studies have involved the quantitation of blood flow to inflammatory lesions [2]. This study is concerned with a better standardization of the assay, as applied to cutaneous blood flow measurements. The workable range of the assay, the reproducibility, treatment of the data, and applications to studies on mediators of inflammation, inflammatory stimuli and pharmacological inhibitors are considered.

Materials and methods

Animals

Female, New Zealand white rabbits, 2.5 to 3 kg, were used.

Microsphere distribution

The back of each rabbit was shaved at least 1 h prior to injection and the sites to be injected were dotted with a marker pen. One rabbit's back can accommodate approximately 36 intradermal injection sites (6 rows of 6 sites). The animals were anesthetized with urethane (BDH Chemicals, Poole, England) and the carotid artery was cannulated, as described previously [2].

Volumes of 0.2 ml of 5 μg/ml solution of PGE₂ (Upjohn Co. of Canada, Don Mills, Ont.) were injected per site, 12 sites per animal; a similar volume of sterile physiological saline was injected into another 12 sites per animal,

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with the remaining 12 sites serving as controls. All injection sites were randomized on each animal's back. Various doses of $^{113}$Sn-labelled microspheres were infused via the carotid artery exactly 45 min after injection of the prostaglandin E$_1$. The microspheres (Nen-Trac microspheres, New England Nuclear, Boston, Mass.) were of $10.5 \pm 1.1 \text{ mm}$ size, suspended in 10% dextran. The specific activity was 10.31 mCi/g and 0.599 c.p.m./sphere, as determined by counting on a hemocytometer and gamma counter. The animals were sacrificed with an overdose of nembutal and the back skins removed. Injection sites were punched out using a 15.9 mm diameter cork borer, and the radioactivity localized in the skin sites was determined on a CG-30 Intertechnique gamma spectrometer. Spleen, kidneys, liver, heart and lungs were excised and the radioactivity in these organs determined.

In all subsequent procedures, only the kidneys and spleen were removed and the radioactivity per organ counted.

**Early hyperemia time course**

The rabbits were anesthetized and cannulated as described above. Solutions of 5 $\mu$g/ml of histamine (Fisher Scientific, Fairlawn, N.J.) and bradykinin (courtesy of Dr H.Z. Movat) were injected in volumes of 0.2 ml at 20 min, 10 min, 5 min and 30 sec before infusion of microspheres. Similar time course studies were performed using normal saline, 0.02 $\mu$g/site adrenalin chloride (Parke Davis Co., Brockville, Ont.) and experimentally produced scratches, 5 mm in length. These were sufficiently deep to draw blood. All animals were given 1.3 $\times$ 10$^7$ microspheres via the carotid catheter and the injection sites were processed as described above.

**Acute inflammation**

Various agents known to induce acute inflammation when administered intraperitoneally, intraleurally, or intrasynovially, were assayed for their ability to induce acute alterations in dermal blood flow. Volumes of 0.2 ml each of 1% sodium caseinate (Eastman Kodak Co., Rochester, N.Y.), 250 $\mu$g/ml endotoxin (lipopolysaccharide W – E. coli, Difco Laboratories, Detroit, Michigan), 1% viscarin carrageenan (Marine Colloids, Springfield, N.J.), 500 $\mu$g/ml purified protein derivative (Connaught Laboratories, Toronto, Ont.), 1% glycogen (Fisher Scientific Co., Fairlawn, N.J.) and 1% calcium pyrophosphate (ICN – K & N Laboratories Inc., Plainview, N.Y.) were injected into 36 sites (12 sites in each of 3 rabbits). The animals were injected with microspheres 100 min after injection of the sample.

**Chronic inflammation following injection**

Three rabbits were injected with 0.1 ml of Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan) in 4 sites at 336 h, 264 h, 96 h and 24 h prior to administration of microspheres. The lesions were punched out with an 18 mm cork borer.

**Inhibition studies**

Groups of animals (3 rabbits per group) were pretreated with various agents known to attenuate the classical signs of acute inflammation. The animals were subsequently injected with casein, histamine, bradykinin and prostaglandin E$_1$ in the dosages stated above.

The first group of rabbits was treated with the anti-histamine triprolidine-HCl (Actidil – Burroughs Wellcome and Co., Sydney, Australia) 30 min prior to injections. The triprolidine (1 mg/kg body weight) was injected with 2.0 ml of 2% Evans' blue intravenously. Histamine and casein were injected 100 min prior to administration of 1.34 $\times$ 10$^7$ microspheres, PGE$_1$ at 30 min and bradykinin at 5 min.

The second group of rabbits was treated with indomethacin, as described by CHAHL and CHAHL [3]. Indomethacin (Sigma Chemical Co., St. Louis, Mo.) was administered in a dose of 20 mg/kg i.p., 45 min prior to injections. The temporal sequence of injections was identical to that described for triprolidine except that the histamine was injected 5 min before administration of microspheres.

The third group of rabbits was pretreated with 0.2 mg/kg dexamethasone sodium phosphate (Decadron-Merck Sharp and Dohme Canada Ltd., Kirkland, Que.) given i.v. 3 h prior to injections. The sequence of injections was identical to that described for the indomethacin-treated group.

**Results**

When the total number of microspheres injected into the heart is known, the proportion trapped in any organ or tissue can be expressed as a percentage of the cardiac output. Table 1 compares the organ distribution of 10 $\mu$m particles with values obtained previously using 15 $\mu$m particles. The results indicate comparable distribution of the smaller microspheres in the major organs. The values given represent the mean $\pm$ the standard error from a total of 20 animals.

**Table 1**

Comparison of the distribution of 10 $\mu$m and 15 $\mu$m microspheres as a percentage of total cardiac output.

<table>
<thead>
<tr>
<th>Organ</th>
<th>10 $\mu$m Microspheres</th>
<th>15 $\mu$m Microspheresa</th>
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<tbody>
<tr>
<td>Spleen</td>
<td>0.588 $\pm$ 0.183</td>
<td>0.32 $\pm$ 0.11</td>
</tr>
<tr>
<td>Kidneys</td>
<td>14.720 $\pm$ 1.980</td>
<td>13.46 $\pm$ 2.25</td>
</tr>
<tr>
<td>Heart</td>
<td>6.544 $\pm$ 1.711</td>
<td>4.37 $\pm$ 1.65</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.393 $\pm$ 0.068</td>
<td>–</td>
</tr>
<tr>
<td>Lungs</td>
<td>3.216 $\pm$ 0.287</td>
<td>4.99 $\pm$ 1.02</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>0.035 $\pm$ 0.004</td>
<td>–</td>
</tr>
<tr>
<td>Liver</td>
<td>2.141 $\pm$ 0.153</td>
<td>2.79 $\pm$ 0.99</td>
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a From HAY and HOBBS (1977) [8].

The total number of microspheres injected was varied over a 10-fold range to test whether the number trapped in the standardized skin sites was proportional to the total injected. These results are summarized in Figure 1. It was found that 177 mm$^2$ of skin received 0.023 $\pm$ 0.001% of the total number of microspheres injected and that this fraction was a constant proportion in