Assessment of Antiinflammatory Agents Using ^{125}I-Labeled Human Serum Albumin to Quantify Footpad Edema Volume in the Rat

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Intravenously injected human serum albumin, labeled with radioactive iodine ([^{125}I]HSA), accumulates at inflammatory foci in proportion to the volume of the exudate, making it possible to quantify the volume of edema. This paper describes the use of [^{125}I]HSA to measure edema formation in the carrageenin rat-footpad model, both continuously and at a single time point. In assessing antiinflammatory agents, the method was shown to be more sensitive than the most commonly used technique of thickness measurement. Because anesthetics are known to suppress inflammation, the comparative effect of five anesthetic agents on the inflammatory response was determined. Ether was the only anesthetic tested that did not substantially inhibit the accumulation of edema. The technique overcomes many of the limitations of previously used procedures and has the potential to become the method of choice when assessing edema in the rat footpad.

KEY WORDS: carrageenin edema; rat; measurement; radioisotope; antiinflammatory drugs; anesthetics.

INTRODUCTION

The hind footpad of the rat has routinely been used for the assessment of edema induced by a variety of chemical and biological agents (1–4). The edema caused by the subplantar injection of such compounds has provided a model of inflammation which has been used extensively in the assessment of antiinflammatory compounds. Footpad thickness, measured using an engineer's thickness gauge, is the simplest and most commonly used method of assessing edema. However, this measurement provides a one-dimensional estimate of a three-dimensional phenomenon and is prone to subjective operator error. Intravenously administered, radioactively labeled, human serum albumin ([^{125}I]HSA) has been used previously to measure edema in experimentally induced cutaneous inflammation (5–8). However, to the best of our knowledge, [^{125}I]HSA has not been used to quantify changes in footpad volume in response to drug administration. This report details an evaluation of the use of [^{125}I]HSA to measure carrageenin-induced footpad edema and the effects of commonly used anesthetics on the response. Additionally, the ability of the procedure to delineate the edema-reducing properties of antiinflammatory agents was determined.

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MATERIALS AND METHODS

Animals

Twelve-week-old male or female Dark Agouti (DA) rats were used in all experiments.

Carrageenin-Induced Footpad Edema

Inflammatory edema was induced by the subcutaneous injection of 0.1 ml of 2% kappa-carrageenin (Sigma Type III) into the plantar region of each hind footpad (1). All injections and measurements were carried out under anesthesia as described under Results.

Assessment of Edema Using a Thickness Gauge

An engineer's pocket thickness gauge (No. 7308, Mitutoyo Mtg. Co., Japan) was used to measure footpad thickness. The gauge was modified by reducing the spring tension and surface area of the striker. The increase in footpad thickness was expressed as a percentage of the baseline thickness.

Assessment of Edema with ^{125}I-Labeled Human Serum Albumin

Continuous Monitoring of Footpad Edema. Each rat was injected intravenously, into the ventral tail vein, with 1 μCi [^{125}I]HSA (Amersham Australia Ltd, Auckland). After allowing 10 min for the isotope to distribute, a baseline reading of the radioactivity in both feet was obtained. This was accomplished by securing the foot of a lightly anesthetized rat in the well of a dismounted, lead-shielded, solid-crystal scintillation detector (Packard Selektronik) and counting for 1 min. At the time of counting, the terminal 2 mm of the tail was snipped off and a 50-μl blood sample collected in a heparinized microhematocrit tube. Carrageenin was then injected into the footpads and the counts in each foot determined at timed intervals. Blood samples were taken coincident with each measurement. Plasma was separated in a microhematocrit centrifuge, and the radioactivity in 25 μl of plasma determined. The level of [^{125}I]HSA in the plasma decreased linearly with time and a mean half-life of 7.5 hr was calculated. Conversely, edema formation is accumulative. Edema was therefore calculated as follows: a regression line was fitted to the [^{125}I]HSA decay data and the predicted values for the individual time points, over the period of the experiment, were determined. The baseline volume of plasma in the footpad was calculated by dividing the counts per minute (cpm) per foot by the predicted cpm per microliter of plasma. To determine subsequent volumes, the foot counts at each time point were subtracted from the counts for the preceding time point to obtain the change in radioactivity. This value was then divided by the mean of the two predicted plasma counts for the current and previous time points to give the increase or decrease in footpad volume. By adding/subtracting the change in foot volume to/from the previous measurement, the accumulated volume of edema was obtained.

Single-Time Point Measurement of Footpad Edema. At

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the time of footpad injection, each rat was injected intravenously with 1 μCi of [^{125}I]HSA. Four hours later, 2 ml of blood was obtained by heart puncture from anesthetized animals and transferred into a tube containing EDTA. Following euthanasia, the hind feet were removed at the tarsal joint. Blood was separated by centrifugation and 200 μl of plasma diluted in 800 μl of distilled water. The level of radioactivity in each foot and in the plasma samples was then determined.

The volume of the foot is expressed as microliters of plasma equivalents, calculated as follows:

$$\frac{\text{cpm/foot} - \text{background cpm}}{\text{cpm/μl plasma} - \text{background cpm}} \times \text{correction factor (0.7; see Results)}$$

Statistical Evaluation

Results were compared using the Wilcoxon sum of ranks test for nonparametric data.

RESULTS

Continuous Monitoring of Footpad Edema Volume Using [^{125}I]HSA

Time Course. Three DA rats were injected intravenously with 1 μCi of [^{125}I]HSA, followed 10 min later by the subcutaneous injection, into both hind footpads, of 0.1 ml of 2% carrageenin. The volume of the foot was determined just prior to stimulation, 15 and 30 min later, and then at 30-min intervals up to 5 hr. A biphasic response to carrageenin was observed (Fig. 1A). In subsequent single-time point experiments, which were designed to assess the method as a screening assay for antiinflammatory drugs, edema was measured at 4 hr. Because plasma radioactivity decreased with time, data obtained by simply dividing the counts in the foot at 4 hr by the plasma count at 4 hr would have given erroneously high values. A correction factor, based on the difference between the actual volume determined as above and the footpad volume calculated using the 4-hr values, was therefore applied. In practice the corrected result was obtained by multiplying the 4-hr single-time point edema volume by 0.7.

Comparative Effect of Different Anesthetics. The effect of ether on the course of inflammation over a 3-hr period was compared with that of four other agents commonly used as small-animal anesthetics. The agents used were chloral hydrate (250 mg/kg i.p.), Nembutal (pentobarbitone sodium, 30 mg/kg i.p.), urethane (1200 mg/kg i.p.) and Innovar-Vet (fentanyl/droperidol, 150 μl/kg i.m.). Thirty animals were divided into five groups of six, one for each anesthetic to be evaluated. All animals were given 1 μCi [^{125}I]HSA i.v. and carrageenin by subplantar injection as in the previous experiment. Footpad volume was determined at baseline (before challenge) and at 30-min intervals thereafter (Fig. 1B). When ether was used as the anesthetic, the profile of edema accumulation followed the biphasic curve shown previously. However, the response in those animals anesthetized with the four parenterally administered agents was almost completely abrogated.

Use of [^{125}I]HSA to Assay Putative Antiinflammatory Agents

Two putative agents (mussel extract and cyclosporin A) were tested in the carrageenin footpad assay, using both the accumulation of radioactive albumin and the increase in footpad thickness to measure edema. Eighteen rats were divided into three groups of six. Individuals in one group were injected i.p. with green-lipped mussel extract (McFarlane Laboratories Ltd., Auckland, New Zealand), at 300 mg/kg, 2 and 17 hr before challenge, while those in another group were treated i.m. with 50 mg/kg cyclosporin A (CsA, Sandoz Pharma Ltd, Auckland, New Zealand), 2 hr and 1, 2, and 3 days before carrageenin injection. The green-lipped mussel extract inhibited edema formation by 80% when volume was employed as the index of edema and by 50% when thickness was used (Fig. 2). In both cases the difference was significant at the $P < 0.01$ level. CsA pretreatment also resulted in a reduction in swelling, but to a lesser degree (24 and 26% for the volume and thickness parameters, respectively). Statistical analysis showed that, when thickness was used as the index of edema, the inhibition of swelling was only marginally significant ($P < 0.05$). However, if the [^{125}I]HSA-determined volume was used the results for CsA were highly significant ($P < 0.01$). Thus, the use of [^{125}I]HSA to assess footpad swelling revealed an antiinflammatory effect which was not obvious when footpad thickness was used as the index of edema.

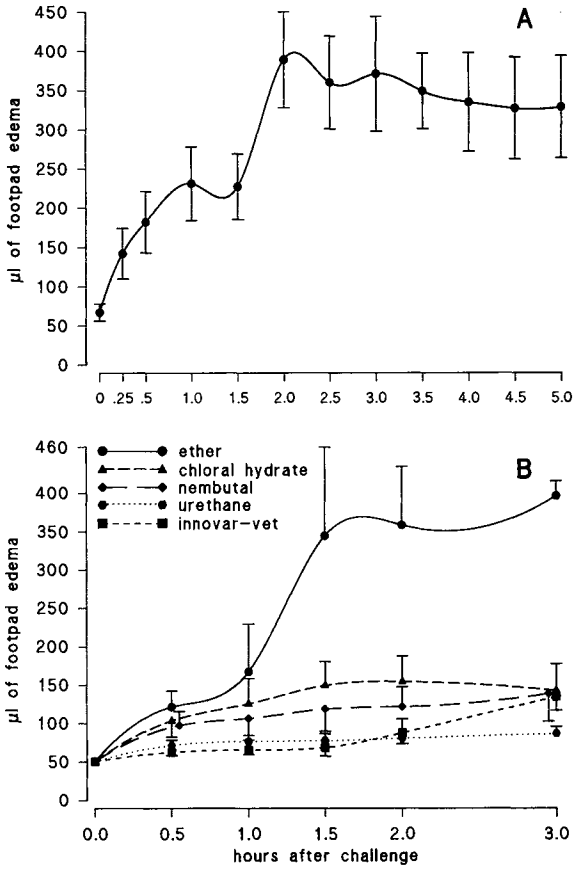

Fig. 1. (A) Accumulation of inflammatory edema in the footpads of rats injected subcutaneously with 0.1 ml of 2% carrageenin. Points are the mean of three rats (six footpads); error bars represent one standard deviation. (B) Comparative effects of five anesthetics on the response to carrageenin in the rat footpad. For each anesthetic, the accumulation was monitored at selected intervals in the same animals. $n = 12$ for each data point.

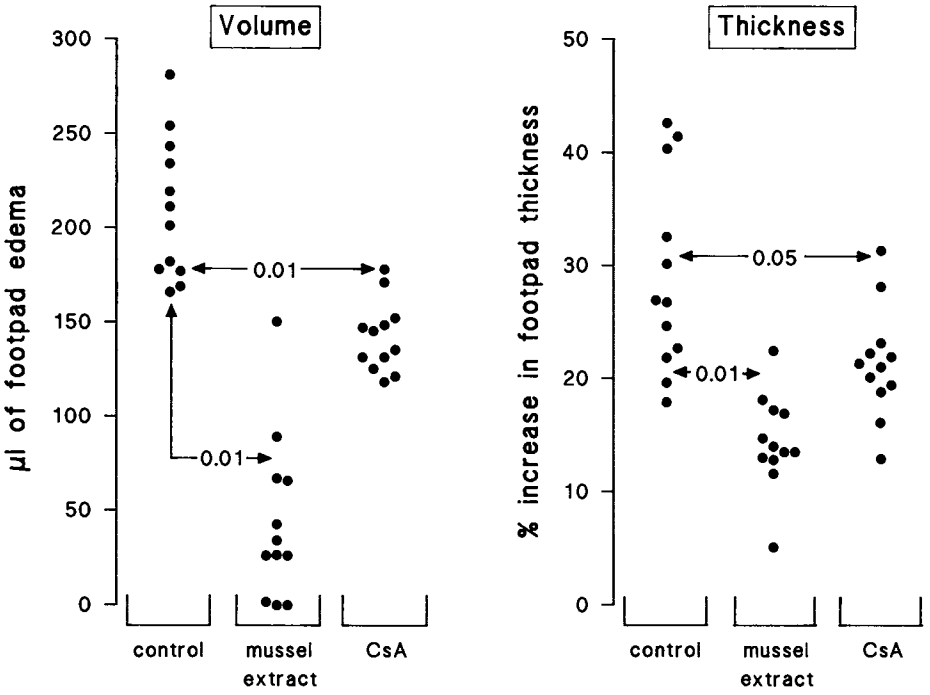

Fig. 2. Demonstration of the increased sensitivity achieved using the isotopic method to measure footpad edema. The antiinflammatory activity of two putative agents, mussel extract (300 mg/kg i.p. at −17 and −2 hr) and cyclosporin A (CsA; 50 mg/kg i.m. at −3, −2, and −1 day and −2 hr), was evaluated using both the isotopic technique (left) and ventral–dorsal footpad thickness measurement (right). Levels of significance are *P* values obtained when treated groups are compared with controls using the Wilcoxon Sum of Ranks Test.

DISCUSSION

The rat hind paw is the site most frequently challenged when animal models are used to evaluate potential antiinflammatory drugs (1–4). The end point of the assay is the assessment of paw edema and two approaches have been used to quantify this response. The first involves the use of plethysmography, in which the volume of the footpad is quantified by displacement of fluid. Although quantitative, and reasonably accurate, the plethysmograph is an uncommon piece of laboratory equipment and highly operator dependent. In the second and, most frequently cited approach, ventral–dorsal footpad thickness is measured using a modified engineer's thickness gauge. This method is simple and inexpensive but lacks sensitivity and is again operator dependent.

A major benefit of the procedure described here is that edema is measured independently and does not include the nonfluid components of the foot, such as bone and tissue. When other techniques are used the percentage increase in footpad volume following carrageenin stimulation is between 50 and 100%. In fact, the true increase in fluid volume, as demonstrated using the [^{125}I]HSA localization, is between 500 and 700%. This is reflected in an increase in sensitivity, as illustrated in Fig. 2. An additional and important advantage of the method is that it can be readily adapted to allow the time course of edema formation to be followed. The continuous monitoring technique allows a comparative assessment of the effect of drugs on the different phases of the response to be carried out and should prove useful in identifying the site of action of individual antiinflammatory agents.

The continuous monitoring procedure requires a subject to be anesthetized for a 3- to 4-hr period and the effect of the anesthetic agent on the inflammatory process needs to be considered. Five anesthetics were evaluated, and all except ether markedly inhibited the inflammatory response. A suppressive effect of anesthetic agents on host defenses has previously been observed and attributed to altered granulocyte function (9). The inflammatory response to carrageenin is known to be neutrophil dependent (10) and this may account for the suppressive effects of the parenteral anesthetics on carrageenin-induced edema.

In conclusion, the use of [^{125}I]HSA to measure edema accumulation in the rat footpad has been shown to be an accurate and simple means of directly assessing the ability of antiinflammatory drugs to suppress carrageenin induced edema. This technique is the only truly nonsubjective method available for measuring exudate volume and significantly enhances the sensitivity of a widely used and effective drug evaluation protocol.

REFERENCES

1. C. A. Winter, E. A. Risley, and G. W. Nuss. Carrageenan-induced edema in the hind paw of the rat as an assay for antiinflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 111:544–547 (1962).
2. K. Fukawa, T. Kanezuka, S. Ohba, O. Kawano, M. Hibi, N. Misaki, and T. Sawabe. Studies on an anti-inflammatory agent. III. Pharmacological investigations of a new non-steroidal anti-

inflammatory agent: 2-Oxo-3-(4-(1-oxo-2-isoindolinyl)-phenyl)-butanamide (GP 650). *Arzneim-Forsch/Drug Res.* **32**(I):225–230 (1982).
3. T. E. Miller and G. Findon. Experimental candidosis: Paw oedema in the analysis of a local infection. *J. Med. Microbiol.* **20**:283–290 (1985).
4. D. K. Gemmell, J. Cottney, and A. J. Lewis. Comparative effects of drugs on four paw oedema models in the rat. *Agents Actions* **9**:107–116 (1979).
5. K. Udaka, Y. Takeuchi, and H. Z. Movat. Simple method for quantitation of enhanced vascular permeability. *Proc. Soc. Exp. Biol. Med.* **133**:1384–1387 (1970).
6. A. C. Issekutz and T. B. Issekutz. Cellular and vascular phenomena in inflammation. *Methods Enzymol.* **162**:301–319 (1988).
7. N. S. Doherty and B. V. Robinson. The inflammatory response to carrageenan. *J. Pharm. Pharmacol.* **27**:701–703 (1975).
8. D. A. A. Owen. Vascular changes during acute inflammatory responses in rat hindpaws. In D. A. Willoughby, J. P. Giroud, and G. P. Velo (eds.), *Perspectives in Inflammation: Future Trends and Developments*, University Park Press, Baltimore, 1977, pp. 491–495.
9. W. D. Welch. Effect of enflurane, isoflurane, and nitrous oxide on the microbicidal activity of human polymorphonuclear leukocytes. *Anesthesiology* **61**:188–192 (1984).
10. R. Vinegar, J. F. Traux, J. L. Selph, P. R. Johnston, A. L. Venable, and K. K. McKenzie. Pathway to carrageenan-induced inflammation in the hind limb of the rat. *Fed. Proc.* **46**:118–130 (1987).