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ORIGINAL PAPER

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The evaluation of rat prostate blood flow using a laser speckle flowmetry: a comparative study using the microsphere method in castrated and androgen-replenished rats

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Abstract To evaluate the accuracy and reliability of laser speckle blood flowmetry in the measurement of prostate blood flow, we performed a comparative study with the ordinary intra-arterial radioactive microsphere injection method using the well-known castrate-rat model. Adult male Wistar strain rats were used for the study. The rats were either only castrated or subcutaneous testosterone replenishment was followed subcutaneously 6 h after castration. The prostate blood flow was measured at different time courses after castration. The effect of a single androgen replenishment on prostate blood flow was also evaluated. The measurement of prostate blood flow was performed by both the radioactive microsphere injection method and laser speckle blood flowmetry, and then the outcomes were compared. Rapid reduction in prostate blood flow to 30% of the normal level was observed 24 h after castration in the measurements with the microsphere injection technique. The prostate blood flow decreased gradually after 24 h post-castration to 23 and 21% of the normal level at 48 and 72 h after castration, respectively. The laser speckle blood flowmetry also detected the decrease in prostate blood flow well, but in a more gradual manner. The prostate blood flow was 70, 52 and 35% of the normal level at 24, 48 and 72 h after castration, respectively. The effect of a single administration of testosterone to castrated rats had recovered the prostate blood flow to 74 and 98% of the normal level by measurement with the microsphere injection technique and laser speckle blood flowmetry, respectively. The different outcome in blood flow rate change between the methods can be explained according to their different mechanism of measurement, thus suggesting the capillary vessels are the early and most responsive portion for hormonal manipulation. In conclusion, the laser speckle blood flowmetry is a convenient and reliable method for evaluating prostate blood flow, especially when the organ is required for other biological and molecular assays, since the method does not require the excision of the organ for the measurement.

Keywords Blood flow · Laser speckle · Microsphere · Castration · Rat

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Introduction

Recently, the study on prostate blood flow regulation is becoming more important to clarify the distinct mechanism underlying the phenomenon. The method used for evaluating prostate blood flow in vivo in most studies was the intra-arterial injection of radioactive microspheres followed by the excision of the organ for the measurement of radioactivity [1]. The microsphere injection technique can be performed steadily, but it has weak points, e.g., the procedure should be performed with the radioactive substances in place, the harvested prostate cannot be used for other assays due to its captured radioactivity and the individual evaluation of the response to a certain substance is impossible since the procedure requires resection of the organ. An ideal alternative method for the prostate blood flow measurement requires the capability of measurement without excision of the organ. The laser speckle blood flowmetry enables measurement of organ blood flow by placing a small probe on the organ's surface. Since it does not require radioactive substances or resection of the prostate, a real-time monitoring of the prostate blood flow can be performed. This is very important for investigating the effect of certain substances on prostate blood flow as it decreases the effect of individual differences between animals. The purpose of this study was

to evaluate the accuracy and reliability of the outcome by laser speckle blood flowmetry on prostate blood flow measurement, and to compare with an ordinary radioactive microsphere injection method.

Materials and methods

Instrument

Laser speckle blood flowmeter ALF21R with the NS type ALF probe (Advance, Tokyo, Japan) was used for the study (Fig. 1). The laser speckle blood flowmeter uses a beam of low-intensity monochromatic infrared light at 780 nm that is emitted from a laser diode within the flowmeter. The beam travels through an optical light fiber and illuminates the organ surface from the tip of the probe. A fraction of the light beam is reflected by moving red blood cells within the tissue, and due to the Doppler effect, undergoes a frequency shift, which is proportional to the average red cell velocity. The reflected signal is processed in real time to give a measurement of tissue perfusion in milliliters of blood per minute per 100-g tissue [2, 3]. The target of the measurement is approximately 1–3 mm^3 at a distance of 1 mm from the probe tip. The probe used in the present study had a 3-mm diameter with a 1-mm hole in the tip center where laser light passed through. The physical principles of the laser speckle blood flowmeter are shown in Fig. 2.

Chemicals

Testosterone was purchased from Sigma Chemical (St. Louis, MO). ^{51}Cr-labeled microspheres (15-μm diameter, 740–3330 MBq/g) were purchased from MEN Life Science Products (Boston, MA.).

Animal protocol

All surgical and experimental procedures were approved and conducted in accordance with the guidelines of the Institutional Laboratory Animal Care and Use Committee of Gunma University School of Medicine. Ten-week-old male Wistar strain adult rats were used for the study. The animals were purchased from Japan SLC (Shizuoka, Japan) and housed under controlled conditions and given water and food pellets ad libitum for at least 1 week before and during the experiments. Operations were performed under intraperitoneal pentobarbiturate anesthesia (50 mg/kg). The rats were randomly divided into the following groups (four rats per group): (1) normal, (2) 24 h post-castration, (3) 48 h post-castration, (4) 72 h post-castration, (5) 24 h post-castration with 1 mg of testosterone replenishment subcutaneously at 6 h (6) 7 days post-castration. The animals were treated exactly the same for both analysis by laser speckle blood flowmetry and the microsphere injection technique.

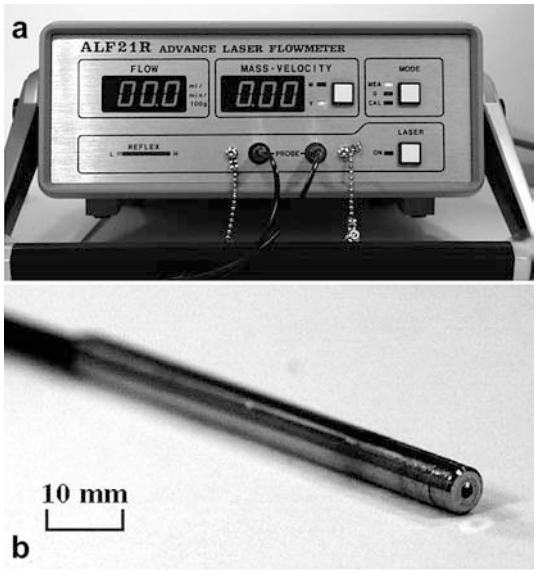

Fig. 1a, b Laser speckle blood flowmeter ALF21R (**a**) and NS type ALF probe (**b**). The laser light comes out from a 1-mm hole on the tip of the probe

Evaluation of the relative blood supply of the prostate by the radioactive microsphere injection technique

The blood supply of the prostate was represented by the uptake of intra-arterially injected radioactive microspheres. The uptake of microspheres in the prostate was reported to parallel the organ blood flow [1, 4]. Under pentobarbiturate anesthesia, the left common carotid artery was cannulated with a 0.96-mm single lumen polyethylene catheter (SP45, 0.58-mm internal diameter, Natsume Seisakusho, Tokyo, Japan). The catheter was inserted 2 cm and fixed, so the tip of the catheter was located near the aortic arch. Then, 15 μCi of radioactive microspheres, suspended in 0.35 ml saline containing 0.01% Tween-80, were injected via the catheter; this was followed by 0.4 ml saline for the complete injection. The ventral prostate and unilateral kidney were excised and weighed 2.5 min after the injection. The radioactivity of the ventral prostate and kidney were measured using a gamma scintillation counter. The prostate blood supply was corrected by the radioactive uptake of the kidney, the androgen-independent organ, for the elimination of a minor difference in each animal during the procedure. The final blood flow of the ventral prostate was calculated using the following formula: radioactivity of the ventral prostate per milligram tissue weight/radioactivity of kidney per milligram tissue weight.

Blood flow measurement by laser speckle blood flowmetry

Each rat was fixed in a supine position and operated on by a mid-abdominal incision. Then ventral lobes of the prostate were exposed carefully, and the probe tip of the laser speckle blood flowmeter was gently placed on the organ (Fig. 3). The probe was set still for 30 s to stabilize measured blood flow values on the display, and the value was recorded. The measurement was repeated three times on randomly selected organ surfaces, and then the values were averaged. Blood flow of the kidney was measured similar to the method for the prostate. The final blood flow of the

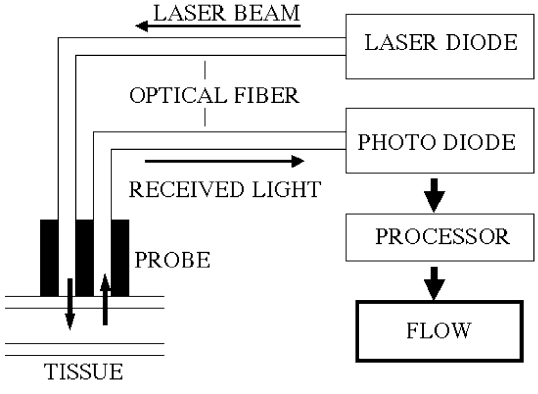

Fig. 2 The physical principles of laser speckle blood flowmetry

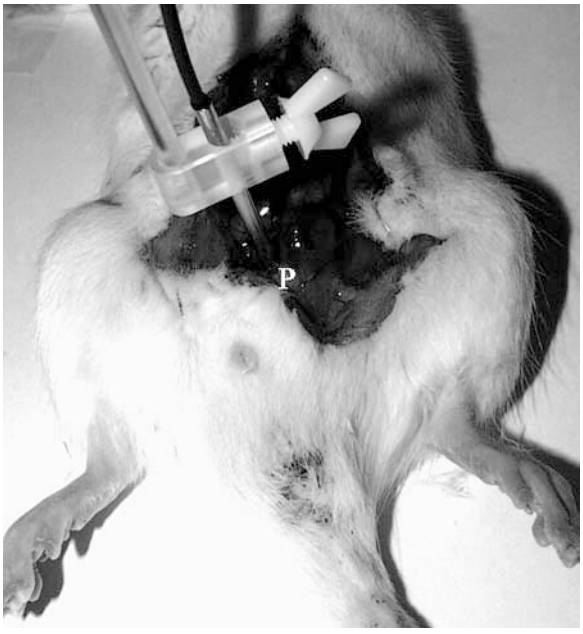

Fig. 3 The actual scene for the measurement of rat prostate blood flow with the NS type probe connected to ALF21R laser speckle blood flowmeter. *P* Ventral prostate

ventral prostate was expressed using the following formula: blood flow of ventral prostate per 100 g tissue weight/blood flow of kidney per 100 g tissue weight.

Statistical analysis

The statistical significance was determined by the Mann-Whitney U test using StatView J-4.51.2 (Abacus Concepts, Berkeley, CA) with a Macintosh computer (Apple Computer, Cupertino, CA). Differences were considered significant when *P* was less than 0.05.

Results

The change of relative prostate blood flow after castration

Rapid reduction in prostate blood flow to 30% of the normal level was observed 24 h after castration in measurements with the microsphere injection technique (Table 1). The prostate blood flow decreased gradually after 24 h post-castration to 21% of the normal level at 72 h after castration. The prostate blood flow recovered to 63% of the normal level at 7 days after castration (Fig. 4A).

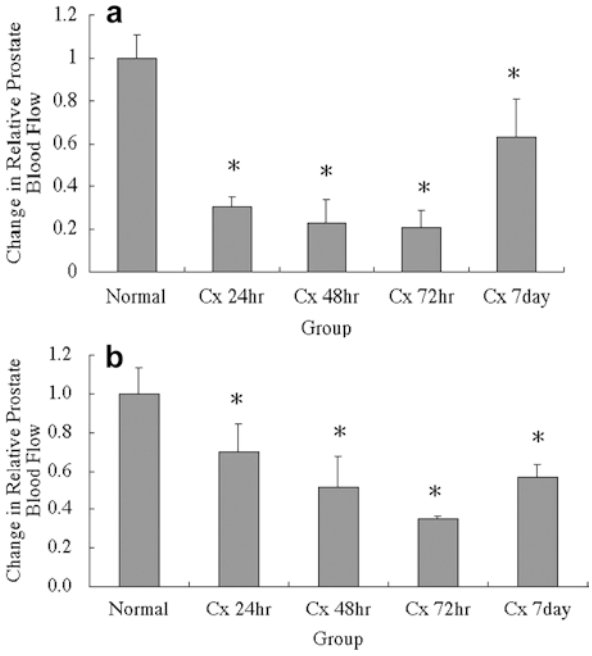

Fig. 4a, b The change of relative prostate blood flow after castration measured using radioactive microsphere injection (**a**) and laser speckle blood flowmetry (**b**). *Cx* Castration, $*p < 0.05$ vs. Normal rats

The decrease in prostate blood flow was also observed in castrated rats measured by laser speckle blood flowmetry but in a slower manner (Table 2). The prostate blood flow was 70, 52 and 35% of the normal level at 24, 48 and 72 h after castration, respectively. The prostate blood flow recovered to 57% of the normal level in 7-day-castrated rats (Fig. 4B).

The change of relative blood flow after castration with sub-cutaneous testosterone replenishment at six hours

The prostate blood flow was recovered in response to testosterone replenishment at 6 h after castration to 74% of the normal level by measurement with the microsphere injection technique (Fig. 5A). The complete recovery of the prostate blood flow was observed in 6-h testosterone-replenished castrated rats using the blood flow measurement with laser speckle blood flowmetry (Fig. 5B).

Table 1 Change in rat organ blood flow in the prostate and kidney after castration were measured by microsphere injection technique

	Radioactivity (cpm/mg)				
	Normal	Cx 24 h	Cx 48 h	Cx 72 h	Cx 7 day
Prostate	3.7 ± 0.85	1.36 ± 0.66	1.38 ± 0.78	0.95 ± 0.30	2.92 ± 0.65
Kidney	47.16 ± 10.71	47.05 ± 14.24	64.76 ± 10.18	60.04 ± 7.58	59.99 ± 10.71

Data presented as the mean ± SD

Table 2 Change in rat organ blood flow in the prostate and kidney after castration measured by laser Doppler blood flowmetry

	Organ blood flow (ml/min/100 g)				
	Normal	Cx 24 h	Cx 48 h	Cx 72 h	Cx 7 day
Prostate	60.1 ± 10.1	34.2 ± 5.0	29.3 ± 4.8	20.0 ± 3.8	24.6 ± 11.8
Kidney	49.1 ± 7.6	40.6 ± 8.8	47.8 ± 9.3	51.5 ± 2.2	34.4 ± 12.9

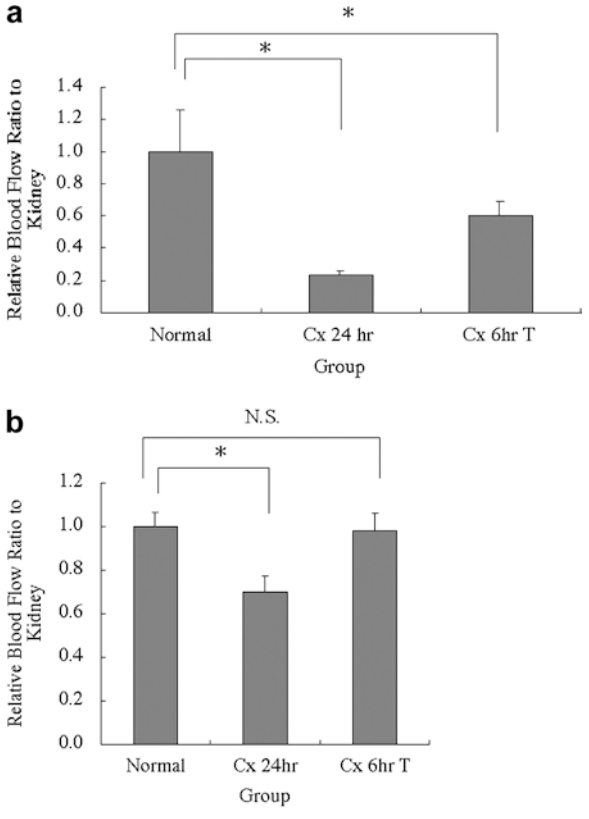

Fig. 5a, b The change of relative prostate blood flow after castration with subcutaneous testosterone replenishment at 6 h measured using radioactive microsphere injection (**a**) and laser speckle blood flowmetry (**b**). *Cx* Castration, *T* Testosterone, $*p < 0.05$

Discussion

The rapid decrease in prostate blood flow after castration precedes prostate volume regression [5], suggesting the androgenic regulation of the prostate through influence on blood flow. The decreasing effect of an antiandrogen, finasteride, on the prostate blood flow [6] also supports the hypothesis of androgenic regulation of prostate through blood flow. Other factors such as vascular endothelial growth factor (VEGF) and nitric oxide that have an influence on the prostate blood flow are also being studied closely.

The evaluation of prostate blood flow in vivo was performed in most studies using the intra-arterial injection of radioactive microspheres [1, 5, 6, 7]. We also studied the changes in prostate blood flow by castration, androgen replenishment and various factors concerning the blood flow regulation such as vascular endothelial growth factor (VEGF) by means of the microsphere injection technique. During the experiment, we always had difficulties in handling the radioactive material, which may have restricted the accuracy of the findings. The contamination of prostates with radioactivity restricts further need for biochemical and molecular assays of the organ. Further, the measurement of prostate blood flow with microspheres is an impractical method for a real-time monitoring of blood flow change, since it measures the radioactivity of captured microspheres in peripheral capillaries that require the excision of the organ. The method of blood flow measurement of tissue in vivo with a laser speckle blood flowmeter was first reported in the late 1970s [8, 9], but its application on prostate blood flow measurement was not performed until recently. Kozlowski et al. first used a laser speckle blood flowmeter for the measurement of rabbit prostate blood flow in 2001 [10]. In the present study, we measured the blood flow in the rat ventral prostate using both the microsphere technique and laser speckle blood flowmetry to insure the reliability of the latter method for the application in the experiments that were performed using the ordinary microsphere method. Both methods clearly showed the prostate blood flow reduction after castration and its recovery in response to testosterone replenishment, but the extent of the response differed between the methods. The reduction in prostate blood flow was more rapid in measurements with microsphere injection than the changes observed by laser speckle blood flowmetry. The difference was greater during the early period after castration, which was 30 vs. 70% of the normal level at 24 h post-castration; then, the difference decreased with the time to 21 vs. 35% at 72 h (Fig. 4). The difference between the methods was also observed in the prostate blood flow measurement after androgen replenishment to castrated rats. The complete recovery of prostate blood flow was observed with laser speckle blood flowmetry, in contrast to 74% recovery with the microsphere injection method. The different outcome between the methods should be due to their mechanism of measurement. The microsphere injection method represents the prostate blood flow indirectly though the radioactivity of the captured microspheres in the prostate microvessels. The diameter of the microsphere is 15 μm, almost twofold the diameter of red blood cells, and it cannot pass through the true capillaries. This suggests that the blood flow measurement with microsphere injection technique represents the blood flow regulation at the point that is proximal to true capillaries [11]. In contrast, the blood flow measurement with laser speckle blood flowmetry detects the movement of blood cells in capillaries and translates it to the blood flow rate according to Doppler's effect. Thus, the outcome with laser speckle blood flowmetry should represent the blood flow rate at the point that is more peripheral compared with the outcome with the microsphere injection technique.

According to this theory, the different outcome between the methods can be explained. The blood flow in proximal vessels represents the blood flow of huge peripheral capillaries beyond them. Castration induces gradual blood flow reduction in peripheral capillaries paralleling prostate androgen content as measured by laser speckle blood flowmetry. This leads to the rapid blood flow reduction at the upstream vessel proximal to them, which was detected by the microsphere injection technique. The observation of quick and full recovery in blood flow of prostate capillary vessels by androgen replenishment to castrated rats using laser speckle blood flowmetry suggested a quicker response to androgen replenishment in peripheral capillaries than more proximal vessels, whichwere represented by the microsphere technique. These results suggest the hormonal regulation of prostate blood flow through peripheral capillaries. The similarity of the outcomes in both methods was seen at 7 days after castration, when hormonal status reached a plateau and blood flow in the overall prostate was balanced.

In conclusion, the results of present study confirmed that both methods were useful in evaluating the tendency of change in prostate blood flow in response to hormonal manipulation. The differences in outcome value between the methods represent the prostate blood flow regulation at different points. Although the methods should be carefully selected according to the aim and style of the experiment, the employment of laser speckle blood flowmetry in experiments that require prostate blood flow evaluation is useful and reliable, especially when the experiment requires harvesting of the organ for further experimentation.

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