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Rate of urinary bladder blood flow evaluated by ^{133}Xe washout and radioactive microspheres in pigs

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Summary. Urinary bladder blood flow as measured by the washout of locally injected ^{133}Xe and by the simultaneously performed radioactive microsphere reference-sample method was studied in pigs. The washout curves were analyzed according to the initial slope, the corrected initial slope and the total curve. The corrected-initial-slope flow rates were not statistically significantly different from the microsphere whole-wall flow rates. The variability between the methods shown by the test-retest difference revealed only a minor lack of agreement. The bladder blood flow tended to decrease after the intravesical pressure had been increased to 20 cmH_2O, but the difference was not statistically significant. Washout of locally injected ^{133}Xe can be used for the evaluation of urinary bladder blood flow in humans and in longitudinal animal studies. The corrected-initial-slope method is recommended for the analysis of washout curves.

Key words: Reference-sample microsphere method – Injection trauma – Urinary bladder blood flow – ^{133}Xe washout – Distension

From experimental in vitro studies in different animals, it is obvious that the detrusor muscle is sensitive to changes in the oxygen/blood supply and that ischemia may result in irreversible changes in detrusor function [13, 21]. Bladder necrosis has been described following urinary retention and overdistension [19]. The question as to whether the increased functional demand on the detrusor muscle in different diseases of the lower urinary tract causes a change in the blood supply to the bladder remains unanswered.

Studies on the rate of urinary bladder blood flow in different animals are few with regard to absolute values and tissue distribution as well as to the effect of bladder filling. Various methods have been used, including washout of locally injected tracers [8, 10, 20], transillumination [22], the radioisotope-labelled microsphere technique [11, 23] and measurements of the venous outflow by a drop counter [2].

To develop a method for measuring the rate of urinary bladder blood flow in a longitudinal animal study and in humans, we compared the results obtained following the washout of ^{133}Xe that had been locally injected in the pig detrusor muscle with those obtained using the simultaneously performed radioactive microsphere technique in the same pigs. Furthermore, the local injection method was compared with atraumatic local labelling of the urinary bladder.

Materials and methods

Surgical and experimental procedure

Four male landrace pigs weighing 32–42 kg were starved for 12 h but were given free access to water. The animals were premedicated with 5 mg/kg azaperone (Sedaperone). Anesthesia was induced with 12.5 mg/kg i.v. thiomebumal. An endotracheal tube was introduced and the animals were mechanically ventilated with atmospheric air. Anesthesia was maintained using pentobarbital when necessary. Arterial oxygen saturation, oxygen and carbon dioxide tensions and pH were measured repeatedly. Fluid was replaced with isotonic saline. The heart rate and arterial blood pressure (Statham pressure transducer) were continuously recorded. The hematocrit was determined. A catheter (6-F pigtail and end curve) was inserted into the left ventricle of the heart via the right carotid artery; the correct position of the catheter tip was secured by pressure-wave recording. Another catheter (6-F, no end curve) was advanced into the abdominal aorta via the left carotid artery for reference blood sampling; its position was controlled by X-ray. A catheter (6-F, no end curve) was advanced via the right jugular vein to the right hypogastric vein; the position of the catheter was controlled by sight and palpation.

A low paramedian abdominal incision was made to expose the bladder. A polyethylene catheter (8-F) was inserted into the bladder through a cystotomy. To measure the intravesical pressure, the catheter was connected to a water column equipped with three-way stopcocks at 5, 10, 15 and 20 cm above the bladder level, enabling constant bladder pressures at these levels. Isotonic body-warm saline was used to distend the bladder. The temperature of the pigs was not measured, but the animals were kept warm by heated blankets.

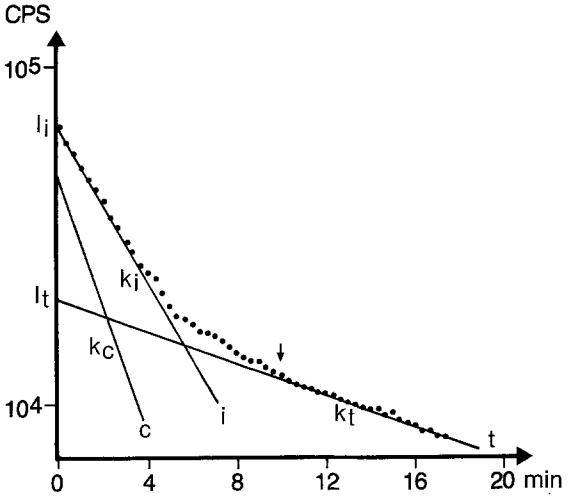

Fig. 1. Representative ^{133}Xe washout curve in a semilogarithmic plot. *I*, initial slope; *t*, tail slope; *c* corrected initial slope; K_i, K_t, K_c, rate constants (min^{-1}) measured from the washout curves; I_i, intercepts of the initial slope; I_t, intercepts of the tail slope; ↓, injection of microspheres

In another study examining functional and morphological changes in the urinary bladder in relation to alterations in the blood supply in mini-pigs with a partial bladder-outlet obstruction (Nielsen et al., unpublished data), the local injection method was compared with atraumatic local labelling of the urinary bladder in five animals. The surgical and experimental procedure described above was used.

The radioactive microsphere method

The microsphere method with reference blood sampling [7, 12, 15] was used to measure the rate of regional blood flow. Microspheres (15 μm; Nentrac, New England Nuclear) labelled with ^{113}Sn, ^{141}Ce, ^{103}Ru and ^{46}Sc were applied. The procedure has been described in details elsewhere [20]. Simultaneously with the reference blood sample withdrawn from the abdominal aorta, a reference sample was hand-drawn from the hypogastric catheter at a flow rate of about 2 ml min^{-1} to obtain information on arteriovenous shunts in the bladder wall. Bilateral samples of tissue from the kidneys were obtained from the first two pigs to check the bilateral symmetry of microsphere distribution. The regional blood-flow rate was calculated as described by Hales [12].

The local isotope washout method

^{133}Xe (0.05 ml) dissolved in isotonic saline (370 MBq ml^{-1}) was slowly injected with great care into the lateral part of the detrusor muscle through a 26-gauge needle. The needle was introduced almost parallel to the surface of the bladder and was then advanced 1 cm. Xenon was delivered to a depth of 2 mm from the surface such that it would be confined to the muscle layer. The needle was left in situ for 10–15 s after the injection before it was removed. No aspiration was performed. ^{133}Xe activity was measured with a collimated sodium iodide detector placed 10 cm above bladder level. A 20% window around the 81 KeV peak of ^{133}Xe was used. The counting of radioactivity began immediately after the injection. The counts were accumulated over 20 s, corrected for background activity and plotted in semilogarithmic diagrams. The biological half-time was determined by fitting a straight line to the recorded points by eye.

For atraumatic labelling, a gas-tight Mylar membrane (diameter 3 cm; thickness 20 μm) was made to adhere to the surface of the urinary bladder. Using an angled needle, 0.05 ml isotope solution was placed under the membrane near the center to ensure uniform distribution over an area of the bladder. The membrane was removed after 1 min and the surplus ^{133}Xe was immediately blown away. The counting of radioactivity was then begun.

The tissue/blood partition coefficient (λ) for xenon in the urinary bladder was calculated for each landrace pig after measurement of the hematocrit and analysis of the tissue composition [24]. In the mini-pigs, the λ value was calculated from the measured hematocrit and from the median result of the tissue-composition analysis in the landrace pigs [24].

Experimental procedure

The animals were allowed to stabilize for 30 min following surgery. In the four landrace pigs, the ^{133}Xe washout after local injection into the detrusor muscle was compared with the simultaneously performed radioactive microsphere technique. Following the injection of ^{133}Xe, the abdomen was temporarily closed with towel clips. After about 10 min of counting, the microspheres were injected. At the end of each ^{133}Xe washout, the bladder content was tested for radioactivity. The measurements were performed at intravesical pressures of 0–3 and 20 cmH_2O; about 15 min prior to the measurements, the bladder was distended to the intravesical pressured desired.

In five mini-pigs, the ^{133}Xe washout was evaluated after both atraumatic labelling and local injection. Paired values were obtained by injecting ^{133}Xe into the muscle layer as soon as the washout curve resulting from atraumatic labelling had reached background levels.

Analysis of ^{133}Xe washout curves

Initial-slope method. From the initial slope of the washout curve, the rate of capillary blood flow was calculated using the following equation (Fig. 1):

$$f = k_i \times \lambda \times 100\ [ml \times (100\ g \times min)^{-1}],$$

where k_i is the rate constant measured from the initial monoexponential washout curve and λ is the partition coefficient ($ml \times g^{-1}$) between the tissue and the blood [17, 18].

Corrected-initial-slope method. The high solubility of ^{133}Xe in fatty tissue, resulting in the trapping of ^{133}Xe in perivesical and perivascular fat, could influence the initial washout rate. Thus, subtraction of the tail part of the curves might be justified. From the subtracted (corrected) initial slope, the blood-flow rate is calculated by the following equation (Fig. 1):

$$f = k_c \times \lambda \times 100\ [ml \times (100\ g \times min)^{-1}],$$

where k_c is the rate constant measured from the corrected washout curve.

Stochastic analysis

In a biexponential curve, the area *A* under the washout curve can be calculated by applying the resulting two (fast and slow) exponentials (Fig. 1):

$$A = \frac{I_t}{k_t} + \frac{I_i}{k_i},$$

where I_t and I_i are the intercepts of the slow and fast slopes,

Table 1. Urinary bladder blood flow: comparison of the results obtained using microspheres and the different analyses of ^{133}Xe washout curves

	^{133}Xe Washout			Microspheres			
	Initial slope	Corrected initial slope	Stochastic analysis	Whole wall	Muscle	Mucosa	Ratio $\left(\frac{\text{muc}}{\text{mus} + \text{muc}}\right)$
Pig 1	13.5[a]	26.5	11.8	24.7			
	11.5[a]	49.3	6.9	45			
	20.9[a]		20.9				
Pig 2	17.3[a]	29.9	11.8	104.2[c]	75.4[c]	21.2[c]	
	18.8[a]	41.1	3.5	92.6[c]	68.6[c]	11.4[c]	
	9[b]	19.4	2				
Pig 3	29.8[a]	53.4	16.7	37.8	40.7	47.6	0.54
	23.6[a]	38.7	12.2	45.2	50.5	62.4	0.55
	18.9[b]	37.8	10.4	30.7	21	42.4	0.67
	24.9[b]	42.9	12.2	35.1	23.4	43.8	0.65
Pig 4	21.1[a]	31	12	29.4	22	48.9	0.69
	31.5[a]	52.7	25.6	56	31.9	72.9	0.7
	5.85[b]	28.2	2.2	12.6	7.6	28.7	0.79
	12.2[b]	34.4	10.3				

Values are expressed in milliliters per 100 g per minute. muc, Mucosa; mus, Muscle
[a] Intravesical pressure = 0–3 cmH_2O
[b] Intravesical pressure = 20 cmH_2O
[c] Failures

respectively, and k_f *and* k_i are rate constants of the slow and fast exponentials, respectively, and the using the equation:

$$f = \lambda \times \frac{H}{A} \quad [\text{ml} \times (100\ \text{g} \times \text{min})^{-1}],$$

where H is the peak count rate [28].

Statistics

The values are expressed as absolute figures. Comparison of ^{133}Xe washout with the microsphere method was performed using the Mann-Whitney rank-sum test for unpaired data and Student's *t*-test (unpaired). Values of $P < 0.05$ were considered to be significant. The assessment of agreement between the ^{133}Xe washout and the microsphere method was evaluated by calculating the test-retest difference [4].

Results

During the experiments, no changes were observed in the haemodynamic or respiratory variables. A median of 4.7% (range 2.8%–11.4%) of the counts obtained in arterial blood samples were found in blood samples from the hypogastric vein. None of the microsphere injections caused any changes in blood pressure or heart rate. The results obtained using microspheres in pig 2 were extremely high in relation to those found in the other pigs. These findings were considered to represent failures and were therefore omitted from the statistical calculations. The exceptionally high values might be attributable to dislocation of the cardiac catheter, resulting in inadequate

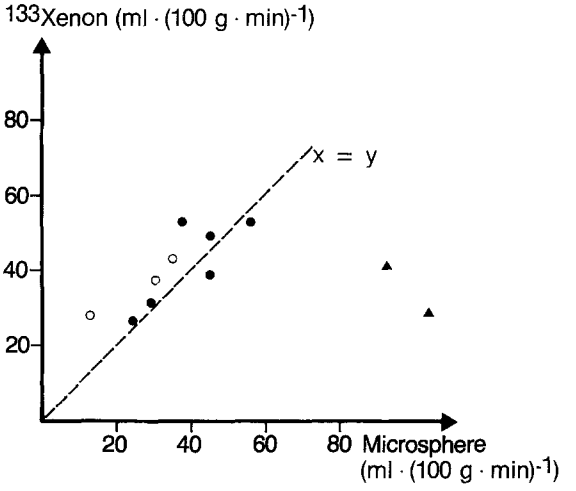

Fig. 2. Rate of urinary bladder blood flow evaluated using the microsphere whole-wall blood flow and the corrected-initial-slope method. ●, Intravesical pressure = 0–3 cmH_2O; ○, intravesical pressure = 20 cmH_2O; ▲, failed microsphere results

mixing of the injected microspheres, or to a local anatomical variation in the blood supply of the tissue sample. As no significant difference was found in the rate of blood flow to the two kidneys, a dislocation of the cardiac catheter seems unlikely.

The statistical calculations showed no significant difference between the whole-wall blood-flow rates determined using microspheres and the results obtained from the corrected initial slope of the ^{133}Xe washout curves (Table 1, Fig. 2). To assess the agreement of the two

methods, a plot of the difference in the results found by the two methods against their mean values was constructed [4]. The limits of agreement, i.e. the mean difference [4.9 ml × (100 g × min)$^{-1}$) ± 2 SD, were calculated from − 9.4 to 19.2 ml × (100 g × min)$^{-1}$. The 95% confidence interval for bias was calculated from − 0.58 to 10.4 ml × (100 g × min)$^{-1}$. This means that the corrected-initial-slope method tends to give values that are between − 0.58 and 10.4 ml × (100 g × min)$^{-1}$ higher than those obtained for the whole-wall blood-flow using microspheres.

There was not statistically significant difference between the muscle blood-flow rate found using microspheres and the results obtained from the initial slope (Table 1); however, the calculated test-retest difference showed considerable variation. The other results of ^{133}Xe washout were statistically significantly different from those obtained by the microsphere method. The results found by corrected-initial-slope method indicated that the blood-flow rate decreased after the intravesical pressure had been increased to 20 cmH_2O, but the difference did not reach statistical significance. The results obtained using microspheres showed that the rate of mucosal blood flow was higher than that in the muscle layer in all cases (Table 1). After bladder distension, the results indicated a further increase in the mucosa: muscle blood-flow ratio.

In the comparative study in mini-pigs using atraumatic vs traumatic application of ^{133}Xe, all of the washout curves in semilogarithmic diagrams were bent. Rates of blood flow were calculated by the corrected initial slope. The mean blood-flow rate following atraumatic labelling was 39.1 ± 2.8 ml × (100 g × min)$^{-1}$ and that after traumatic application was 37.3 ± 1.2 ml × (100 g × min)$^{-1}$; the difference was not statistically significant.

Discussion

The arterial supply of blood to the urinary bladder is extremely complex. It has been reported that seven branches of the anterior division of the hypogastric artery may supply the bladder, but the only constant sources are the superior vesical, vesiculodeferential and inferior vesical arteries [5]. Since the hypogastric artery also supplies many other structures in the pelvic cavity, determination of the rate of total blood flow in the bladder is impossible by direct methods other than the microsphere technique. Reportedly, only about 1% of microspheres sized 15 μm bypass the systemic circulation [12]. Therefore, the use of 15- μm microspheres results in a measure of the capillary blood-flow rate. The median shunt of microspheres in the present study was 4.7%. This could be attributable to the hand-drawn reference sampling of blood. The hypogastric vein receives blood from many structures other than the urinary bladder. Overall, this means that there are only few arterio venous shunts in a normal bladder wall. The number of spheres per 2 g tissue in the present study was estimated to be 1,400. According to Buckberg et al. [6], this means that the number of spheres used in the present study were sufficient to measure the blood-flow rate with an accuracy of about 5%.

All ^{133}Xe washout curves except one exhibited a curving course; with the use of simple graphics they could be described by a slow and a fast exponential component. The bent curves in the present study might be attributed to both counter-current exchange [3, 26] and the distant, wide collimation [14, 25] showing possible uptake and slow washout from perivascular and perivesical fat and connective tissue [9, 14].

In the present study there was no significant difference between the flow rates calculated by the corrected initial slope and the whole-wall blood-flow rates obtained using microspheres; thus, it seemed justifiable to subtract the tail part of the washout curve. The corrected initial slope of ^{133}Xe washout can give useful physiological results, but the variation in the mean test-retest difference ± 2 SD indicates that only relatively large changes in blood-flow rate that might be caused by alterations in the experimental procedure (overdistension, bladder-outlet obstruction) can be detected using the corrected initial slope of ^{133}Xe washout curves. The rate of blood flow in undistended bladders in the present study was higher than those reported elsewhere [2, 8, 10, 11, 23] but in accordance with the results we previously obtained in the urinary bladder of dogs [20].

It has been confirmed that the rate of blood flow in the mucosa is higher than that in the muscle [10, 16, 23]. Most authors have reported a decrease in the blood-flow rate on bladder distension [8, 10, 22, 23], whereas Andersson et al. [2] found a sustained increase. In the present study, the blood-flow rate decreased following distension to 20 cmH_2O, but the difference was not statistically significant. It probably mainly reflected the low number of animals examined. An essential passive property of the detrusor muscle enables elongation of the bladder wall to occur without causing a significant rise in pressure despite an increase in bladder-wall tension [1]; however, the rise in active tension is slow relative to the increase in muscle length [27]. It seems necessary that the intravesical pressure be increased even more to assess the relationship between blood flow and distension in a normal bladder. We found no significant difference in blood-flow rates following atraumatic and traumatic labelling by the injection of 0.05 ml isotope solution. No difference could be observed in the slopes of the curves, indicating that local oedema or tissue damage did not significantly influence the rate of blood flow determined using this technique.

In conclusion, washout of locally injected ^{133}Xe can be used for the evaluation of urinary bladder blood flow in humans and in longitudinal animal studies. The corrected-initial-slope method is recommended for the analysis of washout curves.

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