Lymphology 14 (1981) 133–137

Microspheres in Cardiac Lymph: Control and Ischemic States

L.H. Michael, J.R. Hunt, R.M. Lewis, M.L. Entman

Department of Medicine, Section of Cardiovascular Sciences, Baylor College of Medicine and the Fondren-Brown Cardiovascular Research and Demonstration Center of The Methodist Hospital, Houston, Texas 77030

Summary

The cardiac lymph from conscious animals was monitored for the presence or absence of radiolabelled 15 micrometer microspheres during: a) control periods after left atrial injection of microspheres and b) after circumflex coronary artery (CFX) occlusions (10–20 min total or < 40 mins 50% of control flow) followed by full reperfusions. Microspheres (15 μ) numbering 7–150 were present in the lymph within 2 hrs after the occlusions; and in three experiments, the presence of a small number in the lymph on the following day implied continual release overnight. No microspheres were present in 8–48 hr lymph samples prior to occlusions. This study suggests that some microspheres escape from the intravascular spaze of the myocardium and are channeled into the cardiac lymphatics; this is apparent even after short-term ischemic events.

Introduction

Myocardial ischemia initiated by occlusion of coronary vessels promotes changes in cell membrane permeability as evidenced by release of large enzyme components into cardiac lymph even during short periods (< 20 mins) of ischemia (1, 2). Alterations may occur in the microvascular system of the myocardium which more freely permit the movement of intravascular components into the interstitial spaces. This report will describe the examination of cardiac lymph in conscious dogs to determine if components as large as radiolabelled microspheres, escape the vasculature. Radioactive microspheres have been widely used to measure regional myocardial blood flow, map time-course of changes in coronary collateral flow, and assess infarct state, a method relying on the entrapping of microspheres in the myocardium (3, 4, 5). Although it has been reported that microspheres remain entrapped in the myocardium even during infarction and necrosis, several studies have suggested, in fact, that there may be loss of microspheres by: a) shunting of microspheres with elevated perfusion pressure (6) and b) loss of microspheres during ischemia within 24 hrs of occlusion (7). The mechanism of this loss, however, has not been studied and we hypothesized that it might be partially reflected by microvascular leakage. In the present study, we investigate the appearance of microspheres in cardiac lymph samples from awake, unanesthetized dogs before and after short periods of myocardial ischemia.

Materials and Methods

The cardiac lymphatic duct in dogs was cannulated and the lymph collected in the conscious state as previously described (8). The cardiac lymph was monitored, before and after occlusion of the circumflex coronary artery (CFX), for radiolabelled microspheres ($15 \pm 3\,\mu$ and $9 \pm 1\,\mu$ size) which were injected into the left atrium via an indwelling catheter.

The microspheres (^{103}Ru, ^{85}Sr, ^{141}Ce) were obtained (3M Company, Boston, Mass. and New England Nuclear, St. Paul, MN.) as 1 mCi (10 mCi/g) of nuclide in 10 μl of physiological saline with 0.05% Tween -80. Before injection, the microspheres (2–4 million) were mixed by agitation in an ultrasonic bath and vortex agitator for 10 mins. The injection was given through a left atrial catheter during 10–20 sec and flushed with 5 ml saline.

0024–7766/81 1500–0133 $ 02.00

Table 1 Microsphere (15±3 μ) content of cardiac lymph. Occlusion length refers to total time of occlusion; amount refers to percent decrease from mean Doppler flow. Preocclusion is the period between microsphere injection to left atrium and actual occlusion (8–48 hrs.): post occlusion is the reperfusion state after CFX occlusion (0–2 hrs and 16–18 hrs).

Experiment	Occlusion length, amount (minutes, % flow reduction)	Microspheres Quantity		
		Preocclusion	Post Occlusion 1–2 hrs	16–18 hrs
1	10, 100	0	15	0
*2	20, 100	0	30	9
3	40, 25–75	0	7	14
**4	30, 25–75	–	116	0
***5	20, 100	–	15	13
6	15, 25–75	0	150	0

*same animal as experiment 1, except following day
**same animal as experiment 3, except following day
***same animal as experiment 3, except 36 hrs after experiment 3

Once collected, cardiac lymph samples were centrifuged at 8,000 xg and both supernatant and pellets monitored for microsphere radioactivity by a TRACOR gamma counter at appropriate energy levels. A series of dilutions of the stock microspheres allowed calculation of the cpm/microsphere based on the estimated number of microspheres/mg, i.e. 1.5–2,000,000/mg–9 μ size and 450,000/mg–15 μ size. After appropriate background subtraction, the number of microspheres/lymph sample or tissue sample was calculated.

Results

Cardiac lymph was collected in periods ranging from 8–48 hrs after injection of 15 μ microspheres into the left atrium. Analysis revealed no radioactivity in either pellet or supernatant of centrifuged lymph samples. However, CFX occlusion followed by reperfusion resulted in appearance of microspheres in the lymph. In experiments 1, 2 and 5 (Table 1), total occlusions of 10 and 20 mins caused 15–30 microspheres to appear in cardiac lymph within the first 2 hours; 16–18 hrs after these occlusions there were still microspheres appearing in the lymph in experiments 1 and 5. In experiments 3, 4 and 6, partial occlusions averaging 50% control flow for 15–40 mins followed by reperfusion gave rise to 7–150 microspheres during the first 2 hrs afterward; also in experiment 3, release apparently continued with 14 recorded during collections made 16–18 hrs later. In four animals 9 ± 1 μ microspheres were injected as a bolus into the left atrium. No occlusions were given; however, it is apparent (Table 2) that some of these microspheres (14–839) gained entrance to the lymph within 2 hrs after injection. The distribution of the microspheres within the left ventricle and other tissues is shown in Table 3 where 4 million ^{85}Sr (9 μ) and 4 milion (15 μ), ^{141}Ce microspheres were placed in the atrium. The animal was totally occluded for 10 mins and sacrificed after 24 hrs of reperfusion. The quantity of microspheres/gram of tissue is shown in Table 3 for 2 left ventricular regions: the anterior and posterior papillary muscles.

Table 2 Microsphere (9 ± 1 μ) content of cardiac lymph within 2 hrs post-injection of microspheres into left atrium

Experiment	Microsphere Size	Microsphere Quantity
1	9 ± 1	839
2	9 ± 1	14
3	9 ± 1	15
4	9 ± 1	174

Table 3 4 million microspheres of each kind were injected into the left atrium of a dog and a 10 minute CFX occlusion was initiated after 1 hour the dog was sacrificed at 24 hours and 0.5–1 g of tissue was excised in triplicate and cpm/g calculated after gamma counting. In the heart, 3 samples (midmyocardium, epicardium and endocardium) were excised for each area and mean values are presented

Microsphere and size		Distribution of microspheres per gram of tissue					
		Anterior papillary muscle	Posterior papillary muscle	Spleen	Liver	Lungs	Kidney
^{141}Ce	15 ± 3 μ	1204	976	611	1276	18	7777
^{85}Sr	9 ± 1	1127	923	1127	1205	4283	4314

The number of microspheres shown in Table 1 and 2 only represents those present in the sampled cardiac lymph; only a fraction of the total cardiac lymph during a 24 hr period was collected, hence substantial quantities of microspheres may have been lost. The normal procedure was to collect lymph for at least 2 hrs immediately after injecting microspheres and also after the CFX occlusion; subsequently lymph was collected at arbitrary intervals for 24 hrs. There were no overnight lymph collections because of the probability that the cannula would be dislodged.

Discussion

In conscious animals, efflux from the heart interstitial space was collected after cannulation of the cardiac lymph duct. The escape of intravascular components such as red blood cells into the cardiac lymph was reported after total occlusion of the CFX coronary artery (9); in fact, red blood cell counts increased 418% in the lymph coincident with large protein elevations. In the present study, we monitored the cardiac lymph for radiolabelled microspheres and detected varying quantities in 7 of 12 animals. Fifteen micron microspheres were detected in cardiac lymph after CFX occlusions lasting for short intervals and followed by reperfusion. This experimental manipulation was carried out 8–48 hrs post-microsphere infusion. It should be stressed that lymph collected prior to the occlusions never contained microspheres. Microspheres were recorded in the cardiac lymph in one animal during the actual occlusion. The slower lymph flow (8) and very short periods of ischemia most likely led to inability to monitor microsphere outflow during the occlusion. The mechanism for the movement of some smaller microspheres into the cardiac lymph without initiating an ischemic state is unknown. However, apparently it is not abnormal for red blood cells (approximately 8 μ) to gain access to the cardiac lymph in the normal state, by leaving the bloodstream through endothelial cell junctions (10). It is accepted that white blood cells (7–8 μ) migrate through blood vessel walls into the interstitium and return again into the vascular space (11). In addition, other living cells, motile microfilariae (40 x 5 μ) and non-motile pneumococci travel from capillary blood to lymph (12). The microvascular mechanisms allowing these processes may also be operative in the case of smaller microspheres.

The significance of the appearance of microspheres in the cardiac lymphatic vessel may be exemplified by considering anatomic pathways which whould be traversed. Blood capillaries in tissues under normal function are approximately 8 μ (13) in diameter, but distinct narrowing or dilatation may occur depending on the functional state of the organ. Lying adjacent to the blood capillaries, small arterioles and venules are lymphatic capillaries distinghished by microscopic appearance as terminating in rounded or swollen ends with endothelial cell overlap to form a valve-like arrangement (14). The wall of the lymphatic capillary is formed of a single layer of endothelial cells, as are the blood capillaries, but being slightly larger and thinner. In order for a microsphere to gain access to the interstitial space it may: a) pass between the endothelial cells of the blood capillary, venule or arteriole, b) physically rupture the endothelial membrane

(microvascular hemorrhage), or c) extravasate by an unknown mechanism. The 15 μ (and possibly 9 μ) microspheres are most likely too large for passage through the lumen of a normal capillary. Apparently these spheres become lodged in slightly larger vessels. However, once the CFX occlusion initiates an ischemic state, the physical-chemical characteristics of the microvascular endothelium may be altered to foster the microsphere passage. Additionally, it is well known that a hyperemic response ensues after myocardial ischemia which is effected by a vasodilatory response to cardiac metabolites. We have shown (8) a similar augmentation in cardiac lymph flow following the release of coronary occlusion. Increased size and/or stretch on the vessels with altered permeability may be responsible for this augmented lymph flow and may foster the more easy escape of microspheres through microvascular junctions into the interstitial space and ultimately into the terminal lymphatics. Some microspheres may also be forced through the lumina of capillaries and gain entrance to the lymph through venule junctions. *Shirley* et al. (15) have shown that plasma volume expansion, in general decreases the resistance of capillary walls to the passage of macromolecules or increases the size of the capillary "pores".

The ischemic state may also intrinsically alter the vasoactive state of the myocardium. Local and diffuse alterations in vascular permeability for other tissues beds have been indicated for vasomotor agents such as histamine, epinephrine and bradykinin; histamine caused extravasation of carbon particles in post capillary venules (16); and bradykinin caused separations (0.08–1.4 μ) of endothelial cells of vessels (17).

We have shown that microspheres gain access to lymphatic channels in the heart and appear in lymph after being placed in the blood. Microspheres of the 15 μ dimension require only a short ischemic state to promote their extravasation into the lymph. The basic mechanisms involved in these microvascular alterations may be important considerations to our understanding of the ischemic states and the effect of reperfusion.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Ms. *Jennifer Pocius* and the excellent secretarial assistance of Ms. *Concepcion Mata* and Ms. *Diana Wilson.*

This work was supported by the American Heart Association, Texas Affiliate and grants HL 23,161, HL 13,870, and Hl 17,269 of the Myocardial Biology Section of the National Heart and Blood Vessel Research and Demonstration Center, Baylor College of Medicine, Houston, Texas.

References

1 *Nordbeck, H., H. Kahles, C. Preusse, P. Spieckermann:* Enzymes in cardiac lymph and coronary blood under normal and pathophysiological conditions. J. Mol. Med. 2 (1975) 225–263

2 *Michael, L., R. Lewis, M. Entman:* Release of phosphorylase into cardiac lymph in the conscious dog. J. Mol. Cell. Cardiol. (Suppl. I) II (1979) Abst. 40

3 *Becker, L., R. Ferreira:* Mapping of left ventricular blood flow with radioactive microspheres in experimental coronary artery occlusion Cardiovasc. Res. (1973) 391–400.

4 *Domenech, R., J. Hoffman, M. Noble, K. Saunders, J. Henson, S. Subijantō:* Total and regional coronary blood flow measured in conscious and anesthetized dogs. Circ. Res. 25 (1969) 581–596

5 *Rivas, F., F. Cobb, R. Bache, J. Greenfield, Jr.:* Relationship between blood flow to ischemic regions and extent of myocardial infarction. Circ. Res. 38 (1976) 439–447

6 *Crystal, G., R. Boatwright, H. Downey, F. Bashour:* Shunting of microspheres across the canine coronary circulation. Am. J. Physiol. 236 (1979) H7–H12

7 *Capurro, N., R. Boldstein, R. Aamodt, H. Smith, E. Epstein:* Loss of microspheres from ischemic canine cardiac tissue. Circ. Res. 44 (1979) 223–227

8 *Michael, L., R. Lewis, T. Brandon, M. Entman:* Cardiac lymph flow in conscious dogs. Am. J. Physiol. 237 (1979) H311–H317

9 *Feola, M., G. Glick:* Cardiac lymph flow and composition in acute myocardial ischemia in dogs. Am. J. Physiol. 229 (1975) 44–48

10 *Clark, E.R., E.L. Clark:* Observations on living mammalian lymphatic capillaries – their relation to blood vessels. Am. J. Anat. 60 (1937) 253–298

11 *Nishi, M., N. Hamada, H. Nomura, M. Mastueda, T. Aido:* The migration of lymphocytes across the vascular endothelium in lymph nodes: a scanning electron microscopic study. Lymphology 12 (1979) 9–10

12 *Augustine, C., C. Drinker:* The migrations of microfilariae from the blood vessels to the lymphatics. Trans. R. Soc. Trop. Med. Hyg. 29 (1935/6) 303–306

13 *Bloom, W., D. Fawcett:* A Textbook of Histology. 10th Ed. (1975) 386–426, W.B.Saunders, Philadelphia
14 *Leak, L., J. Burke:* Ultrastructure of the lymphatic capillaries. J. Appl. Physiol. 36 (1965) 2620–2621
15 *Shirley, H., C. Wolfram, K. Wasserman, H. Mayerson:* Capillary permeability to macromolecules: Stretched pore phenomenon. Am. J. Physiol. 190 (1957) 189–193
16 *Majno, G., V. Gilmore, M. Leventhal:* On the mechanism of vascular leakage caused by histamine-type mediators. Circ. Res. 21 (1967) 833–847
17 *Arfors, K.E., G. Rutili, E. Svensjo:* Microvascular transport of macromolecules in normal and inflammatory conditions. Acta Physiol. Scand. Suppl. 463 (1979) 93–103

L.H. Michael, Ph.D., Department of Medicine, Section of Cardiovascular Sciences, Baylor College of Medicine, 1200 Moursund Avenue, Houston, Texas 77030 (713) 790–3146