MICROVASCULAR RESEARCH 31, 110–114 (1986)

In Vivo Size of Leukocytes in the Spontaneously Hypertensive Rat

ROBERT A. MAYROVITZ, RONALD SAMPSELL, AND HARVEY N. MAYROVITZ

Research Division, Miami Heart Institute, Miami Beach, Florida 33140

Received July 26, 1984

INTRODUCTION

A microvascular hemodynamic impact of circulating leukocytes (mainly polymorphonuclear granulocytes, PMN) due in part to plugging and adherence within microvessels is strongly suggested by recent findings (Bagge *et al.*, 1980; Engler *et al.*, 1983; Braide *et al.*, 1984; Mayrovitz *et al.*, 1984). The extent of the leukocyte effect in a given vessel depends on factors such as the size, mechanical properties, and state of activation of the PMN and the vessel, as well as the prevailing local hemodynamics. For any set of fixed conditions the size of the leukocyte in relation to the vessel lumen likely affects the extent and frequency of plugging, blood flow velocity (Mayrovitz, 1982), the initiation of PMN adherence to vascular endothelium (Schmid-Schönbein *et al.*, 1975), and the distribution of leukocytes at branch points (Mayrovitz and Rubin, 1984). In spite of the significance of the size of the circulating PMN, little systematic work in characterizing this parameter has evolved. The work herein described summarizes our measurements of the *in vivo* size of circulating PMN as observed in the cremaster microvasculature of the widely studied spontaneously hypertensive rat (SHR).

MATERIALS AND METHODS

Male SHR (N = 6, age = 6–8 weeks, wt = 85–110 g) of the Okamotto and Oaki strain (1963) were used in this study. Each animal was initially anesthetized with a single dose of Nembutal (5.0 mg/100 g ip), placed on a heated mat, and had the trachea cannulated. The cremaster muscle was prepared for microscopic observation using the Baez method (1973) with slight modifications (Mayrovitz and Roy, 1983). After this preparation, the animal, already secured to a mounting board, was placed on the stage of a Leitz Laborlux 12 HL trinocular microscope equipped with a 150 W Xenon light source for transillumination. After a 1-hr stabilization period the microvascular field was observed (50×, 1.0 numerical aperture water immersion objective and 16× oculars) to locate postcapillary venules in which leukocytes could be seen rolling along the endothelium. The postcapillary sites were selected for leukocyte size measurements because the cells are most clearly visualized in these vessels. This is because they tend to

0026-2862/86 $3.00

marginate toward the wall
for example, in arteriolar

The images of these rollir
consisting of a Newvicon
tronically controlled magn
a 19-inch TV monitor (R
trinocular port of the mic
image that was horizontal
located in the tissue at va
measurements of leukocyt
images using frame-by-fran
the coordinates of the vide
electronically generated vic
Inc, Model 321). The inters
a unique voltage character
of the image field used fc
micrometer with 10-μm lir
cal calibration factors we
measurements.

Cell diameters (Horizon
those cells with the best vis
ascertained. For these cell
time and there was no att
flowing cells. For any given
to have optimal visual clari
20% nor more than 70%.
determined. A mean spheri
was used to calculate cell su
cell shape with diameter of
of D_x and D_y were used to
(V_e) of a prolate spheroid h
respectively. Assuming tha
the associated equivalent sph
with D_m.

Accuracy of the dimensic
standard having opaque an
with an absolute error gua
Linewidth and Calibration
standard indicated we coul
Though the system was cap
depth, the *in vivo* measure
thin interference ring (0.3
causes some ambiguity in th
and because the outer ring i
to present all results base
interference ring. If the cer
the cell wall location, then

marginate toward the wall and they move slowly. This is not generally the case, for example, in arteriolar vessels.

The images of these rolling cells were recorded using a closed-circuit TV system consisting of a Newvicon camera (MTI-model VC-65) equipped with a 2× electronically controlled magnifier, a video tape recorder (JVC, Model 6060U) and a 19-inch TV monitor (RCA Model TC1119). The TV camera affixed to the trinocular port of the microscope, was rotated to produce a recorded vessel image that was horizontal as viewed on the monitor. When observed, leukocytes located in the tissue at various distances from vessels were also recorded. All measurements of leukocyte size were done off-line by processing the recorded images using frame-by-frame analysis. This process was done by first calibrating the coordinates of the video image on the monitor using manually controlled and electronically generated video cross-hairs from a video analyzer (Colorado Video Inc, Model 321). The intersection of the horizontal and vertical cross hairs define a unique voltage characterizing each x–y coordinate. Calibration of the region of the image field used for analysis was established using a calibrated stage micrometer with 10-μm line spacing (Leitz, Model M9). Horizontal and vertical calibration factors were determined and thereafter used for the *in vivo* measurements.

Cell diameters (Horizontal, D_x, and vertical, D_y) were determined only for those cells with the best visual clarity in which the cell borders could be optimally ascertained. For these cells the diameters were determined at a single instant in time and there was no attempt to make multiple measurements of individual flowing cells. For any given video field, the percentage of cells which we considered to have optimal visual clarity was quite variable but was generally not less than 20% nor more than 70%. The sizes of a total of 300 cells in six animals were determined. A mean spherical diameter, D_m, was calculated as $(D_x + D_y)/2$ and was used to calculate cell surface area (A_m) and volume (V_m) assuming a spherical cell shape with diameter of D_m. In addition, for comparative purposes, the values of D_x and D_y were used to directly calculate the surface area (A_e) and volume (V_e) of a prolate spheroid having D_x and D_y as the larger and smaller diameters, respectively. Assuming that V_e was distributed within the volume of a sphere, the associated equivalent spherical diameter, D_e, was then determined and compared with D_m.

Accuracy of the dimension measuring system was evaluated using a calibrated standard having opaque and transparent line widths varying from 3.0 to 12.0 μm with an absolute error guaranteed to be within ± 0.10 μm (Gold Arc Precision Linewidth and Calibration Standard No. 1562). Multiple measurements of the standard indicated we could routinely obtain the stated value within ± 0.12 μm. Though the system was capable of this accuracy for structures with infinitesimal depth, the *in vivo* measurement of cells of the order of 10 μm is affected by a thin interference ring (0.3 to 0.4 μm) surrounding the cell. This phenomenon causes some ambiguity in the precise location of the cell border. For consistency, and because the outer ring is better visualized in our preparation, we have elected to present all results based on measurements taken at the outer edge of the interference ring. If the center of interference ring were to be a better index of the cell wall location, then the dimensions we report may be as much as 0.3–

0.4 μm larger than actual. However, the choice of which criteria is more accurate—ring edge or center, is not certain.

RESULTS

In Table 1 the mean and standard deviation obtained for the 300 intravascular cells studied is presented. As revealed by the entries in the first two columns a small (0.47 μm), but significant ($P < 0.001$, paired t test) differences between D_x and D_y was found. The calculated values of surface area and volume depended very little on whether a spherical or spheroidal shape was used. The equivalent spherical diameter, D_e, obtained from the spheroidal volume calculation was only 0.1 μm less than the mean spherical diameter, D_m, obtained from the average of D_x and D_y. The mean spherical diameter distribution of the rolling cells is presented in Fig. 1 as a histogram with 0.5-μm bin sizes. Separate calculations show that the median value for this distribution is 7.92 μm. No statistical difference between this diameter distribution and a normal distribution could be detected using either a χ-square test ($P = 0.221$), or a Kolmogorov–Smirnov test ($P = 0.516$).

DISCUSSION

Documented reports of *in vivo* PMN size are quite limited. Observations of cell size made *in vivo* in humans apparently vary considerably, but a value of about 9 μm is quoted as appropriate for the majority of cells (Bagge and Brånemark, 1977). Extensive measurements of human cells *in vitro* have shown that freely suspended leukocytes, (e.g., neutrophils) are essentially spherical and have diameters (about 7.55 μm) that are much less than those obtained using standard blood smears in which the cells are somewhat squashed (Schmid-Schönbein *et al.*, 1980b). Measurements based on photographs of rolling and adherent leukocytes in venules of rabbit omentum indicate a diameter range of 5.0 to 9.5 μm for 31 cells (Schmid-Schönbein *et al.*, 1975). From this data a mean cell diameter of about 7.5 μm may be calculated. The present results based on 300 measurements in six rats indicate a mean spherical diameter of rolling cells of about 8 μm with a small variance from animal to animal. This value is thus similar to that obtained for the rabbit cells obtained under very similar *in vivo* conditions but somewhat less than that reported for the *in vivo* value in humans. In the present case, however, it was clear that under the conditions of our experiments the horizontal

TABLE 1
GEOMETRIC DATA FOR ROLLING LEUKOCYTES

	Diameter (μm)				Area (μm)^2		Volume (μm)^3	
	D_x	D_y	D_m	D_e	A_m	A_e	V_m	V_e
Mean	8.20 *	7.72	7.96	7.86	200	197	268	260
SD	0.63	0.67	0.55	0.57	28	29	57	58

* Horizontal and vertical diameter difference is significant, $P < 0.001$.

FIG. 1. Frequency

diameter of the rollin
diameter. We feel th
shear in the directio
vertical dimensions
For these nonmoving
to the mean of the rol
of leukocyte impact
sizes considerably le

Though the mean s
applicable to the hyp
size in this strain is
for example that the
SHR as compared w
there is no known li
which would suggest
in a genetically simil
SHR cell sizes are c
man, suggests that s
main point of the p
various rat strains,
concerning a widely
ability of this informa
hemodynamic interac

The research support p
AG813 is gratefully ackn

BAEZ, S. (1973). An open
microscopy. *Microvasc*

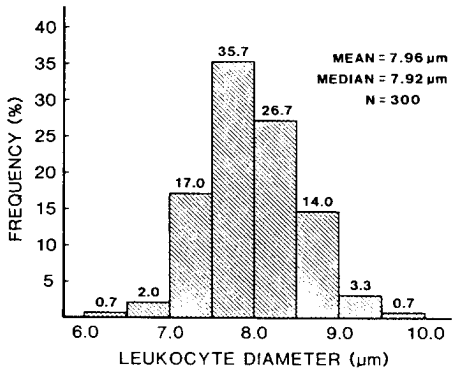

FIG. 1. Frequency distribution of the mean spherical diameter of rolling leukocytes.

diameter of the rolling cells was slightly, but significantly, greater than the vertical diameter. We feel that this is due to a slight cell deformation due to the blood shear in the direction of flow (horizontal). Differences between horizontal and vertical dimensions were not present in 38 cells which were within the tissue. For these nonmoving cells a mean diameter of 7.9 μm was found which is close to the mean of the rolling cells. The present results confirm the fact that estimations of leukocyte impact within the living microcirculation must be based on cell sizes considerably less than quoted by most hematology textbooks.

Though the mean spherical diameter of about 8 μm herein detemined is specifically applicable to the hypertensive rat, we have no reason to believe that the leukocyte size in this strain is different from other rat strains. Previous work has shown for example that there is no difference in leukocyte counts or blood viscosity in SHR as compared with normotensive controls (Mayrovitz *et al.*, 1982). Further, there is no known link between leukocyte size and hypertension development which would suggest that cell size in the SHR would be different from cell size in a genetically similar but normotensive counterpart. Indeed, the fact that the SHR cell sizes are close to those in different and much larger species including man, suggests that significant differences across rat strains are not likely. The main point of the present study was not to compare leukocyte sizes between various rat strains, but rather to provide basic *in vivo* cell size information concerning a widely studied and important experimental model. Given the availability of this information, it is felt that a more accurate estimation of the possible hemodynamic interactions of these cells within the microvasculature can evolve.

ACKNOWLEDGMENT

The research support provided by the American Heart Association, Florida Affiliate, Inc., Grant AG813 is gratefully acknowledged.

REFERENCES

BAEZ, S. (1973). An open cremaster muscle preparation for the study of blood vessels by *in vivo* microscopy. *Microvasc. Res.* **5**, 384–394.

BAGGE, U., AND BRÅNEMARK, P-I. (1977). White blood cell rheology. An intravital study in man. *Adv. Microcirc.* **7**, 1–17.

BAGGE, U., AMUNDSON, B., AND LAURITZEN, C. (1980). White blood cell deformability and plugging of skeletal muscle capillaries in hemorrhagic shock. *Acta Physiol. Scand.* **108**, 159–163.

BRAIDE, M., AMUNDSON, B., CHIEN, S., AND BAGGE, U. (1984). Quantitative studies of the influence of leukocytes on the vascular resistance in a skeletal muscle preparation. *Microvasc. Res.* **27**, 331–352.

ENGLER, R. L., SCHMID-SCHÖNBEIN, G. W., AND PAVELEC, R. S., (1983). Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. *Amer. J. Pathol.* **111**, 98–111.

MAYROVITZ, H. N. (1982). The relationship between leukocyte and erythrocyte velocity in arterioles. In "White Blood Cells, Morphology and Rheology Related to Function" (U. Bagge, G. V. R. Born, and P. Gaehtgens, eds.), pp. 82–88. Nijhoff, The Hague.

MAYROVITZ, H. N., LUCIO, J. C., AND DURDOCK, G. J. (1984). Predicted leukocyte occupancy and potential impact in coronary capillaries. In "Proc. Cardiovascular System Dynamics Society 6th International Conference," pp. 160–163. Univ. of Pennsylvania Press.

MAYROVITZ, H. N., POLANI, A., TATARSKY, S., AND ROY, J. (1982). Blood viscosity: A non-factor in spontaneous hypertension. *Proc. ACEMB* **24**, 169.

MAYROVITZ, H. N., AND ROY, J. (1983). Microvascular blood flow: Evidence indicating a cubic dependence on arteriolar diameter. *Amer. J. Physiol.* **245**, 1031–1038.

MAYROVITZ, H. N., AND RUBIN, R. (1984). Leukocyte distribution to arteriolar branches: Dependence on microvascular blood flow. *Microvasc. Res.* **29**, 282–294.

OKAMOTO, K., AND OAKI, K. (1963). Development of a strain of spontaneous hypertensive rats *Japan. Circ. J.* **27**, 282–293.

SCHMID-SCHÖNBEIN, G. W., FUNG, Y-C., SKALAK, R., AND ZWEIFACH, B. W. (1975). Vascular endothelium–leukocyte interaction. Sticking shear forces in venules. *Circ. Res.* **36**, 173–184.

SCHMID-SCHÖNBEIN, G. W., SHIH, Y. Y., AND CHIEN, C. (1980b). Morphometry of human leukocytes. *Blood* **56**, 866–875.

SCHMID-SCHÖNBEIN, G. W., USAMI, S., SKALAK, R., AND CHIEN, S. (1980a). The interaction of leukocytes and erythrocytes in capillary and postcapillary vessels. *Microvasc. Res.* **19**, 45–70.