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STOCHASTIC PROPERTIES OF LEUKOCYTE TRANSIT IN ARTERIOLES

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INTRODUCTION

Circulating leukocytes that become entrapped within the microvasculature during or following ischemia can significantly effect the extent of the resultant tissue injury [1-3]. Though adherence of activated leukocytes to postcapillary venules may occur in these and other inflammatory-like conditions the principal site of mechanical entrappment is in the capillaries. Entry of the leukocytes into the capillary network is most directly dependent on the flux of these cells in the supplying terminal arteriole. Observations of the microvasculature reveal that leukocyte movement in the arterioles [4,5] and their entry into the capillaries [6-8] is a time dependent process influenced by a variety of factors. In the present paper we treat the single file flow of leukocytes in the supplying arteriole as a stochastic point process in an attempt to develop quantitative data concerning the statistical properties of leukocyte delivery into the capillary network and thereby gain basic information on the nature of this dynamic process.

METHODS

Experimental Preperation

The animal preparation and experimental setup is the same as used previously [4]. Syrian golden hamsters (n=8) were prepared for microscopic observation of the cheekpouch following anesthetization with pentobarbital (0.6 ml/100 g body weight ip). The left femoral vein was cannulated and the animal placed on the stage of a fluorescence microscope equipped with a 50-W ultrahigh-pressure mercury lamp used as an epiilumination system. Most measurements were made using a 50X water immersion objective (N.A = 1.0) and a 5X or 10X ocular was used in the trinocular tube of the microscope to obtain video data via a closed circuit low-light level camera and video recording system. Acridine orange (50 ug/ml filtered and pH balanced to 7.4) was infused via the cannulated femoral vein at a constant rate of 0.00866 ml/min. Leukocytes stained with the acridine orange and brilliantly fluorescing, were observed traveling along the arteriole. Arteriolar vessels chosen for observation needed to satisfy the following criteria: (1) the diameter, Da, was within the range of 8-12 um; (2) single-file leukocyte flow could be observed, and (3) the vessel had no branches within the segment of interest. Data was recorded through the video system for periods not less than 10 min.

Data Acquisition

The parameters measured were; (1) arteriole diameter D_a, (2) leukocyte velocity V_{wbc}, (3) time intervals between successive leukocytes x_i and (4) leukocyte flux here defined as the number of leukocytes detected in contiguous 10 second intervals. D_a was measured at several sites along the vessel through a reticule in a 16x ocular previously calibrated with a stage micrometer. V_{wbc} was measured by placing two cursors (axially seperated by a known distance) on the video image of the observed arteriole. As the leukocytes passed each cursor, a change in the optical density was recorded and displayed on a dual-channel chart recorder. As each cell passed by the

cursors, an optical density peak was generated and recorded to determine the transit time between cursors. The distance between the cursors divided by the transit time yielded V_{wbc}. The time interval between the arrival of successive leukocytes at a given cursor site (x_i) was determined from the chart recording. Last, the entire series was broken up into 10 second intervals and the number of cells in each intervals were counted and defined as leukocyte flux.

Statistical Analysis

We treat the time interval (x_i) between successivly arriving leukocytes as a random variable which we hypothesize to be a stochastic point process. We test for stationarity in the sequence and its conformance to a renewal and Poisson process. Tests employed herein examine weak or wide sense stationarity. The procedures used to test for stationarity in the present analysis are the Wald-Wolfowitz runs test, regression analysis and the Cox-Lewis U-statistic. This latter procedure assumes a Poisson model and tests for a trend in the rate of occurrence represented by a smooth change in time. If stationarity cannot be established, then a non-homogeneous (time-dependent) stochastic point process is inferred. The simplest generalization of the stationary Poisson process is the non-homogeneous Poisson process in which the rate is a function of time due to some observed explanatory variable v(t). Tests for a renewal process are essentially those which establish the independence of the x_i. Primarily we are concerned with the serial correlation coefficients which characterize the second order, joint probabilities of the intervals between leukocyte arrivals, and tests based on the periodogram. The serial correlation coefficients are the Fourier coefficients of the spectral density function and provide a means of testing for the presence of serial correlation provided the marginal distributions of the x_i is not too highly skewed. The expected value of the serial correlation should be approximately zero if the x_i come from a renewal process. A special case of the renewal process is the Poisson process which in addition to the requirements of a renewal process, have the requirement that the number of events in any set of non-overlapping intervals are independent random variables with a Poisson distribution and the intervals between events are independent random variables with an exponential distribution. Tests for a Poisson hypothesis, based on the uniform distribution of times to events (y_i) and the Durbin modification of the x_i, was carried out by using two distribution free goodness-of-fit tests (Kolmogorov-Smirnov (K-S) and Anderson-Darling (A-D)). Another index of the x_i's conformity to a Poisson process is the coefficient of variation (CV). For a Poisson process the CV should be 1; with a range of $0.8 <= CV <= 1.2$ being acceptable. The non-homogeneous Poisson process has primarily the same requirements as the homogeneous process with the following differences: (1) non-stationaity of the series of x_i, (2) the x_i are not independent but the number of cells in any non-overlapping intervals are independent with a Poisson disrtibution, and (3) the x_i no longer have the simple exponential distribution.

RESULTS AND DISCUSSION

Results of the analysis show that leukocyte transit through arterioles, en route to the capillary network, can be suitably modeled as a stochastic point process. When the celluar velocity is relatively constant over the obeservation time of interest, the sequence of arrival times between consecutive leukocytes comforms to that of a renewal process and more

specifically to that of a Poisson process. If cellular velocity has a significant trend over the observation time of interest, non-stationarities in the series of leukocyte arrivals are to be expected, and the sequences are best characterized by a non-homogeneous Poisson process.

Previous applications of stochastic point process models to study the properties of other physiological processes have yielded varying results. The properties of spontaneous transmitter release at the crayfish and frog neuromuscular junction was studied using stochastic point process theory [9,10]. These workers rejected the Poisson hypothesis as describing the spontaneous release of neurotransmitters for two reasons; (1) the intervals between end-plate potentials were found not to be independently and exponentially distributed and (2) there was a possibility of simultaneous occurrence of events. In our analysis we successfully showed that the intervals between leukocyte transit are independently and exponentially distributed if the sequence is stationary and we eliminated the potential for simultaneous occurrances by analyzing arterioles <12 um in diameter thus eliminating simultaneously passing leukocytes.

Cermile and co-workers [11] in their investigation of erythrocyte passage in capillaries concluded that neither a renewal nor a Poisson process described these events because of non-stationarities in transit times probably due to microvascular vasomotion. We have also found some non-stationarities (in two of eight experiments). The source of the non-stationarity in these two cases was not likely caused by vasomotion as no diameter modulations were observed. However, a measured linear trend in the blood velocity was found to correlate with the observed non-stationariy in the intra-arrival sequence. Though the cause of the velocity change is not known, the incorporation of its perturbing effect into the model to produce a detrended sequence allowed us to estimate the properties of the detrended sequence. In the absence of the confounding effect of velocity changes the analyses revealed that the series conformed to a renewal and Poisson process.

Our demonstration of the applicability of a Poisson model to describe leukocyte delivery into the capillary network may now lead to better understanding of the patho-physiological occurrences related to leukocyte impact in capillaries. Tissue damage due to ischemia has been linked to entrapment of leukocytes in the microvasculature. It may be argued that this may occur during the onset of an ischemia producing vascular occlusion (either experimentally or spontaneously), during the maintained ischemic phase , or during a reperfusion phase if present. Knowledge of the nature of the process distributing leukocytes into the capillary network may lead to better understanding of these events.

In spite of our successful use of stochastic theory to characterize the leukocyte dynamics our findings do not necessarily imply that leukocyte delivery to the capillary mesh is governed by random principles. Clearly the number of leukocytes delivered to any given terminal arteriole depends on all the physical, topographical and hemodynamic factors which influence the cellular distribution within the microvasculature. For any terminal arteriole, the source of leukocytes is the immediately proximal parent arteriole in which there is present a concentration of leukocytes (leukocrit). This leukocrit is itself time dependent and some portion of the cells are distributed to the arteriolar branch under observation. The amount distributed depends, in part, on the blood flow in the observed vessel,

relative to the blood flow in the segment of the parent vessel which is immediately distal to the site of branching. For a given set of hemodynamic conditions the leukocyte distribution to the terminal arteriole is also importantly influenced by the details of the branch junction and the radial position of the leukocyte as it approaches the junction. The size of the leukocyte, either its absolute value or relative to the branch diameters, also plays some role in determining distribution selectivity. The presence of a broad statistical distribution of circulating leukocyte sizes could thus effect the temporal sequence of leukocyte entry into the observed branch. Similarly, a statisical distribution in the physical and mechanical properties of the circulating leukocytes would likely effect the observed sequence since these features influence distribution at branch points.

The above statements concerning events within the observed terminal arteriole and its parent vessel apply to varying degrees to each branch site along the vascular path which joins this branch to the main entering arteriole, thus influencing the dynamic leukocrit at all distal sites. The consequence of this vast number of interacting factors (many of which may be deterministic at a local level) is to give rise to an observed sequence which exhibits the stochastic features herein described. The stochastic characterization thus represents a consise formulation which allows us to deal with leukocyte dynamics within the terminal microvasculature.

REFERENCES

1. ENGLER RL, SCHMID-SCHONBEIN GW, PAVELEC RS. Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. Am. J. Pathol. 111 98-111, 1983.
2. BRAIDE M, BLIXT A, BAGGE U. Leukocyte effects on the vascular resistance and glomerular filtration of the isolated rat kidney at normal and low flow states. Circulatory Shock 20, 71-80, 1986.
3. LEWIS DH, MENSCHIK A, ERIKSDOTTER BEHM K, BENGTSSON MB. The role of polymorphonuclear leukocytes in ischemia and injury. Prog. appl. Microcirc. 12, 231-235, 1987.
4. MAYROVITZ HN, RUBIN R. Leukocyte distribution to arteriolar branches: dependence on microvascular blood flow. Microvasc. Res.29, 282-294, 1985.
5. MAYROVITZ HN. The relationship between leukocytes and erythrocytes velocity in arterioles. In: White Blood cells, Morphology and Rheology as Related to Function. U. Bagge, G.V.R. Born, P. Gaehtgens (eds.), The Hauge, Martinus Nijhoff, 1982.
6. BAGGE U, AMUNDSON B, LAURITSEN C. White blood cell deformability and plugging of skeletal muscle capillaries in hemorrhagic shock. Acta Physiol. Scand. 108, 159-163, 1980.
7. SCHMID-SCHONBEIN GW, SKALAK R, USAMI S, CHIEN S. Cell distribution in capillary networks. Microvasc. Res. 19, 18-44, 1980.
8. MAYROVITZ HN, KANG S-J, HERSCOVICI B, SAMPSELL RN. Leukocyte adherence initiation in skeletal muscle capillaries and venules. Microvasc. Res 33, 22-34, 1987.
9. COHEN I, KITA H, VAN DER KLOOT, W. The intervals between miniature end-plate potentials in the frog are unlikely to be independently or exponentially distributed. J. Physiol. 236, 327-339, 1974. J. Physiol. 236, 341-361, 1974.
10. COHEN I, KITA H, VAN DER KLOOT, W. Stochastic properties of spontaneous transmitter release at the crayfish neuromuscular junction. J. Physiol. 236, 363-371, 1974.
11. CERIMELE BJ, GREENWALD EK. Stochastic aspects of erythrocyte transit in capillaries. Microvasc. Res. 2, 139-150, 1970.