Viscous Resistance to Blood Flow in Solid Tumors: Effect of Hematocrit on Intratumor Blood Viscosity

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ABSTRACT

Blood flow rate in a vascular network is proportional to the arteriovenous pressure difference and inversely proportional to the geometric and viscous resistances. We have recently shown that the geometric resistance to blood flow increases with increasing tumor size and/or decreasing arterial pressure. In this study, the viscous resistance to blood flow within tumor microvasculature was determined by alternately perfusing mammary adenocarcinoma, R3230AC, N = 12; tumor weight, 2.2 ± 1.6 (SD) at ex vivo with Krebs-Henseleit solution and with RBC suspensions at hematocrits between 1 and 60%. Our results demonstrate that: (a) intratumor blood viscosity increases with increasing hematocrit; and (b) for fixed hematocrits between 10 and 60%, the intratumor blood viscosity is significantly reduced (P < 0.0001) compared to bulk viscosity measured at shear rates of 460 s⁻¹ using a cone/plate viscometer. However, this reduction of intratumor blood viscosity is not as pronounced as in a previous study of skeletal muscle. Further comparison shows that as arterial pressure is lowered, intratumor blood viscosity increases at a greater rate and at lower hematocrits than in normal tissues. We attribute the increased viscous resistance in tumor microvasculature to (a) a less pronounced Fahraeus effect (i.e., reduction in hematocrit in small vessels) and a less pronounced Fahraeus-Lindqvist effect (i.e., reduction in blood viscosity in small vessels) in dilated tumor microvessels compared to normal microvessels; (b) low shear rates (i.e., velocity gradients) associated with tumor vessels which may facilitate rouleaux formation at moderate pressures and even at low hematocrits; and (c) vascular fluid losses of 5–14% which may also increase microvesSEL hematocrit. We also propose that intratumor blood viscosity may be even higher in vivo than ex vivo due to the presence of WBC and cancer cells in vivo; considerably more rigid than RBC, these cells may cause increased viscous resistance and transient vascular stasis in tumors. The implications of these results in tumor blood flow modulation using chemical and physical agents are discussed.

INTRODUCTION

Blood perfusion rate, q (cm³/h/g), in a tissue is given by the following relationship:

\[ q = \frac{\Delta p}{\eta z} \tag{A} \]

where \( \Delta p \) (mm Hg) is the arteriovenous pressure difference, \( \eta \) (g/m/s) (1 g/m/s = 2.08 × 10⁻³ mm Hg·h·cm³) is the apparent blood viscosity, and \( z \) (g/cm³) is the extrinsic geometric resistance. The product of \( \eta \) and \( z \) is referred to as the FR⁴ (mm Hg·h·g/cm³). Despite the importance of blood flow: \( \Delta p \), \( \eta \), and \( z \) (2). Recently, using an isolated tumor preparation, we have measured the geometric resistance in tumors as a function of tumor size and perfusion pressure. Below 40 mm Hg, we have shown that the intratumor geometric resistance, \( z \), increases with decreasing perfusion pressure and, above 40 mm Hg, it reaches a constant value, \( z_0 \), which is dependent upon tumor size (3). Here we report the first measurements of apparent blood viscosity, \( \eta \), in a solid tumor.

The apparent viscosity of blood is governed primarily by hematocrit and shear rate. As hematocrit increases, blood viscosity also increases. At low shear rates, RBC aggregate to form rouleaux, causing an increase in blood viscosity; as shear rates increase, rouleaux are disrupted and blood viscosity approaches a constant value. Furthermore in blood vessels RBC migrate due to their deformability toward the center of microvessels, leaving a cell-free marginal layer at the vessel wall. Since the RBC in the center of the vessel travel faster than the cell-free layer near the wall, the hematocrit in small vessels (<500 μm) is lowered (Fahraeus effect) and blood viscosity is consequently reduced (Fahraeus-Lindqvist effect). As vessel diameter decreases below 500 μm, the cell-free marginal layer constitutes a greater portion of the microvessel flow and the reductions in hematocrit and apparent blood viscosity become more pronounced. However, as the vessel diameter becomes comparable to RBC diameter, both vessel hematocrit and apparent viscosity begin to increase.

As a result of these phenomena, the viscosity of blood measured in vitro using a cone/plate viscometer is higher than in several normal tissues (4–11). However, in solid tumors, experimental evidence of (a) increased caliber of microvessel diameter, (b) sluggish blood flow or flow reversal, and (c) significant vascular fluid losses suggests that the Fahraeus-Lindqvist effect may be less pronounced in tumors, rouleaux formation may be prevalent in tumor vessels, and local homoconcentration may increase viscous resistance in solid tumors (for a review, see Ref. 2). On the basis of these experimental data, Jain (2) hypothesized that the intratumor blood viscosity, \( \eta \), may be higher than the blood viscosity in normal tissues. However, whether this hypothesis is true and to what extent viscosity in normal tissues is lower than that in solid tumors is not known.

The objective of this study was, therefore, to directly measure the intratumor blood viscosity as a function of arterial hematocrit using the methodology of alternate perfusion developed by Whittaker and Winton (4). In this method (Fig. 1) an isolated tissue is first perfused ex vivo with an acellular medium of known Newtonian viscosity to calculate the constant geometric resistance, \( z_0 \). The ratio of the reciprocal slope of the linear pressure-flow curves. Assuming that the geometric resistance remains unchanged with change of perfusate, a second, alternate perfusion with an RBC suspension of known hematocrit permits calculation of the blood viscosity within the tissue, \( \eta \). This approach to determine blood viscosity in vascular preparations under ex vivo conditions allows one to extrapolate to the in vivo situation. A prerequisite to measure intratumor viscosity using this methodology is the availability of an isolated tumor connected to the host by a single artery and a single vein. As
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**Table 1** lists the tumor weights and perfusate viscosities for 5 of tumor weight.

Where $L_1, L_2, and L_3$ are the three characteristic lengths of an ellipsoid.

**Materials and Methods**

**Animals and Tumor.** Mammary adenocarcinoma R3230AC (obtained from Biomeasure Laboratories, Hopkinton, MA) was carried s.c. in female Fischer 344 weanlings (Harlan Sprague-Dawley, Inc., Madison, WI) up to the 20th generation. The rats were fed ad libitum a standard diet (Purina, Ralston, NC) and subjected to alternating light and darkness for 12 h.

**Preparation of "Tissue-Isolated" Tumors.** Ovarian tissue-isolated tumors were prepared following the method of Cullino and Grantham (12, 13) to measure the geometric resistance of tumors. In the present investigation, by perfusing mammary adenocarcinoma ex vivo with an acellular KH medium of known Newtonian viscosity, $\eta_{\text{acell}}$, to obtain the geometric resistance, $z_{0\text{known}}$. In the second step, an alternate perfusion with a RBC suspension allows calculation of the blood viscosity, $\eta_{\text{intra}}$, if it is assumed that $z_0$ remains constant with change of perfusate.

**Discussion.** We have adapted the isolated tumor preparation of Gullino and Grantham (12, 13) to measure the geometric resistance of tumors. In the present investigation, by perfusing mammary adenocarcinoma ex vivo with an acellular KH medium of known Newtonian viscosity and alternately with RBC suspensions of varying hematocrit, the geometric resistance, $z_0$, and the intratumor blood viscosity, $\eta_1$, are directly obtained. Results of intratumor blood viscosity are compared with those available for normal tissues in the literature, and clinical implications are discussed.

**Materials and Methods.** The viscosities of all perfusates as well as that of tumor-efferent samples were measured at 37°C at a shear rate of 460 s$^{-1}$ using a cone/plate digital viscometer (Model LVTDCP, Brookfield Engineering Laboratories, Stoughton, NJ). The viscosity of KH Perfusion 1 was found to be 0.9 g/m/s. Intratumor viscosity determinations were made from ratios of the linear pressure-flow slopes and the measurement of KH Newtonian viscosity as depicted in Fig. 1.

**Volume Determination.** Hematocrit measurements of tumor-effluent samples were measured using the microhematocrit capillary technique (Fisher Scientific, Pittsburgh, PA). Hemoglobin measurements were also obtained spectrophotometrically (ABL3; Radiometer, Copenhagen, Denmark).

**Tumor Volume Determination.** After perfusion, tumor volume was determined from three tumor dimensions using the formula

$$ V = \pi L_1L_2L_3/6 $$

where $L_1, L_2, and L_3$ are the three characteristic lengths of an ellipsoid. Tumor weight was calculated assuming the wet weight density of 1 g/cm$^3$. Injection of polymer materials used for vascular casting and detection of vascular leaks prevented direct and accurate measurement of tumor weight.

**Results.** Verification of alternate perfusion technique for viscometry. Table 1 lists the tumor weights and perfusate viscosities for 5 tumors (range, 0.6–9.8 g) alternately perfused with viscous KH
(1.2–2.1 cP). \textit{In vitro} (bulk) and intratumor perfusate viscosities were measured with a cone/plate viscometer and calculated from the ratio of the linear pressure-flow slopes, respectively. These two viscosities are not significantly different \((P = 0.163, \text{Student's} \ t\text{ test})\). Fig. 2A is an example of a typical flow-pressure \((Q-\Delta p)\) behavior of alternate tumor perfusion with 0.9 and 2.1 cP Krebs-Henseleit solutions. Fig. 2B illustrates the FR \((\text{mm Hg} \cdot \text{h} \cdot \text{g/cm}^3)\), calculated by dividing the arteriovenous pressure difference, \(\Delta p\), by the perfusion rate, \(q\), plotted against \(\Delta p\). The RFRs, calculated by dividing FR by \(z_0q\), obtained from the linear slope of the flow-pressure curve, are shown for the two alternate perfusions in Fig. 2C.

\textbf{Blood Flow Resistances at Varying Hematocrit.} Twelve tumors [2.2 ± 1.6 (SD) g; range, 0.5–6.6 g] were perfused with RBC suspensions of hematocrits between 1 and 60%. Fig. 3 is a plot of the tumor FR, \(\Delta p/q\) \((\text{mm Hg} \cdot \text{h} \cdot \text{g/cm}^3)\), versus venous hematocrit \%. For comparison, values obtained from \textit{in vivo} perfusion of DS carcinosarcoma implanted in rat kidneys (15, 16) are also shown.

\textbf{Intratumor Blood Viscosity at Varying Hematocrit.} Fig. 4A shows the typical flow-pressure \((Q-\Delta p)\) behavior at 0, 11, 35, and 57% HCT for a 2.1-g tumor and Table 2 lists the ratio of \textit{in vitro} and intratumor blood viscosities measured with a cone/plate viscometer and calculated from the linear pressure-flow slopes, respectively. The composite of the apparent normal tissue and intratumor blood viscosities as a function of hematocrit for all 12 alternate perfusions is shown in Fig. 5. Paired Student \(t\) test analysis indicates that the intratumor blood viscosity is significantly lower \((P < 0.0001)\) than that measured \textit{in vitro}. To compare this reduction of intratumor blood viscosity with those for normal tissues, blood viscosities from previous studies of isolated dog hindlimb (4), rabbit hindlimb (6), rabbit heart (11), and dilated cat calf muscle (8) are included in Fig. 6A. Fig. 6B shows the 95% confidence level fits to the Casson model (18) for the current measurements of intratumor blood viscosity as well as for the original data taken from a previous study of the skeletal muscle of the dog hindlimb (4):

\[
\eta = \frac{1}{(1 - \text{HCT})^\alpha}
\]

where HCT is expressed as a fraction. For intratumor measurements \(\alpha = 1.4 ± 0.2\), and for intramuscle measurements \(\alpha = 0.7 ± 0.3\) \((±95\%\) confidence interval). Above hematocrits of 20\%, intratumor blood viscosity is elevated in comparison to that in skeletal muscle.

\textbf{Intratumor Blood Viscosity as a Function of Perfusion Pressure.} Fig. 4B illustrates the flow resistance, FR \((\text{mm Hg} \cdot \text{h} \cdot \text{g/cm}^3)\), plotted against arteriovenous pressure difference, \(\Delta p\) (mm Hg), for the typical alternate blood perfusion shown in Fig. 4A. The RFRs, calculated by dividing the flow resistances with the reciprocal slope of the linear pressure-flow curve \((i.e.,\text{, with the product of } z_0 \text{ and } \eta \text{ as depicted in Fig. 1}), do not collapse upon one another for alternate blood perfusion as shown in Fig. 4C as they do for alternate KH perfusion (Fig. 2C). For the same typical alternate tumor perfusion, the ratio of the FR interpolated from blood to FR interpolated from KH alternate perfusions is plotted against arteriovenous pressure difference, \(\Delta p\) (mm Hg), in Fig. 7. Assuming that geometric resistance remains unchanged with perfusate, Fig. 7 represents the relative intratumor blood viscosity, \(\eta\), versus arteriovenous pressure difference, \(\Delta p\).

\textbf{DISCUSSION}

Several investigators have suggested changes in intratumor blood viscosity as a possible mechanism for tumor blood flow modifications due to physical \((e.g.,\text{, heat, hemodilution})\) and
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Accuracy of Methodology. Unlike blood, the viscosity of a Newtonian fluid (e.g., KH, KH with high molecular weight dextran) is independent of shear rate or vessel diameter. Therefore, the viscosity of KH with high molecular weight dextran solutions measured using an alternate perfusion method should be equal to that obtained using a cone/plate viscometer, as shown in Table 1. Furthermore, when the FR (Δp/q) is divided by the Newtonian perfusate viscosity (η) and constant geometric resistance, z₀, the resulting quantity, referred to as the RFR should be the same for perfusates of differing Newtonian viscosities and should reflect changes in geometric resistance with perfusion pressure (3). Only if this condition is satisfied can the alternate perfusion method be used to measure intratumor viscosity, η. As shown in Fig. 2C, the RFR curves for the viscous perfusates collapse upon one another; this observation confirms that the tissue preparation remains intact during the course of the experiment, and that geometric resistance and the dependence upon perfusion pressure (3) remain unchanged with the change of perfusate.

Blood Flow Resistances in Neoplastic Tissues. Since tumors exhibit poor perfusion characteristics, intratumor flow resistance (FR = Δp/q) is expected to be elevated in comparison to normal tissues. Fig. 3 shows the FR obtained from the linear portion of the flow-pressure (Q-Δp) curves at moderate to high perfusion pressures. Using in vivo perfusion of kidneys implanted with DS carcinosarcoma, Vaupel and Mueller-Klieser (16) also demonstrated increased intratumor FR. However, the values of FR in DS carcinosarcoma are significantly higher than those in R3230AC mammary adenocarcinoma; whether these differences are due to intertumor variations or due to differences in methodology is not known. In ex vivo perfusion, WBC and cancer cells are absent, whereas in in vivo perfusion, these cells are present. WBC and cancer cells are more rigid than RBC; as a result, their presence in perfusate would increase both intratumor blood viscosity, η, and flow resistance, FR (2).

Blood Viscosity in Normal and Neoplastic Tissues. The bulk viscosity of blood decreases with increasing shear rates and asymptotically reaches a constant value above shear rates of 100 s⁻¹. Furthermore, shear rate in a blood vessel is proportional to the blood velocity, v, and inversely proportional to the vessel diameter, D; since both v and D vary throughout the vasculature, it is not possible to ascribe a shear rate for a whole vascular bed. How, then, does one estimate the asymptotic viscosity for isolated tumor perfusion? In the work of Sevick and Jain (3) and in Fig. 2C, we have shown that the intratumor geometric resistance becomes a constant, z₀ above perfusion pressures of 20–40 mm Hg; since the flow-pressure (Q-Δp)

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**Fig. 3.** Composite of tumor blood flow resistances (mm Hg-h-lg/cm³) versus arteriovenous pressure drop (mm Hg) for alternately perfused tumors (N = 12; tumor weight, 2.2 ± 1.6 (SD) g., current data; A and B, average vascular resistances of tissue-isolated DS carcinosarcoma perfused in vivo from Vaupel (16) and Vaupel and Mueller-Klieser (17), respectively.

**Fig. 4.** Typical alternate perfusion with 0, 11, 35, and 57 volume % RBC suspensions in a 2.1-g tumor. (A) Perfusion rate (cm³/h/g) versus arteriovenous pressure difference (mm Hg) for the alternate RBC perfusions; ---, least squares fit of the linear pressure-flow behavior. (B) Flow resistance (mm Hg-h-lg/cm³) versus arteriovenous pressure difference (mm Hg) for the alternate RBC perfusions; ---, least squares fit of the linear pressure-flow behavior. (C) Relative flow resistance versus arteriovenous pressure drop (mm Hg) for this tumor.
Therefore, by dividing the FR from RBC perfusion by the FR at a fixed pressure can be attributed to increased intratumor T-
that, at a fixed pressure, z remains unchanged with change of
viscosity, coupled with increased geometric resistance, is respon-
sible for the poor perfusion characteristics of solid tumors. Therefore, one would expect intratumor j) to increase as the
arterial pressure and, hence, intravascular shear rates are low-
ner, however, is that intratumor r¡ is greater than r¿ in
previous normal tissue studies and is not surprising. What is
interesting, however, is that intratumor r¡ as a function of perfusion
pressure, the greater is the intratumor y. Using a similar
method, Gustafsson et al. (9, 10) calculated the relative blood
viscosity at varying perfusion pressures in the vasodilated dog
hindlimb; their results are reproduced in Fig. 7, inset. This
limited comparison indicates that the intratumor blood viscosity
may substantially increase at higher perfusion pressures and
lower hematocrits as compared to the blood viscosity in normal
tissues. In fact, as shown for a typical tumor perfusion in Fig.
7, rouleaux formation may even prevent intratumor blood vis-
cosity from reaching the asymptotic value at arterial pressures
as high as 60 mm Hg at a hematocrit of 57%.

Mechanisms of Increased Viscous Resistance in Solid Tumors. As discussed in detail by Jain (2), there are at least three
mechanisms responsible for the increased viscosity in tumors:
(a) since tumor microvessels have relatively large diameters
(25-27), the Fahraeus-Lindqvist effect (i.e., a reduction in blood
viscosity in small vessels) may be less pronounced in tumors
than in normal tissues; (b) due to larger vessel diameter and
sluggish flow, the low shear rate in tumor vessels should be
reflected in an increased intratumor blood viscosity; and (c) as
blood passes through the tumor microvasculature, plasma
losses between 5 and 14% cause an increase in intravascular
hematocrit (14, 28, 29). This increase in microvessel HCT may
also contribute towards increased y in tumors. There are two
factors which may contribute to a decrease y in tumors: (a)
reduction in systemic hematocrit with tumor growth; and (b)
presence of RBC-free plasma channels (2). Our hypothesis that
the factors contributing to an increase in viscosity dominate
those causing a decrease in viscosity is substantiated by the
current results. Some investigators have proposed that systemic
hematocrit in cancer patients be increased through transfusion
of erythrocytes to improve tumor oxygenation and radiosensi-
tivity (30). While an increase in hematocrit would indeed en-

chave demonstrated that within a certain range of hematocrits,
the oxygen availability in tumors actually increases with de-
creasing hematocrit, suggesting hemodilution may improve ra-
diosensitivity. This interplay between increased oxygen carrying
capacity and viscosity may be responsible for the lack of radi-
osensitization with homocentration seen in some studies
(32).

We also propose here a fourth mechanism for increased
intratumor y involving the vascular morphology of tumors. It
is evident from the microvascular casts resulting from polymer
infusion that tumor microvessels are strikingly tortuous and
winding as compared to the skeletal muscle microvessels (33).
The secondary flow patterns in the tortuous tumor microvessels
may tend to “mix” the two-phase flow described above thereby
reducing the cell-free marginal layer and increasing microvessel
hematocrit. Furthermore, several investigators have shown that
the vessel length decreases with tumor growth (25-27). The
decrease in vessel lengths effectively diminishes the develop-
ment of two-phase flow in solid tumors. In normal tissues, as
blood passes through the microvascular tree, a consistent re-
duction in hematocrit in daughter branching microvessels oc-
curs due to the uneven partitioning of RBC resulting from the

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<th>Hematocrit (%)</th>
<th>Intratumor blood viscosity</th>
<th>In vitro blood viscosity</th>
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<tr>
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<td>Intratumor KH viscosity</td>
<td>In vitro KH viscosity</td>
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<tr>
<td>11</td>
<td>1.15 ± 0.29</td>
<td>1.38 ± 0.09</td>
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<tr>
<td>35</td>
<td>1.85 ± 0.01</td>
<td>2.48 ± 0.08</td>
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<td>57</td>
<td>3.58 ± 0.05</td>
<td>3.62 ± 0.09</td>
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* SD derived from propagation of SD arising from least squares regression of q — Ap curve.
* N = 3-5 measurements in a cone/plate viscometer at 460 sec⁻¹.
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Fig. 6. (A) Composite of relative intratumor blood viscosity measured at high perfusion pressures in the current study and in previous studies of normal tissues (see legend for details) versus perfusate hematocrit (%). (B) 95% confidence regions for the least squares fit to the Casson model (Equation C) which describes relative blood viscosity as a function of hematocrit. For the current measurements of intratumor blood viscosity $\alpha = 1.4 \pm 0.2$, and for those from a previous study of skeletal muscle $\alpha = 0.7 \pm 0.3$.

Fig. 7. Relative intratumor blood viscosity of the tumor perfusion illustrated in Fig. 4 versus perfusion pressure (mm Hg) for 11, 35, and 57% hematocrits. Inset, relative blood viscosity versus perfusion pressure in a skeletal muscle perfusion (9, 10).

The extent to which each of these five mechanisms (the effect of glycolcalyx on the luminal side of the endothelium on the intratumor viscosity is not known) outlined above contributes to increased viscous resistance in solid tumors is not known. Presumably, these contributions vary spatially, temporally, and 5A. Sasaki, R. K. Jain, A. A. Maghazachi, R. H. Goldfarb, and R. B. Heberman. Low deformability of lymphokine activated killer cells: a possible determinant of in vivo distribution, Cancer Res. (in press).
from one tumor to another, giving rise to the dynamic and heterogeneous blood flow characteristics which have made the study of tumor blood flow difficult.

Implications. The efficacy of various therapies depends crucially upon tumor blood flow. For example, in radiotherapy, chemotherapy, and immunotherapy, the delivery of blood-borne substances to the tumor (e.g., oxygen, cytotoxic agents, antibodies, killer cells) would be improved if tumor blood flow rate could be increased and/or made spatially and temporally more uniform. On the other hand, the ability to heat a tumor during hyperthermia could be facilitated by reducing the tumor blood flow and thereby reducing the convective transport of heat. We have presented here an approach which can be used to measure the three determinants of tumor blood flow: \( \Delta P \), \( z \), and \( \eta \). This methodology can be now used to screen various physical and chemical agents which modulate blood flow by changing one or more of these parameters. An improved understanding of the mechanisms of tumor blood flow modification is likely to lead to novel strategies for management of solid tumors.

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