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Nitric oxide reduces tumor cell adhesion to isolated rat postcapillary venules

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Adhesion of circulating tumor cells to microvascular endothelium plays an important role in tumor metastasis to distant organs. The purpose of this study was to determine whether nitric oxide (NO) would attenuate tumor cell adhesion (TCA) to naive or lipopolysaccharide (LPS)-treated postcapillary venules. A melanoma cell line, RPMI 1846, was shown to be much more adhesive to postcapillary venules isolated from rat mesentery than to corresponding precapillary arterioles. Although venules exposed to LPS for 4 h demonstrated an increased adhesivity for the melanoma cells, TCA to LPS-treated arterioles was not altered. Isolated venules exposed to DETA/NO (1 mM), an NO donor, for 30 min prior to tumor cell perfusion prevented the increment in adhesion induced by LPS and attenuated TCA to naive postcapillary venules. While L-arginine (100 μM), an NO precursor, failed to decrease TCA to naive postcapillary venules, this treatment abolished LPS-stimulated TCA to postcapillary venules. The effect of L-arginine was reversed by administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 μM), an NO synthase (NOS) inhibitor. These observations indicate that both exogenous and endogenous NO modulate TCA to postcapillary venules. To assess the role of NO-induced activation of cGMP in the reduction in TCA produced by DETA/NO, two additional series of experiments were conducted. In the first series, LY-83583 (10 μM), a guanylyl cyclase inhibitor, was shown to completely reverse the effect of DETA/NO on TCA to both naive and LPS-activated postcapillary venules. On the other hand, administration of 8-bromoguanosine 3′,5′-cyclic monophosphate (8-B-cGMP) (1 mM), a cell permeant cGMP analog, mimicked the effect of DETA/NO and reduced TCA to LPS-stimulated postcapillary venules. These data suggest that (a) tumor cells are more likely to adhere to postcapillary venules than to corresponding precapillary arterioles, (b) LPS enhances TCA to postcapillary venules, (c) both exogenously applied (DETA/NO) and endogenously generated (L-arginine) NO attenuate the enhanced adhesion induced by LPS, but only DETA/NO reduced TCA to naive postcapillary venules, and (d) the NO-induced reduction in TCA to LPS-activated postcapillary venules occurs by a cGMP-dependent mechanism.

Keywords: cancer, endothelium, invasion, melanoma cells, metastasis

Introduction

Tumor cell metastasis is a complex, multistep process that involves cell separation from the primary tumor, entry into the vascular and lymphatic systems, transport to and arrest within the microcirculation of distant organs and extravasation [1,2]. The arrest of tumor cells within the microcirculation of distant organs is a key event in the metastatic process and is thought to occur by entrapment in capillaries and/or by forming adhesive interactions between circulating tumor cells and microvascular

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endothelium. The latter mechanism is particularly appealing because it provides a rationale for organ-specific metastasis [2,3]. Thus, an intensive research effort has been directed at defining the factors that contribute to tumor cell–endothelial cell adhesion (TCA). A large number of endogenous proinflammatory factors such as eicosanoid metabolites [4,5], thrombin [6], interferon gamma [7], tumor necrosis factor, interleukin-1, and lipopolysaccharide (LPS) [8–12] have been shown to promote tumor cell adhesion to endothelial cells by promoting the expression of adhesive ligands on the surfaces of both cell types. Comparatively less is known about endogenously produced factors that exert an inhibitory influence on tumor cell adherence. However, a growing body of evidence suggests that nitric oxide (NO) may be a naturally occurring anti-adhesive molecule. For example, NO inhibits platelet aggregation [13,14] and platelet adhesion to both vascular endothelium [15] and subendothelial matrix [16]. NO has also been shown to inhibit neutrophil adhesion to postcapillary venular endothelium [17,18]. More recent experimental evidence indicates that nitric oxide synthase (NOS) activity of tumor cells is correlated with their metastatic ability [19]. It has also been suggested that NO may be able to affect tumor cell metastasis either by inhibiting platelet aggregation [20] or by interfering with the adhesive process that modulates tumor cell adhesion to cultured endothelial cells [21]. Thus, the purpose of this study was to test the effects of both exogenous and endogenous NO on TCA to postcapillary venular endothelium. In addition, since LPS induces the surface expression of adhesive molecules thought to participate in TCA to endothelial cells and upregulates NOS activity, we also sought to determine the influence of NO on TCA to LPS-treated postcapillary venules. A final goal was to examine the mechanisms whereby exogenous NO attenuates TCA to postcapillary venules under these conditions.

Materials and methods

Tumor cell line and culture conditions

RPMI 1846, a melanotic melanoma derived from hamster, was obtained from American Type Culture Collection (Rockville, MD, USA) and grown in McCoy's 5A medium (Gibco, Grand Island, NY, USA) supplemented with 20% fetal bovine serum (Gibco) at 37°C in a humidified 5% CO_2 atmosphere. Once confluent, cells were harvested by brief trypsinization with 0.25% trypsin (Gibco), neutralized with complete culture medium, centrifuged at 100***g*** for 5 min and resuspended at a concentration of 10^6 cells/ml in physiological salt solution (PSS) consisting of 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM $CaCl_2$, 1.17 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer to which fetal bovine albumin was added at a final concentration of 1% (APSS). The solution was buffered to a pH of 7.4 and filtered through P8 filter paper (Fisher Scientific, Pittsburgh, PA, USA) before adding the cells. Cell viability was assessed by trypan blue staining and only cell suspensions with viability greater than 90% were used for experiments.

Isolated vessel preparation

Male Sprague–Dawley rats or hamsters weighing between 50 g and 150 g were administered 10 ml/kg PSS or 10 ml/kg PSS containing lipopolysaccharide (LPS; Sigma, St Louis, MO, USA) at 1 mg/ml by intraperitoneal injection. Four hours later, the animals were anesthetized with Inactin (100 mg/kg i.p.) and the superior mesenteric artery was isolated and cannulated via a midline incision. To visualize microvessels for subsequent extrication, warm porcine gelatin solution containing India ink (1 ml) was injected into the mesenteric vasculature via the superior mesentery artery. The porcine gelatin India ink solution was prepared by dissolving 0.36 porcine skin gelatin (Sigma) and 0.2 ml non-dialysed India ink in 10 ml warm APSS and filtered through P8 filter paper. The solution is liquid at room temperature but gels at temperatures below 20°C. A segment of bowel and attached mesentery was then excised and placed in a dissecting chamber containing ice-cold PSS (pH 7.4). At this temperature, the porcine gelatin India ink solution, which is liquid at room temperature, forms a gel which prevents opposing sides of the vessel wall from rubbing together during the isolation procedure, thereby preserving endothelial integrity. An unbranched segment of an arteriole or venule, 70–100 μm in diameter (OD) and 1.5–2.0 mm in length, was dissected free, transferred to an isolated vessel chamber (Halpern type), cannulated on both ends with glass micropipettes (~50 μm diameter), secured with 11–0 suture, and bathed in 37°C PSS equilibrated with room air. The bath solution was in constant renewal at a rate of 2 ml/min. The vessel lumen was perfused with 37°C APSS via a gravity-fed reservoir connected to the inflow catheter. After cannulation, the vessel chamber was placed on an inverted microscope (Nikon, TMS-F). A television

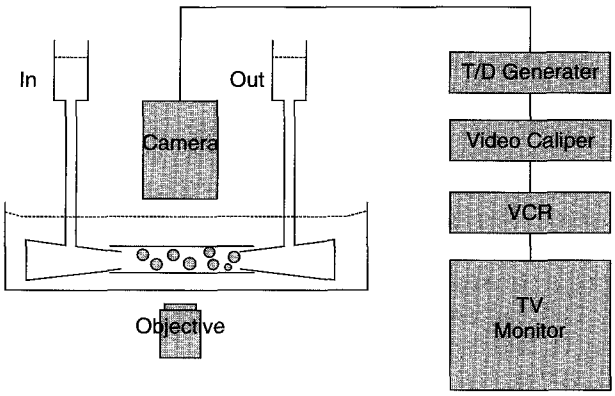

Figure 1. Isolated vessel preparation for tumor cell adhesion. See text for description.

camera (RCA TC1005) mounted on the microscope was used to project the image onto a television monitor (Ultrak, KM-12) and the images were recorded using a videocassette recorder (Mitsubishi, HS-U65). A video time/date generator (Panasonic, WJ-810) projected the time, date, and stopwatch function onto the monitor. A video caliper (Microcirculation Research Institute, Texas A & M University) was used to measure the diameter of the vessel (Figure 1). The flow of tumor cell suspension through the isolated vessel was monitored on the TV screen. By adjusting the pressure gradient between the inflow and outflow reservoir connected to the vessel, the flow rate through the vessel could be modified. Tumor cell adhesive interactions with the endothelium were recorded using a video cassette recorder and analysed off line after the experiment.

Experimental protocols

Pilot experiments indicated that melanoma cells did not adhere to the walls of microvessels under flow conditions. Similar results were recently reported using an *in vitro* parallel plate flow assay [22]. In these latter studies, it was noted that while adhesive interactions did not occur at normal shear rates (related to perfusate flow rate), perfusion for short periods of time at low or absent shear rates allowed adhesive interactions to become established which were then able to withstand subsequent substantial increases in shear (flow) rate. In view of these observations, we adopted a similar protocol in our studies. After a 30 min stabilization period during which the vessels were perfused with APSS, the isolated arterioles or venules were perfused with the tumor cell suspension. Perfusate inflow was then interrupted and the tumor cells were allowed to settle onto the endothelium. The number of cells settling on the microvessel was observed and recorded. Twenty minutes later, perfusate flow was resumed at a pressure gradient of 115 cm H_2O (84.5 mmHg) between inflow and outflow micropipettes. Five minutes later, the number of tumor cells remaining adherent to the vessel wall was counted and recorded. The percentage of adherent cells was calculated by dividing the number of tumor cells remaining adherent to the vessel wall after flow was restored by the total number of cells that settled on the vessel wall when flow was interrupted.

Effect of LPS on TCA to precapillary arterioles and postcapillary venules. Rats in this group were treated with LPS (Sigma) as described above. Control animals received an equivalent volume of PSS but no LPS. Four hours later, the animals were sacrificed and an arteriole or venule was isolated from the mesentery for assessment of tumor cell adhesion, as described above.

Effect of exogenous nitric oxide on TCA to postcapillary venules. The isolated venules were bathed in PSS (37°C, pH 7.4) containing 1 mM DETA/NO ($H_2NCH_2CH_2N[N(O)NO]^-CH_2CH_2NH_3^+$, Chemical Abstracts Service registry number 146724–94–9), an NO donor with a half-life of 20 h under these conditions [23], for 30 min before, and also during, perfusion with the tumor cell suspension. Given the half-life (20 h) of DETA/NO under these conditions, NO was being generated into the bath at the rate of approximately 1 nmol/min/ml. To control for potential effects of the decomposition products of DETA/NO, diethylenetriamine (Aldrich, Milwaukee, WI, USA) and sodium nitrite (Aldrich) were added to the bath at concentrations of 1 mM each, and TCA was assessed as described above.

Effect of L-arginine and L-NAME on TCA to postcapillary venules. To assess the role of endogenous NO on TCA, five experimental protocols were employed. Isolated venules were bathed in PSS (37°C, pH 7.4) containing 0.1 mM L-arginine (Sigma), an NO precursor, 0.1 mM L-arginine methyl ester (L-NAME; Sigma), a competitive NOS inhibitor, 0.1 mM L-arginine plus 0.1 mM L-NAME, or 0.5 mM L-arginine and 0.1 mM L-NAME. A separate group of venules was obtained from LPS-treated animals and bathed in PSS containing 0.1 mM L-NAME. The vessels in each of these groups were then perfused with the tumor cell suspension as described above.

Effect of 8-bromoguanosine 3',5'-cyclic monophosphate (8-B-cGMP) on TCA to postcapillary venules. Isolated venules were bathed in PSS (37°C, pH 7.4) containing 1 mM 8-B-cGMP (Sigma), a cell permeant cGMP analog. Then the venule was perfused with the tumor cell suspension as described above.

Effect of LY-83583 on TCA to postcapillary venules. Since the vascular effects of NO have been shown to involve stimulation of guanylyl cyclase [24], isolated venules were bathed in PSS (37°C, pH 7.4) containing 0.1 mM DETA/NO plus 10 μM LY-83583 (Calbiochem, La Jolla, CA, USA), a guanylyl cyclase inhibitor. Then the venule was perfused with tumor cell suspension as described above.

Statistical analysis

The data were initially analysed by one-way analysis of variance (ANOVA). To determine which groups were statistically different, the Student–Newman–Keuls test was used. A value of $P < 0.05$ was considered to indicate statistical significance. All data are presented as the mean ± SEM.

Results

Figure 2 compares the adhesion of RPMI 1846 melanoma tumor cells to precapillary arterioles and postcapillary venules and illustrates the effects of LPS treatment on TCA to both vessel types. Melanoma cells adhered much more avidly to naive (non-LPS-treated) venules versus untreated arterioles. While postcapillary venules isolated from LPS-treated animals demonstrated a significant increase in TCA relative to naive venules, LPS had no effect on TCA to precapillary arterioles.

As shown in Figure 3, administration of the NO donor, DETA/NO, completely prevented the enhanced TCA induced by LPS and significantly reduced TCA to naive venules. This anti-adhesive effect was not mediated by the decomposition products of DETA/NO since combined administration of diethylenetriamine and sodium nitrite failed to alter TCA to LPS-activated venules (data not shown). To determine whether endogenous NO influenced TCA to naive or LPS-treated postcapillary venules, these vessels were pretreated with the NO precursor, L-arginine, or L-NAME, a NOS inhibitor of (Figures 4 and 5). Treatment of isolated venules with either 100 μM L-arginine or 100 μM L-NAME in the absence of LPS had no effect on TCA. Although NOS inhibition with L-NAME had no effect on TCA

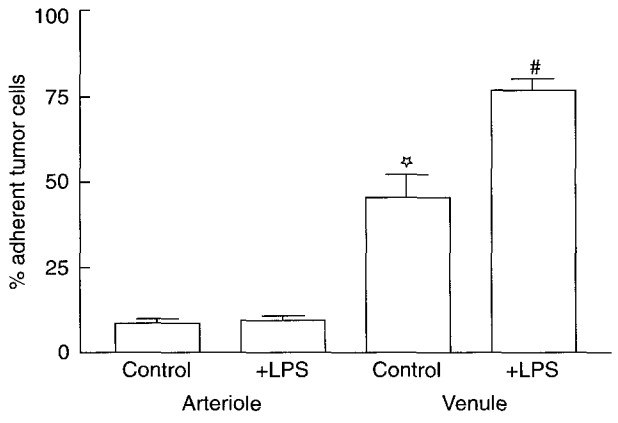

Figure 2. Comparison of RPMI 1846 melanoma cell adhesion to naive and LPS-activated precapillary arterioles and postcapillary venules. Four hours before vessel isolation, some rats were treated with LPS dissolved in PSS (1 mg/ml) at a dose of 10 mg/kg i.p. Control animals were treated with the PSS vehicle (10 ml/kg i.p.). Then the animals were sacrificed and an arteriole or venule was isolated from the mesentery for assessment of TCA as described in the methods section. The data are presented as the mean ± SEM. * and # indicate values that were significantly different (at $P < 0.05$) from corresponding values in arterioles or control venules, respectively. (Control = naive vessels, +LPS = LPS-activated vessels.)

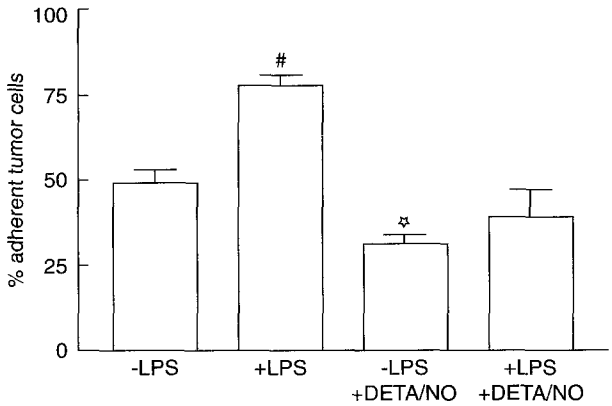

Figure 3. Effect of DETA/NO, an exogenous NO donor, on RPMI 1846 melanoma cell adhesion to naive and LPS-treated postcapillary venules. After isolation and cannulation, the venules were bathed in 37°C PSS or PSS containing 1 mM DETA/NO for 30 min prior to vessel perfusion with the tumor cell suspension. TCA was assessed as described in the methods section. The data are presented as the mean ± SEM. * and # indicate values that were significantly different (at $P < 0.05$) from those in the –LPS or +LPS +DETA/NO groups, respectively. (–LPS = naive venules, +LPS = LPS-activated venules, +DETA/NO = venules treated with 1 mM DETA/NO).

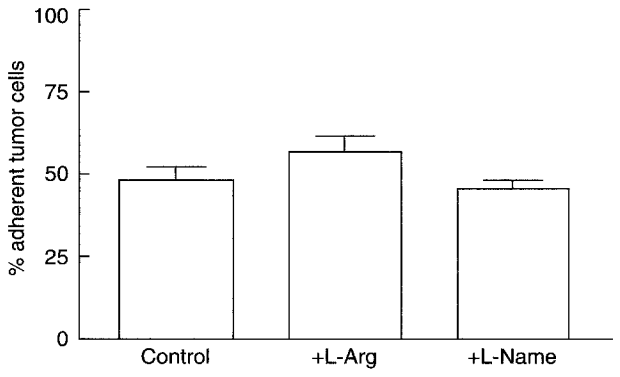

Figure 4. Effect of L-arginine, a NO precursor, and L-NAME, a competitive NO synthase (NOS) inhibitor, on RPMI 1846 melanoma cell adhesion to naive postcapillary venules. The isolated venules were bathed in 37°C PSS containing either 0.1 mM L-arginine or 0.1 mM L-NAME for 30 min prior to venule perfusion with tumor cell suspension. TCA was assessed as described in the methods section. The data are presented as the mean ± SEM. (Control = naive venules, +L-Arg = naive venules treated with 0.1 mM L-arginine, +L-NAME = naive venules treated with 0.1 mM L-NAME.)

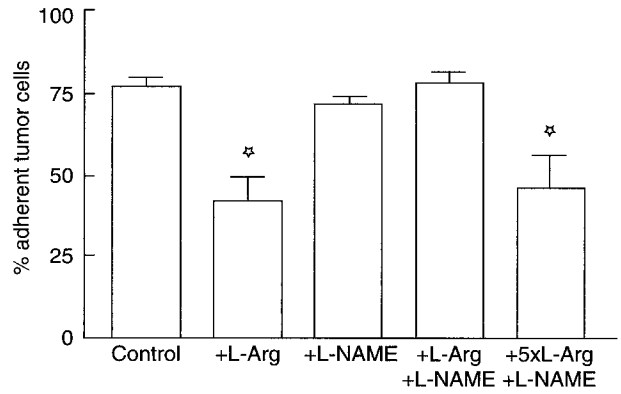

Figure 5. Effect of L-arginine, a NO precursor, and L-NAME, a competitive NO synthase (NOS) inhibitor, on RPMI 1846 melanoma cell adhesion to LPS-treated postcapillary venules. The isolated venules were bathed in 37°C PSS containing either 0.1 mM L-arginine, 0.1 mM L-NAME, 0.1 mM L-arginine plus 0.1 mM L-NAME, or 0.5 mM L-arginine plus 0.1 mM L-NAME for 30 min prior to venule perfusion with tumor cell suspension. TCA was assessed as described in the methods section. The data are presented as the mean ± SEM. The two groups marked with an asterisk were significantly different at the $P < 0.05$ level from the other three groups, but not from each other. (Control = LPS-activated venules, +L-Arg = LPS-activated venules treated with 0.1 mM L-arginine, +L-NAME = LPS-activated venules treated with 0.1 mM L-NAME, +5 × L-Arg = LPS-activated venules treated with 0.5 mM L-arginine.)

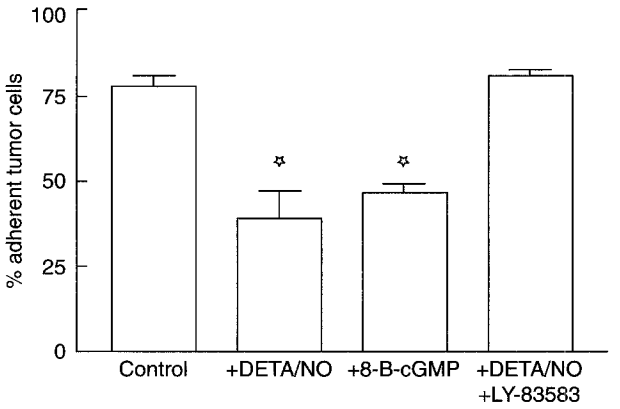

Figure 6. Effects of 8-bromoguanosine 3',5'-cyclic monophosphate (8-B-cGMP), a cell permeant analog of cGMP, and LY-83583, a guanylyl cyclase inhibitor, on RPMI 1846 melanoma cell adhesion to LPS-activated postcapillary venules. The isolated venules were bathed in 37°C PSS containing either 1 mM DETA/NO, 1 mM 8-B-cGMP, or 1 mM DETA/NO plus 10 μm LY-83583 for 30 min prior to venule perfusion with tumor cell suspension. TCA was assessed as described in the methods section. The data are presented as the mean ± SEM. The two groups marked with an asterisk were significantly different at the $P < 0.05$ level from the other two groups, but not from each other. (Control = LPS-activated venules, +DETA/NO = LPS-activated venules treated with 1 mM DETA/NO, +8-B-cGMP = LPS-activated venules treated with 1 mM 8-B-cGMP, +LY-83583 = LPS-activated venules treated with 10 μm LY-83583.)

to LPS-treated venules, the enhanced TCA noted in these vessels was significantly reduced by administration of L-arginine (100 μM). This effect of L-arginine (100 μM) was prevented by co-administration of 100 μM L-NAME in LPS-treated postcapillary venules. However, a 5-fold excess of L-arginine (500 μM) overwhelmed the effect of 100 μM L-NAME and prevented the enhanced TCA to LPS-treated postcapillary venules.

Since it is well known that NO activates guanylyl cyclase and increases cGMP, we examined the influences of a guanylyl cyclase inhibitor on the ability of NO to reduce TCA to LPS-treated venules (Figures 6 and 7). Pretreatment with LY-83583 completely reversed the ability of DETA/NO to reduce TCA to either naive or LPS-activated postcapillary venules. The notion that NO-induced guanylyl cyclase activity contributes to the anti-adhesive effect is further supported by the observation that 8-B-cGMP mimicked the inhibitory effect of NO on TCA to LPS-treated venules. In contrast, treatment of naive venules with 8-B-cGMP failed to reduce TCA to naive postcapillary venules.

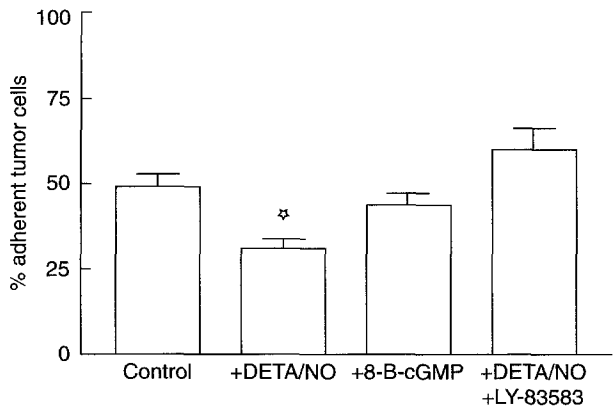

Figure 7. Effects of 8-bromoguanosine 3',5'-cyclic monophosphate (8-B-cGMP), a cell permeant analog of cGMP, and LY-83583, a guanylyl cyclase inhibitor, on RPMI 1846 melanoma cell adhesion to naive postcapillary venules. The isolated venules were bathed in 37°C PSS containing either 1 mM DETA/NO, 1 mM 8-B-cGMP, or 1 mM DETA/NO plus 10 μm LY-83583 for 30 min prior to venule perfusion with tumor cell suspension. TCA was assessed as described in the methods section. The data are presented as the mean ± SEM. The group marked with an asterisk was significantly different from the other groups at the $P < 0.05$ level. (Control = naive venules, +DETA/NO = naive venules treated with 1 mM DETA/NO, +8-B-cGMP = naive venules treated with 1 mM 8-B-cGMP, LY-83583 = naive venules treated with 10 μm LY-83583.)

Discussion

It is clear that the arrest of tumor cells within the microcirculation of distant organs is a critical step in tumor metastasis [1,2]. Most prior studies regarding tumor cell–endothelium interactions have been conducted using static adhesion assays [8,9,25]. While such approaches have provided important insight regarding the mechanisms of TCA to endothelial cells, static assays do not take into account the influence of wall shear rates related to blood flow on TCA. That is, TCA is determined not only by the adhesive forces generated by the interaction of molecules on cell surfaces, but also by hydrodynamic shear forces generated by the flowing blood which tend to sweep the adherent cells away from the endothelium. Since it was possible that TCA to cultured endothelium may not truly reflect *in vivo* events, we sought to develop a model to study tumor cell–endothelium interactions under conditions that more closely mimic the situation encountered by tumor cells flowing through intact microvessels. However, tumor cells failed to adhere at normal or even dramatically reduced (1% of normal) shear stresses. Our results are similar to those reported by Giavazzi *et al.* [22] and Menter *et al.* [26] who used a parallel flow chamber adhesion assay to examine TCA to cultured endothelium under conditions of flow. These investigators demonstrated that TCA to endothelium only occurs when wall shear rate is reduced far below normal physiological levels.

While our results are consistent with these *in vitro* studies, the lack of adhesion under conditions of flow could be due to an alteration in hydrodynamic dispersal forces that displace tumor cells toward the vessel wall and allow adhesive contacts to become established. *In vivo*, these forces are dependent on the presence of red blood cells which act to displace tumor cells towards the vessel wall. To determine whether imposition of such hydrodynamic shear forces would modify TCA in our model, we added glass microbeads (11.4 ± 0.1 μm diameter, 5×10^6 beads per ml perfusate) or erythrocytes (Hct = 1 and 40%) to the perfusate. However, these maneuvers failed to promote tumor cell adhesion. It has also been suggested that platelets, which can adhere to circulating tumor cells, may facilitate TCA by acting as a tether or bridge between the tumor cell and endothelium. Addition of platelet-rich plasma (at a concentration of 10^7 platelets/ml perfusate) to the perfusate also failed to promote adhesion. This latter observation also suggests that the absence of serum from the perfusate normally used in these experiments does not explain the lack of tumor cell adhesion to endothelium under conditions of flow. Administration of LPS, an intervention known to upregulate the expression of endothelial cell adhesion molecules such as ICAM-1 and E-selectin, also failed to promote adhesion under conditions of flow. We also conducted studies in which the melanoma cells were harvested by agitation rather than trypsin. Again, no TCA was observed under conditions of flow. This latter result suggests that potential trypsin-induced modifications in adhesion molecule function do not represent a likely explanation for the lack of TCA adhesion when flow is present. Since the melanoma cell line used in these studies was derived from the hamster, we also evaluated TCA adhesion to mesenteric venules isolated from this species. No adhesion was noted under conditions of flow when hamster microvessels were perfused with the hamster melanoma cell line. Lastly, intravital microscopic examination of TCA in venules perfused *in situ* via the mesenteric arcade arteries with the melanoma cell suspension revealed that melanoma cells did not adhere in any segment of the intact microvasculature, although transient capillary plugging was occasionally observed.

These studies suggest that melanoma cells are unlikely to establish adhesive interactions with the microvascular endothelium when perfused at normal physiological shear rates. However, our results do indicate that if perfusate or blood flow is interrupted for a short period of time, TCA to microvascular endothelium is increased. These adhesive interactions become stabilized over time and are quite strong. That is, once TCA becomes established during low or no flow conditions, the adherent melanoma cell is able to withstand subsequent substantial increases in wall shear rate and remain adherent. There are several physiological and pathophysiological conditions which could result in the reduction or even transient cessation of blood flow in local tissue regions. First, rhythmic intrinsic arteriolar vasomotion, which is a normal physiological activity of vascular smooth muscle, can substantially reduce the blood flow in the microcirculation. Indeed in many peripheral vascular beds, only a portion of capillaries (~25% in the intestine) are open to flow under resting conditions [27]. The pattern of capillary perfusion is not static, but changes over time such that flow in previously perfused capillaries can cease. Other capillaries, previously without flow, can be recruited at this time to maintain adequate oxygen and nutrient delivery to the tissues. Secondly, severe vasospasm associated with sympathetic discharge or other factors may impede blood flow and result in transient ischemia to a limited area. Microembolism can also induce the same effect. The net effect is that circulating tumor cells, which normally are prevented from establishing adhesive interactions by the flow of blood, can now settle onto the endothelial cell surface. During the period of transient ischemia, adhesive interactions become established and stabilized such that the adherent tumor cell is able to withstand subsequent substantial increases in wall shear rate when flow is re-established.

Our results indicate that if flow is transiently reduced, tumor cells can form adhesive contacts that allow the cell to remain adherent even in the face of substantial subsequent increases in shear rate. Similar findings have been reported *in vitro* [22]. Furthermore, our results indicate that tumor cells are much more adhesive to postcapillary venules than to precapillary arterioles. The latter effect is amplified by prior exposure to LPS (Figure 2). These findings support the hypothesis that interaction of circulating tumor cells with microvascular endothelium may be enhanced at sites of inflammation or when blood flow and shear rate are low.

NO, a free radical produced by endothelial cells, macrophages, neutrophils and some tumor cell types [19,28,29], relaxes vascular smooth muscle and vasodilates the microvasculature [30,31], blocks neutrophil adhesion to postcapillary venules [17], inhibits platelet aggregation and adhesion [14–16], and modulates TCA to cultured endothelial cells [21]. Our data indicate that DETA/NO, an exogenous NO donor, significantly reduces TCA to naive as well as LPS-treated venules (Figure 3).

Because L-NAME inhibits both inducible and constitutively expressed NOS, we expected that this agent would increase TCA to naive venular endothelium by inhibiting constitutively expressed NOS, thereby reducing baseline synthesis of NO. However, our results showed that L-NAME had no effect on TCA to naive venules. This effect may have been due to the fact that the perfusing and bathing solutions in our isolated vessel perfusing system were free of L-arginine, the substrate for NO production by NOS. However, addition of L-arginine to the bathing solution also failed to influence TCA to naive venules. These results suggest that the naive postcapillary venular endothelial cells may not be able to produce sufficient NO to modulate TCA.

It is well known that endothelial cells use L-arginine as a substrate for the synthesis of NO, a reaction catalysed by a constitutively expressed NOS. L-Arginine is also a substrate for inducible NOS, which is activated by a variety of proinflammatory agents including LPS or cytokines [28,29]. Therefore, we hypothesized that L-arginine would attenuate TCA to postcapillary venules in which NOS activities were elevated with LPS. Indeed, we observed that L-arginine blocks the stimulatory effect of LPS on TCA to venular endothelium, an effect that was reversed by concomitant administration of L-NAME.

It is now clear that the vascular effects of NO are mediated by activating a guanylyl cyclase-dependent pathway [24]. In our studies, 8-B-cGMP mimicked the effects of NO and reduced TCA to LPS-activated venules but not to naive venules. On the other hand, LY-83583, a guanylyl cyclase inhibitor, blocked the ability of NO to reduce TCA to both naive and LPS-activated venules. The reason that 8-B-cGMP failed to reduce TCA to naive venules but was effective in LPS-treated venules is not clear. One explanation may be that the cell membrane of naive venular endothelium is not as permeable to 8-B-cGMP as the plasmalemma in venules obtained from LPS-treated animals. Whatever the explanation, our results suggest that NO reduces TCA to postcapillary venules by a cGMP-dependent pathway.

In summary, the results of this study indicate that melanoma cell adhesion to venular endothelium is not likely to occur under normal blood flow conditions. However, the likelihood for tumor cells to adhere to the intact blood vessel wall is greatly enhanced by conditions associated with the development of inflammatory reactions or reduced blood flow. Exogenous administration or endogenous generation of NO inhibits TCA to LPS-activated endothelium by a cGMP-dependent mechanism. Furthermore, exogenous NO also reduces adhesion of tumor cells to naive venular endothelial cells. These observations raise the possibility for the potential application of NO as a means to reduce tumor cell metastasis. This is especially appealing in light of the vasodilatory properties of NO, which should enhance wall shear rate and decrease the likelihood that an adhesive interaction can be initiated.

Acknowledgements

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