Lymphology 38 (2005) 181-192

MONITORING OF NICOTINE IMPACT IN MICROLYMPHATICS OF RAT MESENTERY WITH TIME-RESOLVED MICROSCOPY

E.I. Galanzha, P. Chowdhury, V.V. Tuchin, V.P. Zharov

Philips Classic Laser Laboratories (EIG,VPZ), University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA, Saratov State University (EIG,VVT), Saratov, Russia, and Department of Physiology and Biophysics (PC), University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

ABSTRACT

The aim of the study was to evaluate nicotine-induced microlymphatic responses in rat mesentery in vivo with time-resolved transmission microscopy. Male Fisher rats weighing 150-200g were used in all experiments. The experiments revealed that changes of lymphatic function under direct nicotine impact (continuous topical application of nicotine in the concentration ranges of 0.001 mM, 10 mM and 100 mM for 15 minutes) were dose- and time-dependent. A nicotine dose of 10mM caused an immediate short-term constriction of 100% of lymphangions. At the highest concentration of nicotine (100 mM), similar constriction as well as inhibition of lymph flow, local stable constriction of lymph microvessels, stasis in blood microvessels, and disturbances of respiration were observed. Application of chronic nicotine (0.23 ml from a 10 mM solution) injection for 14 days via a mini-osmotic pump did not substantially change the function of lymphatic and blood microvessels. Our data show for the first time that the acute effect of nicotine is accompanied by significant changes in microlymphatic function. We conclude that the observed effects are due to the direct action of nicotine on lymphatics. The mechanism by which nicotine induces this response remains to be investigated further.

Keywords: nicotine, lymph microvessels, lymphangions, lymphatic contractility, microcirculation, endothelium, rat mesentery, time-resolved microscopy

Nicotine, an important component of cigarette smoke, has been shown to be indirectly responsible for damage to many tissues in both humans and animals (1-4). Exposure to cigarette smoking increases the nicotine level in the blood to a maximum in less than 10 seconds (5-8). Therefore, the immediate responses of living tissues to the action of nicotine would appear to be important for development of these acute changes. It is also known that nicotine affects different types of blood macro- and microvessels (e.g., arteries, veins, arterioles, venules, etc.), but the observed effects are controversial and sometimes conflicting. Studies in human and animal vessels conducted *in vitro* and *in vivo* have revealed that nicotine induces an endothelium-dependent or independent dilatation of the microvessels (9-16). These effects have been attributed to the activation of acetylcholine receptors and/or $ß_2$-adrenergic receptors leading to nitric oxide production (9,16). Other studies have reported that nicotine induces the constriction of blood vessels (17-20), and these effects may be due to oxidative stress and acceleration of free radical reactions (21). The effects of nicotine

in human skin vasculature have been shown to enhance norepinephrine-induced vasoconstriction and inhibition of endothelium-dependent vasodilator responses (22). Dynamics of nicotine effects on blood vessels (constriction as well as dilation) are dose- and time-dependent (23,24). In addition to the vascular effects, a rise in blood flow velocity in the microvessels of the brain of warm-blooded animals and frog web have also been reported (25,26). An increase in leukocyte rolling and adhesion in cerebral mouse microcirculation *in vivo* as well as impaired blood rheology in human (e.g., decrease of RBCs deformability) by nicotine show to be dose-dependent (27-29).

Many investigators have postulated that nicotine affects the vascular network through production of NO and oxygen radicals (9,17,22, 24-27,30-35). However, some studies show that nicotine does not modulate relaxation induced by nitroglycerine and sodium nitroprusside, a donor for NO production (22,33). Additionally, the mechanisms by which nicotine induces dysfunction of blood vessels could be mediated by damaged endothelial cells and dysfunction particularly due to apoptosis (36-39). Nicotine also alters the responses to vasoactive substances, causes impairment of vasodilatory action of acetylcholine and ATP, brings about enhancement of the vasoconstrictor effect of noradrenaline, induces the loss of sensitivity to bradykinin, and inhibits the histamine (21,33-35).

In general, in recent years, the pathological effects of nicotine on the blood microcirculation have been reported in detail, but disturbances in the lymphatic system caused by nicotine remain obscure. Therefore, because there is a close functional relationship between the blood and lymph vasculatures and active participation of the lymphatic system in different pathologic conditions, we hypothesized that the lymphatic system could be involved and play an important role in understanding the mechanisms of nicotine induced toxicity.

To our knowledge, effects of nicotine on the lymphatic system are poorly understood. However, the fact that airway edema occurs with smoking (40) raises the possibility that damage to the lymphatic drainage system might be involved. From the available literature only a single study of lymphatic vessels of smokers was found (41), where lymphatic responsiveness (constriction responses) in human cigarette smokers was altered by eicosanoid production (41).

Earlier, we demonstrated the advantages of rat mesentery as an animal model for real time *in vivo* monitoring of acute lymphatic microvessel replay (changes in diameter, phasic contractions, valve activity, lymph flow) in response to the direct action of different vasoactive drugs (sodium nitroprusside, N-Nitro-L-arginine, DMSO, and etc.) (42-44). In particular, sodium nitroprusside led to significant dilatation and inhibition of valve activity; N-Nitro-L-arginine caused stimulation of phasic-contractile activity and significantly increased lymph flow. We showed that staphylococcal toxin significantly changed lymphatic microvessel function and lymph flow including the production of stasis and constriction of small lymphatics leading to obstruction. The rat mesentery model of venous insufficiency, lymphedema and pathologic stress (immobilization plus sound) allows study of the dynamics of microcirculation disturbances in the development of these abnormalities in greater detail, including the latent stage of lymphedema (43-46). The aim of the current study was to identify the nicotine induced response in microlymphatics of rat mesentery *in vivo* as evaluated with transmission digital microscopy.

MATERIALS AND METHODS

Animal Model

Rat mesentery was selected as the animal model based on our own prior experience (42-48) and evidence from other laboratories

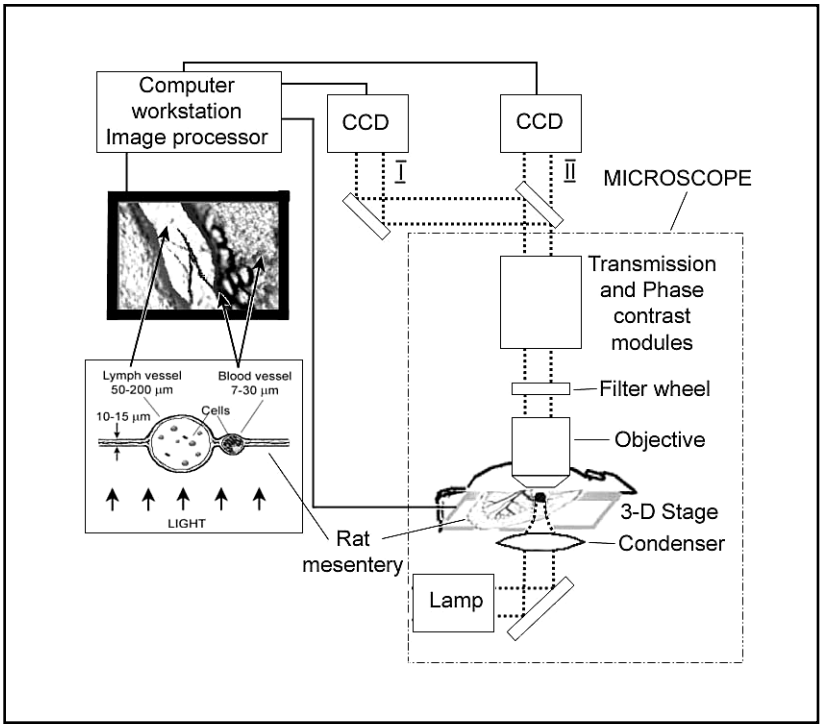

Fig. 1. Schematics of transmission image microscopy in vivo for the study of lymph and blood microcirculation of rat mesentery

that demonstrated its potential advantages as an intravital model for monitoring lymphatic parameters using Transmission Digital Microscopy (TDM) (49-51).

Male Fisher rats weighing 150-200g were used in all experiments (23 lymph microvessels total), in accordance with UAMS protocol #2318, approved by Institutional Animal Care and Use Committee. All rats were housed in an environmentally controlled vivarium with a 12:12-hour light-to-dark cycle. Rats were allowed free access to standard diet and water. Before an experiment, rats were anesthetized by administration of Nembutal (50 mg/kg body weight, intramuscularly), and then a laparotomy was performed. The rat was placed on a heated underlay (37.7°C) of a customized microscope stage with an optical window of an upright Nikon microscope. The mesentery and intestine were maintained in Ringer's solution with 1% albumin (37°C, pH 7.4) during the experiment.

Imaging System

The microlymphatics of the intestinal mesentery of anesthetized rats were studied *in vivo* by TDM *(Fig. 1)*. Images were recorded with black-and-white CCD camera (Cohu 2122, Cohu, Inc., San Diego, CA) with 12 fps, which was sufficient for studying main parameters of microlymphatic function (see below). Scion Image software (Scion Corp., Frederick, MD) systems performed the scanning, processing, capturing, measuring, and editing of images, as well as analysis of the moving and static object images. As mentioned before (48), this is a relatively simple schematic that allows imaging the whole lymphangion, neighboring blood microvessels, and main mesenteric micro-

structures (lymphatic walls, leaflets of valve, and flowing single cells and etc.), as well as allowing monitoring of the dynamics (microvessel wall and valve leaflets motion, cell traffic in flow, etc.) with good resolution, *in vivo*, in real time without any contrast agent in the colorless lymphatic. By TDM, the dynamic of the following quantitative parameters of lymph microvessel function were measured: diameter (μm); proportion of lymphatics with phasic contractions (%); amplitude of phasic contraction (%; the percentage difference between maximum and minimum diameters during contraction: $A = (D_{max} - D_{min}) \times 100\%/D_{max}$); rate per minute of these contractions; proportion of lymphatics with working valves; rate per minute of valve opening; proportion of microvessels with lymph flow. To analyze the heterogeneity of lymphatic wall reactivity we measured diameter and its changes during contraction in at least 3 different locations of non-valvular part of each lymphangion.

Nicotine Administration

The acute effect of nicotine was studied by direct topical application in the concentrations 0.001 mM, 10 mM, and 100 mM solution during 15 min (12 lymphangions). Total volume of nicotine solution for each experiment was ~2ml. We determined microvessel function parameters prior to nicotine administration (intact state), and after nicotine administration for 3 min with continuous monitoring. Subsequent experiments were conducted at time intervals of 5, 10, and 15 min.

Chronic nicotine intoxication was induced in 4 rats via Alza mini-osmotic pumps (Alza Corporation, Palo Alto, California) from a stock 10 mM Nicotine solution at a nicotine delivery rate of 0.5 μl/h for 14 days. The total volume delivered was 0.23 ml with a standard deviation of 0.23 μl. When we were assured that the rat was fully anesthetized, the fur on the back area of the neck was shaved, the skin was pretreated with 70% ethanol and betadine and a small incision was made. The pump was implanted in a prepared subcutaneous "pocket." Then sutures were placed close to the wound and the animals were transferred to their cage. Animals were housed in the Division of Animal Care facility at the University of Arkansas for Medical Sciences and monitored daily. The skin suture was removed on the seventh day after surgery. After 14 days, rats underwent laparotomy and the function of mesenteric small lymph microvessels was analyzed.

To verify results of acute effects of nicotine and avoid the influence of side effects (such as the incision on microcirculation and operative stress), we compared the data from test groups with the control group (15 min topical application with Ringer's solution only; 4 lymphatics) at the same time points. Results of chronic experiments were compared with control groups where a mini-osmotic pump with physiological solution under the same conditions was used (3 rats).

The results were represented as mean ± standard error of the mean (±SE), Student's t test was employed for independent samples to determine the statistical significance. A p value of < 0.05 was considered as statistically significant.

RESULTS

Topical application of Ringer solution during 15 min showed only small variable changes of diameters (dilatation as well as constriction; 7-10 μm on average; *Fig. 2A*). More than 50% of lymphangions had vasomotions in the range ~2-3 %. Spontaneous phasic activity was recorded in half of investigated lymphatics. As we demonstrated earlier (43), this activity is characterized as single contraction cycles (systole of lymphangion, 2.7±0.1s in average) with relaxation periods between them (pause or diastole of lymphangion, 3.8±0.7s in average). For 15 minutes, the phasic contractions did not start in inactive

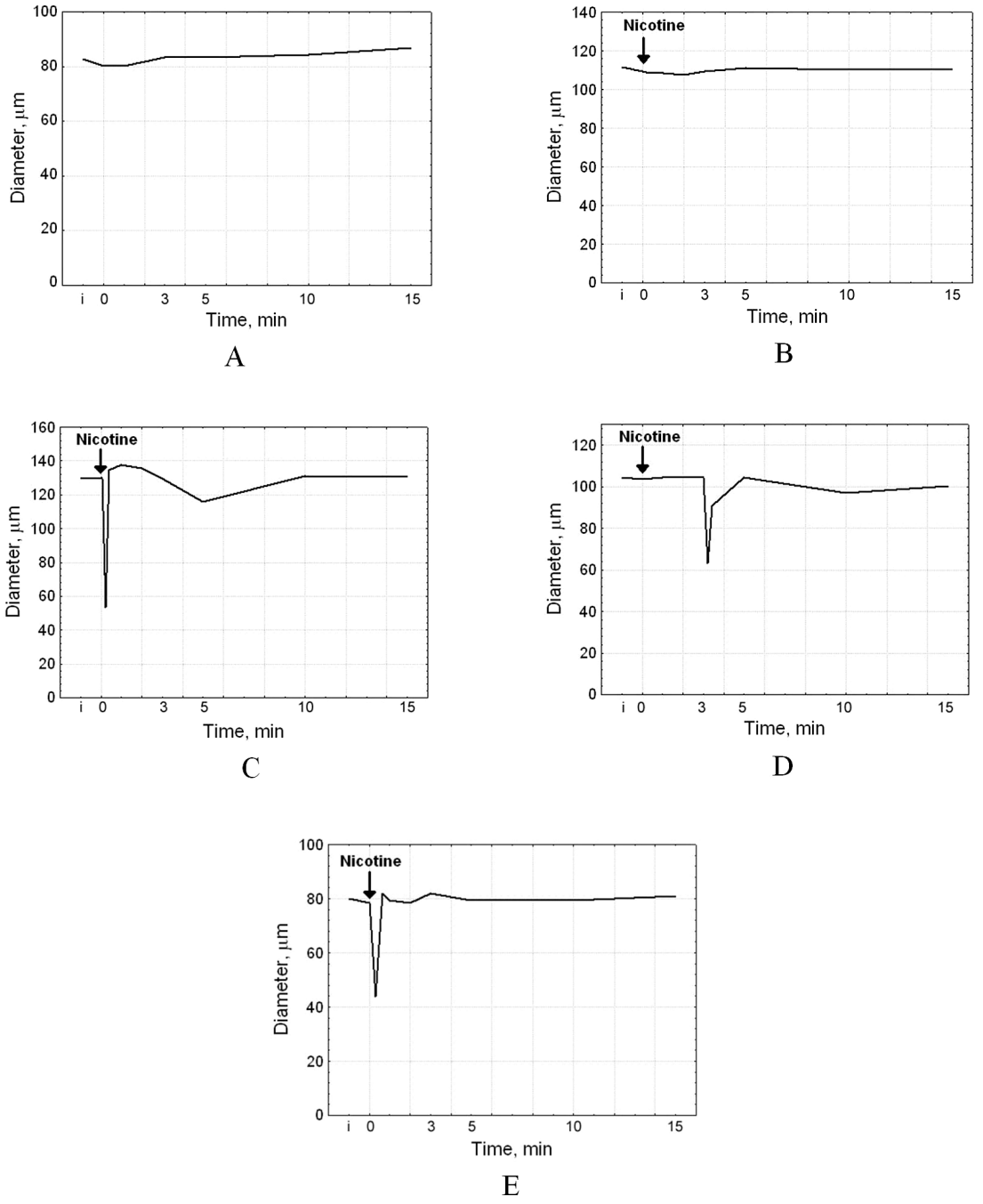

Fig. 2. The time-resolved monitoring of lymphangion diameter. A - Control group, 15 min topical application of Ringer solution; B-E - Test groups, 15 min topical application of different concentrations of nicotine solution: B - application of 0.001 mM nicotine solution, C and D - application of 10 mM nicotine solution and E - application of 100 mM nicotine solution. On all graphs small letter 'i' represents initial state before nicotine application. Data represented as mean ± SEM.

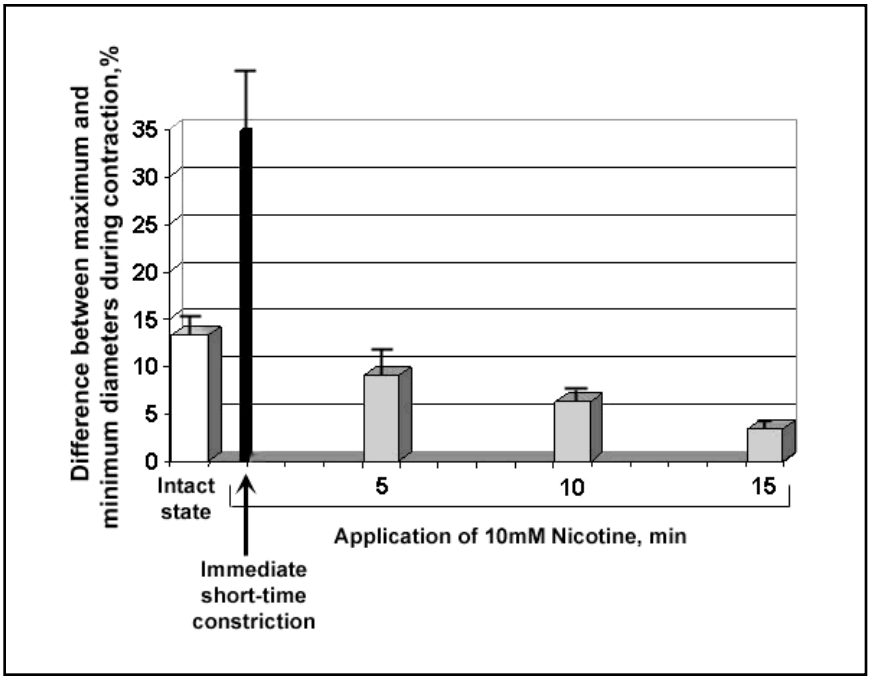

Fig. 3. The dynamic of lymphangion contractile activity during 15 min action of nicotine (10 mM solution): wide columns – amplitude of spontaneous (white) and nicotine-induced (gray) phasic contractions; narrow black column – immediate contractile response of lymphangion to start of nicotine application. Data represented as mean ± SEM

lymphatics. Valve activity was observed in 20-30% of lymphatics. All lymphangions were characterized by lymph flow and remained unaltered. Imaging of moving cells in the lymph flow allowed us to measure absolute cell velocities and the direction of their motion during typical lymph flow oscillations by video recording (frame-by-frame method). Mean cell velocity was ~200–300 μm/sec (43,47,48).

The topical application of nicotine solution at a concentration of 0.001 mM on lymphangions (initial mean diameter 125±5 μm) did not change microlymphatic function significantly *(Fig. 2B)*. However, significant constriction of microlymphatics (initial mean diameter 127±12 μm) on nicotine impact was observed at a dose of 10 mM. In particular, we observed the rapid response of 100% lymph microvessels. The effect started within 3-5 sec of application (67% of cases) or after ~3 min of nicotine action (33% of cases) *(Figs. 2C,D)*. The duration of effect was 12-40 sec. The mean lymphatic diameter decreased from 127.3±11.6 μm to 84.4±12.3 μm ($p<0.05$). The degree of constriction (34±7%) was more than ~2 times the amplitude of spontaneous phasic contractions in the intact state of lymphangions prior to nicotine application (13±2.5%) *(Fig. 3)*. Constricted lymphangions had markedly erratic diameter changes *(Fig. 4)*. In contrast, the lymphangion wall during spontaneous phasic contraction moved relatively uniformly *(Fig. 5)*.

After nicotine-induced constriction, the phasic activity was restored in some cases (in lymphatics which had spontaneous phasic activity before nicotine action) as well as sometimes it started in lymphangions without spontaneous phasic contractions. The nicotine-stimulated phasic activity was different from spontaneous activity. It was characterized by

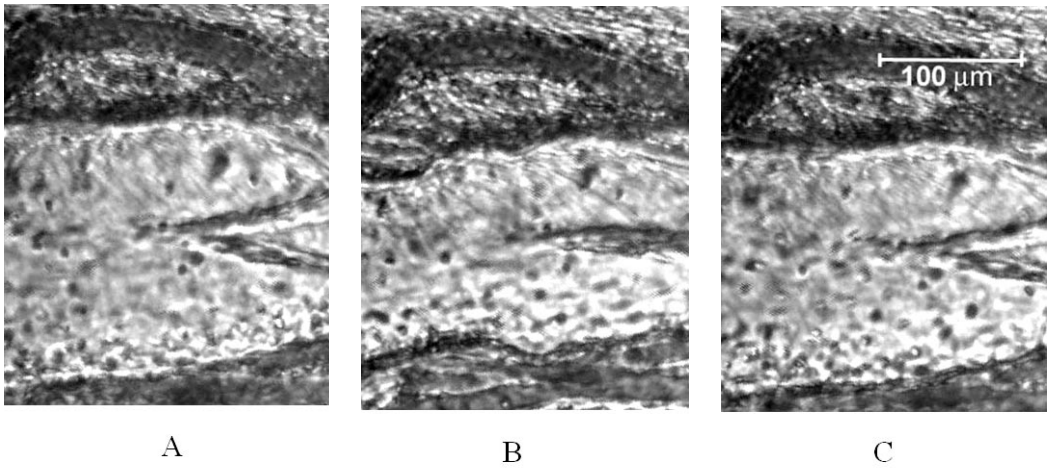

Fig. 4. The response of lymph microvessels at topical application to 10 mM nicotine solution: A – initial state of investigated lymphangions, B - immediate constriction of same region of lymphangion at the 15 sec after nicotine action (marked unequal diameter at the maximum constriction) and C – the relaxation at the 30 sec.

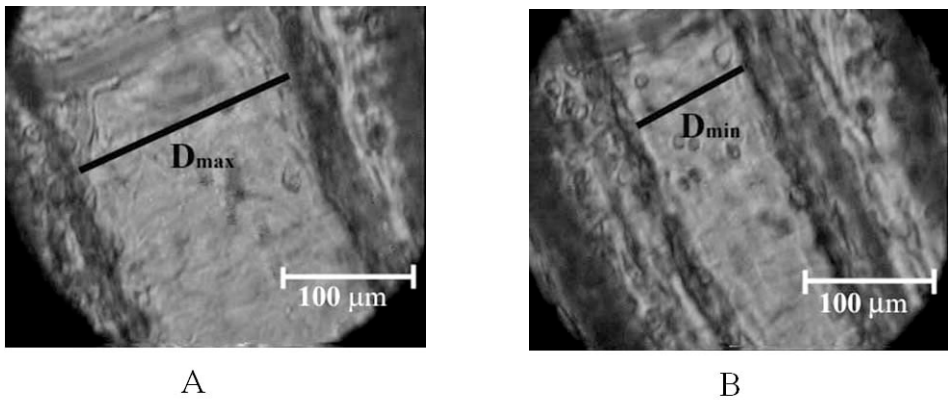

Fig. 5. Transmission images of intact lymphangion (during topical application of Ringer solution) region during spontaneous phasic contraction: A - maximum relaxation in diastole (D_{max}) and B - maximum constriction in systole (D_{min}), the lumen of lymphangion is constricted relatively uniformly.

asynchronous motion of the vessel wall and in some cases by groups of 5-6 contractions together without a period of diastole.

At the 5, 10, and 15 min of nicotine effect, the pathologic characteristics of phasic activity progressed with significant decrease in amplitude: from 13±2.5% in the initial state to 3.4±0.7% at 15 min ($p<0.05$) *(Table 1)*. Other recorded parameters of microlymphatic functions (lymph flow, valve activity, and diameter) did not change markedly from initial ones. Visual changes in blood microcirculation were absent for the observation period.

Upon application of the highest concentration of nicotine (100 mM) (initial mean diameter of lymphangions, 93±6 μm), the marked immediate constriction in all cases appeared as described above. The effect in 100% of lymphatics started within 3-5 sec

TABLE 1
Parameters of Phasic Activity of Lymph Microvessels During Topical Application of 10 mM Nicotine Solution

Time of nicotine effect, min	Proportion of lymphatics with phasic contractions, %	Amplitude of phasic contraction, %	Rate of phasic contraction, min^{-1}
initial state	83	13.2 ± 2.5	6.4 ± 1.6
5	100	9.0 ± 2.6	8.1 ± 2.5
10	67	6.3 ± 1.1*	9.3 ± 2.2
15	50	3.4 ± 0.7*	7.0 ± 2.6

* = significant differences from initial state, $p<0.05$

and continued 20-40 sec *(Fig. 2E)*. Degree of constriction was 28±8%. During the first minute, in parallel with constriction, stasis in blood microvessels and disturbances of respiration were documented.

Observations at 5, 10, and 15 min showed that phasic activity is absent at this dose of nicotine. It was noted that lymphangions of this group did not have spontaneous phasic activity before nicotine application. Additionally, there was a slowing of lymph flow and local stable constriction of lymph microvessels. Individual sensitivity to nicotine action was observed with disturbances of respiration which progressed, remained unchanged, or decreased. Disturbances of microcirculation usually continued during 15 min of observation (the slowing of lymph flow, local stable constriction of lymph microvessels, and stasis in some blood microvessels). In one case, respiratory standstill and the total stasis in blood and lymph microvessels after 5 min of nicotine application led to death of the rat.

Chronic nicotine intoxication in rats did not alter the function of lymphatic and blood microvessels and values did not differ notably from the control group.

DISCUSSION

The current study provides the first experimental evidence of potential active participation of lymphatic microvessels in the mechanism of nicotine effects *in vivo*. The data from our study indicate that nicotine stimulates a significant response of small microlymphatics, and its effects are dose and time dependent. We observed (1) unchanged lymphangion function at the lower nicotine dose, (2) significant immediate short-time constriction and pathologic phasic activity of lymph microvessels with a nicotine dose of 10 mM and (3) extended microlymphatic disturbances (short-time constriction, slowing of lymph flow and local stable constriction of lymph microvessels) in combination with blood microcirculation and respiration disturbances at the higher nicotine concentration (100 mM).

In the control group with Ringer's solution, the disturbances produced by nicotine action (immediate constriction, pathologic phasic activity, etc.) were absent. Additionally, our experiments showed that significant microlymphatic dysfunction appeared with a smaller dose of nicotine

than in the blood microvessels. Lymph microvessels may be more sensitive to nicotine, but this issue needs to be studied in detail in the future.

The immediate nicotine effect on lymphatics could reflect direct action on components of the microvessel wall and/or on free flowing cells inside the lymphangion. By analogy with blood vessels, the effect on lymphatics could induce specific nicotine-induced endothelial cell dysfunction and/or injury of smooth muscle cells of lymphatic wall (16,21,30-32,36-39). One might expect that the pathologic changes in lymphatic contraction are the result of a direct effect on the pacemaker of the lymphangion because there is some indication of disruption of the heart pacemaker function with nicotine (52). The possible mechanisms of the observed effects include also disturbances in eicosanoid production, which was a finding in lymph macrovessels wall of smokers (41). The marked variations in the degree of constriction along the lymphangion could be due to the uneven distribution of microlymphatic cell wall receptors which are sensitive to nicotine.

It was noted that the use of nicotine solution at the concentration of 10 mM during chronic intoxication was ineffective. The same observation has been obtained for peripheral rat blood vessels showing significant acute effect and absence of response at the chronic dose (11). This finding may be the result of adaptation of the microcirculation to the action of nicotine. The more intricate details (e.g., interaction of moving and static cell properties related to blood and lymph flow; interstitial edema; and tissue perfusion) and long-term studies (e.g., long-term intermittent inhalations of nicotine) need to be explored further to understand possible microcirculatory disturbances during chronic nicotine intoxication. The pathologic effects induced by nicotine may be amplified in some instances. For example, it has been demonstrated that nicotine decreases RBC deformability in essential hypertension and inhibits the efficacy of antihypertensive treatment on RBC deformability (28). Therefore, a significant decrease of threshold for microvessel reactivity due to action of nicotine in disease conditions with microvascular disturbances (e.g., cardiovascular disorders, sickle-cell diseases, diabetes, lymphedema) is likely to occur.

The mechanisms underlying cellular and subcellular disturbances of endothelial and smooth muscle microlymphatic cells as well as lymph and blood flow *in vivo* due to direct action of nicotine remain to be investigated further, including the effect of nicotine on NO-synthase pathways, oxidative stress, and cellular apoptosis. In this direction, the focus of our future studies will be on *in vivo* single cell responses to nicotine and the application of selective inhibitors of cell receptors based on the developed optical techniques. We have recently demonstrated that TDM provides high resolution (up to 350-400 nm) and high speed (with short exposure time up to 0.1m sec) real time imaging of rat mesentery at the single cell level *in vivo* without any contrast agent including images of lymph and blood flow (e.g., moving single RBCs, leukocytes, platelets, cell aggregation, cell shapes and sizes) and interstitial structures (e.g., fat cells, fibroblasts, fibers) (47,48). These improvements should allow us to obtain more detailed information on the impact of nicotine. A limitation of TDM technique is low absorption sensitivity, which does not allow imaging of intracellular structures. As shown in our recent data, the promising direction for the detailed study of cell migration (*in vivo* flow cytometry) and fixed cells can be pursued by the application of the combination of TDM and photothermal imaging (53,54).

Thus, we conclude that nicotine induces marked changes of small lymphatic function via an acute direct impact *in vivo*. The observed microlymphatic disturbances due to the action of nicotine may be an important mechanism of complex immediate reaction to cigarette smoking. The acute lymphatic damage from nicotine could be more crucial

in some disease conditions or during treatment regiments, where nicotine may contribute to vascular abnormalities and tissue edema.

ACKNOWLEDGMENTS

This work was supported by a grant from the NIH (EB001858) and by CRDF grant REC-006.

REFERENCES

1. Chowdhury, P, PL Rayford: Smoking and pancreatic disorders. Eur. J. Gastroenterol. & Hepatol. 12 (2000), 869-877.
2. Chowdhury, P, S Macleod, KB Udupa, et al: Pathophysiological effects of nicotine on the pancreas: An update. Exp. Biol. Med. 227 (2002), 445-454.
3. Report of Surgeon General: The Health Consequences of Smoking. Department of Health & Human Services, Public Health Services, Centers for Disease Control & Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office of Smoking & Health, Washington, D.C. 2004.
4. Chowdhury, P, PL Rayford, LW Chang: Induction of acinar cell pathology via inhalation of nicotine. Proc. Soc. Exp. Biol. Med. 20 (1992), 159-164.
5. Armitage, AK, CT Dollery, CF George, et al: Absorption and metabolism of nicotine from cigarettes. Br. Med. J. 4 (1975), 313-316.
6. Feyerabend, C, RM Ings, MA Russel: Nicotine pharmacokinetics and its application to intake from smoking. Br. J. Clin. Pharmacol. 2 (1985), 239-247.
7. Isaac, PF, MJ Rand: Cigarette smoking and plasma levels of nicotine. Nature 236 (1972), 308-310.
8. Russel, MA, C Feyerabend: Cigarette smoking: A dependence on high nicotine boli. Drug Metab. Rev. 8 (1978), 29-57.
9. Lee, TJ, W Zhang, S Sarwinski: Presynaptic beta(2)-adrenoceptors mediate nicotine-induced NOergic neurogenic dilation in porcine basilar arteries. Am. J. Physiol. Heart Circ. Physiol. 279 (2000), H808-H816.
10. Okamura, T, K Ayajiki, M Uchiyama, et al: Neurogenic vasodilatation of canine isolated small labial arteries. J. Pharmacol. Exp. Ther. 288 (1999), 1031-1036.
11. Li, Z, V Barrios, JN Buchholz, et al: Chronic nicotine administration does not affect peripheral vascular reactivity in the rat. J. Pharmacol. Exp. Ther. 271 (1994), 1135-1142.
12. Sarabi, M, Lind L: Short-term effects of smoking and nicotine chewing gum on endothelium-dependent vasodilation in young healthy habitual smokers. J. Cardiovasc. Pharmacol. 35 (2000), 451-456.
13. Sigalet, DL, G Martin: Lymphatic absorption of glucose and fatty acids as determined by direct measurement. J. Pediatr. Surg. 34 (1999), 39-43.
14. Fang, Q, H Sun, WG Mayhan: L-arginine prevents impaired endothelium-dependent cerebral arteriolar dilatation during acute infusion of nicotine. Nicotine Tob. Res. 6 (2004), 1009-1014.
15. Okamura, T, N Toda: Mechanism underlying nicotine-induced relaxation in dog saphenous arteries. Eur. J. Pharmacol. 263 (1994), 85-91.
16. Hui, SC, Z Wang, H Zhang, et al: Arachidonic acid metabolism in nicotine-treated rats and nicotine-incubated rabbit aortic smooth muscle cells. Clin. Exp. Pharmacol. Physiol. 19 (1992), 689-693.
17. Kurahashi, K, H Shirahase, S Nakamura, et al: Nicotine-induced contraction in the rat coronary artery: Possible involvement of the endothelium, reactive oxygen species and COX-1 metabolites. J. Cardiovasc. Pharmacol. 38 (2001), 21-25.
18. Wang, T, Y Gu, G Wu: Injury effects of nicotine on isolated rat common carotid artery. Chung-Hua i Hsueh Tsa Chih [Chinese Medical Journal] 77 (1997), 115-118.
19. Wang, R, Z Wang: Three different vasoactive responses of rat tail artery to nicotine. Can. J. Physiol. Pharmacol. 78 (2000), 20-28.
20. Koide, M, S Nishizawa, S Yamamoto, et al: Nicotine exposure, mimicked smoking, directly and indirectly enhanced protein kinase C activity in isolated canine basilar artery, resulting in enhancement of arterial contraction. J. Cereb. Blood Flow Metab. 25 (2005), 292-301.
21. Mayhan, WG, GM Sharpe: Chronic exposure to nicotine alters endothelium-dependent arteriolar dilatation: effect of superoxide dismutase. J. Appl. Physiol. 86 (1999), 1126-1134.
22. Black, CE, N Huang, PC Neligan, et al: Effect of nicotine on vasoconstrictor and vasodilator responses in human skin vasculature. Regul. Integr. Comp. Physiol. 281 (2001), R1097-R1104.
23. Holden, WE, SS Kishiyama, SP Dong, et al: Endothelium-dependent effects of cigarette

smoke components on tone of porcine intrapulmonary arteries in vitro. Toxicol. Appl. Pharmacol. 104 (1990), 191-199.
24. Okamura, T, M Enokibori, N Toda: Neurogenic and non-neurogenic relaxations caused by nicotine in isolated dog superficial temporal artery. Pharmacol. Exp. Ther. 266 (1993), 1416-1421.
25. Hans, FJ, L Wei, D Bereczki, et al: Nicotine increases microvascular blood flow and flow velocity in three groups of brain areas. Am. J. Physiol. 265 (1993), H2142-H2150.
26. Horimoto, M, T Koyama: Acute microcirculatory response to nicotine in frog web. Jpn. J. Physiol. 32 (1982), 771-782.
27. Yong, T, MQ Zheng, DS Linthicum: Nicotine induces leukocyte rolling and adhesion in the cerebral microcirculation of the mouse. J. Neuroimmunol. 80 (1997), 158-164.
28. Salbas, K, A Gurlek, T Akyol: In vitro effect of nicotine on red blood cell deformability in untreated and treated essential hypertension. Scand. J. Clin. Lab. Invest. 54 (1994), 659-663.
29. Maurel, A, M Apovo, Y Beuzard, et al: Effect of smoking on blood rheology. J. Mal. Vasc. 22 (1997), 239-243.
30. Gerzanich, V, F Zhang, GA West, et al: Chronic nicotine alters NO signaling of Ca(2+) channels in cerebral arterioles. Circ. Res. 88 (2001), 359-365.
31. Miller, VM, WD Clouse, BH Tonnessen, et al: Time and dose effect of transdermal nicotine on endothelial function. Heart Circ. Physiol. 279 (2000), H1913-H1921.
32. Tonnessen, BH, SR Severson, RD Hurt, et al: Modulation of nitric-oxide synthase by nicotine. J. Pharmacol. Exp. Ther. 295 (2000), 601-606.
33. Chalon, S, H Jr Moreno, NL Benowitz, et al: Nicotine impairs endothelium-dependent dilatation in human veins in vivo. Clin. Pharmacol. Ther. 67 (2000), 391-397.
34. Mayhan, WG, GM Sharpe: Nicotine impair histamine-induced increases in macro-molecular efflux: Role of oxygen radicals. J. Appl. Physiol. 84 (1998) 1589-1595.
35. Mayhan, WG, GM Sharpe: Superoxide dismutase restores endothelium-dependent arteriolar dilatation during acute infusion of nicotine. J. Appl. Physiol. 85 (1998), 1292-1298.
36. Neunteufl, T, S Heher, K Kostner, et al: Contribution of nicotine to acute endothelial dysfunction in long-term smokers. J. Am. Coll. Cardiol. 39 (2002), 251-256.
37. Tithof, PK, M Elgayyar, HM Schuller, et al: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a nicotine derivative, induces apoptosis of endothelial cells. Heart Circ. Physiol. 281 (2001), H1946-H1954.
38. Zhang, S, IN Day, S Ye: Nicotine induced changes in gene expression by human coronary artery endothelial cells. Atherosclerosis 154 (2001), 277-283.
39. Zhang, S, IN Day, S Ye: Microarray analysis of nicotine-induced changes in gene expression in endothelial cells. Physiol. Genomics 5 (2001), 187-192.
40. Lundberg, JM, CR Martling, A Saria et al.: Cigarette smoke-induced airway oedema due to activation of capsaicin-sensitive vagal afferents and substance P release. Neuroscience 10 (1983), 1361-1368.
41. Sinzinger, H, J Kaliman, A Oguogho: Eicosanoid production and lymphatic responsiveness in human cigarette smokers compared with non-smokers. Lymphology 33 (2000), 24-31. Erratum in Lymphology 33 (2000), 75.
42. Galanzha, EI, GE Brill, AV Solov'eva, et al: Nitric oxide in the lymphatic microvessel regulation. Ross. Fiziol. Zh. Im. I M. Sechenova 88 (2002), 983-989.
43. Galanzha, EI, GE Brill, Y Aizu, et al: Speckle and doppler methods of blood and lymph flow monitoring. Chap. 16. In: *Handbook of Optical Biomedical Diagnostics*, Bellingham, SPIE Press, PM107, 2002; pp. 875-937.
44. Brill, GE, EI Zakharova (Galanzha): The effect of dimethyl sulfoxide on the changes in the lymph microcirculation induced by staphylococcal toxin. Eksp. Klin. Farmakol. 61 (1998), 54-56.
45. Galanzha, EI, AV Solov'eva, TV Stepanova, et al: Analysis of lymph microcirculation in norm, at the experimental lymphedema and pathological stress on animal model. J. Vasc. Res. 39(Suppl 1) (2002), 30.
46. Galanzha, EI, VV Tuchin, AV Solov'eva, et al: Experimental evaluation on the transmission optical microscopy for the diagnosis of lymphedema. J. X-Ray Sci. and Technol. 10 (2002), 215-223.
47. Galanzha, EI, VV Tuchin, VP Zharov, et al: The diagnosis of lymph microcirculation on rat mesentery in vivo. Proc. SPIE 4965 (2003), 325- 333.
48. Galanzha, EI, VV Tuchin, VP Zharov: In vivo integrated flow image cytometry and lymph/blood vessels dynamic microscopy. J. Biomed. Opt. 10 (2005) (in press).
49. Benoit, JN: Relationship between lymphatic pump flow and total lymph flow in the small intestine. Am. J. Physiol. 261 (1991), H1970-H1978.
50. Shirasawa, Yu, F Ikomi, T Ohhashi: Physiological roles of endogenous nitric oxide

in lymphatic pump activity of rat mesentery in vivo. Gastrointest. Liver Physiol. 278 (2000), G551-G556.
51. Ono, N, R Mizuno, H Nojiri et al: Development of an experimental apparatus for investigating lymphatic pumping activity of murine mesentery in vivo. Jpn. J. Physiol. 50 (2000), 25-31.
52. Mehta, MC, AC Jain, A Mehta et al: Cardiac arrhythmias following intravenous nicotine: Experimental study in dogs. Cardiovasc. Pharmacol. Ther. 2 (1997), 291-298.
53. Zharov, VP, EI Galanzha, VV Tuchin: Photothermal image flow cytometry in vivo. Opt. Lett. 30 (2005), 628-630.
54. Zharov, VP, EI Galanzha, VV Tuchin: Integrated photothermal flow cytometry in vivo. J. Biomed. Opt. 10 (2005) (in press).

Ekaterina I. Galanzha, MD, PhD, DrSc
Philips Classic Laser Laboratories
University of Arkansas for Medical Sciences
4301 West Markham Street #543
Little Rock, Arkansas 72205 USA
Telephone: 501-526-7620
Fax: 501-686-8029
E-mail: EGalanzha@uams.edu