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Direct Vascular Effects of Furosemide in Humans

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Background In humans, hemodynamic changes observed within minutes after systemic administration of furosemide are often referred to as direct vascular effects. However, these immediate changes do not per se imply a direct vascular effect. We examined the genuine direct vascular effects of furosemide on the human forearm vascular bed and dorsal hand vein.

Methods and Results Forearm blood flow in response to infusion of increasing dosages of furosemide into the brachial artery was recorded by venous occlusion plethysmography. Local plasma concentrations of furosemide reached a maximum of 234±40 µg/mL during the highest infused dose but did not significantly affect the ratio of flow in the infused/noninfused arms. Venous distensibility of a dorsal hand vein was measured with a linear variable differential transformer. During precontraction with norepinephrine, five increasing dosages of furosemide (1 to 100 µg/min) were administered locally. Additional experiments using local administration of indomethacin or L-NMMA were carried out to determine whether effects were dependent on local prostaglandin or nitric oxide synthesis, respectively. Also, the effects of systemic administration of furosemide were examined. Local administration of furosemide led to a dose-dependent venorelaxation of 18±6% at the first to 72±16% at the last dose. Indomethacin almost completely abolished furosemide-induced venorelaxation, whereas L-NMMA had no effect. Systemic administration of furosemide resulted in a time-dependent increase of hand vein distensibility, reaching 45±11% after 8 minutes.

Conclusions Furosemide does not exert any direct arterial vasoactivity in the human forearm, even at supratherapeutic concentrations. In contrast, at concentrations estimated to be in the therapeutic range, we observed a dose-dependent direct venodilator effect on the dorsal hand vein that appears to be mediated by local vascular prostaglandin synthesis. (Circulation. 1997;96:1847-1852.)

Key Words • furosemide • pharmacology • vasodilation • blood flow • prostaglandins

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selected abbreviations and acronyms

FBF = forearm arterial blood flow
L-NMMA = Nω-monomethyl-l-arginine
LVDT = linear variable differential transformer
NO = nitric oxide
PG = prostaglandin

restricted. None of the participants smoked or used any medication (including analgesics). All experiments were performed in a single-blind fashion in a temperature-controlled laboratory (23°C to 24°C for the perfused forearm experiments and 28°C to 29°C for the venous distensibility measurements) with the subject in the supine position.

Demographic characteristics of the participants are summarized in the Table.

Arterial Vascular Activity Measurements

Two protocols were conducted in a total of 22 subjects to assess the direct arterial vasoactivity of loop-active diuretics. First, we examined the direct arterial vasoactivity of furosemide and bumetanide and second, the effect of locally administered furosemide on norepinephrine-induced vasconstriction. The perfused forearm technique was used for both protocols. For this technique, the left brachial artery was cannulated with a 20-gauge catheter (Angiocath, Deseret Medical, Becton Dickinson) after induction of local anesthesia (lidocaine 2%). This catheter was used for drug infusion (automatic syringe infusion pump, type STC-521, Terumo) and blood pressure monitoring (Hewlett Packard GmbH). At least 30 minutes after intraarterial cannulation, baseline values of FBF were measured in both arms three times per minute by ECG-triggered venous occlusion plethysmography with mercury-in-Silastic strain gauges (Hokanson EC4, DE Hokanson).11 To ensure that FBF recordings referred predominantly to the forearm skeletal muscle resistance arteries, the hand circulation was occluded during all FBF recordings by a wrist cuff inflated 100 mm Hg above the systolic pressure.12 The upper arm collecting cuffs were simultaneously inflated to 45 to 50 mm Hg with a rapid cuff inflator (Hokanson E-20). In all experiments, we also inserted a catheter into a deep ipsilateral forearm vein, during which time the upper arm collecting cuffs were inflated to 45 mm Hg to occlude venous outflow. Under these conditions, the upper arm collecting cuffs were simultaneously inflated to 45 to 50 mm Hg with a rapid cuff inflator (Hokanson E-20). In all experiments, we also inserted a catheter into a deep ipsilateral forearm vein. During the last minute of each drug infusion period of 10 minutes, a venous blood sample was taken and drug concentrations were measured by high-performance liquid chromatography assay as previously described.13

Direct Arterial Vasoactivity of Loop-Active Diuretics

Dosages of furosemide were normalized to forearm volume (water displacement method). Total infusion rate was kept constant at 100 μL · min⁻¹ · 100 mL forearm volume⁻¹. Furosemide was infused at 1, 3, 10, 30, and 100 μg · min⁻¹ · DL⁻¹ in 8 subjects. In another 6 subjects, furosemide was infused at 1000 μg · min⁻¹ · DL⁻¹ for 6 minutes. In 4 subjects, we administered bumetanide (0.025, 0.075, 0.25, 0.75, and 2.5 μg · min⁻¹ · DL⁻¹) instead of furosemide to perceive possible differences in vasoactivity between these two loop-active diuretics.

Demographic Characteristics of the Study Groups

Effect of Furosemide on Norepinephrine-Induced Vasconstriction

Animal data suggest that furosemide may exert an antivasoconstrictor effect, because the drug did not directly dilate mesenteric resistance vessels but rather inhibited the vasoconstrictor effect of norepinephrine and angiotensin II.14 To study this possible mechanism in humans, we measured the reduction in FBF in response to cumulative intra-arterial norepinephrine infusions in the absence and presence of local furosemide administration. In 4 subjects, norepinephrine was infused at 10, 30, and 100 mg · min⁻¹ · DL⁻¹ before and after local administration of furosemide (10 μg · min⁻¹ · DL⁻¹ for 20 minutes, preceded by a 30-minute interval after the first norepinephrine dose-response curve). Previous experiments revealed that intrabrachial infusion of this dose of furosemide led to clinically relevant concentrations in the infused forearm.

Venous Vascular Activity Measurements

Four protocols were carried out to determine the venous vasoactivity of furosemide. The direct venous effect of locally administered furosemide was examined, after which involvement of vascular prostaglandin and NO synthesis was assessed. Also, the venous vasoactivity of systemic administration of furosemide was examined. All protocols were conducted by the LVDT technique, in which venous distensibility of a dorsal hand vein was measured with the LVDT as described by Aellig15 and evaluated by Alradi and Carruthers.16 A total of 51 experiments were performed in 28 young and 10 elderly subjects. Regression analysis established that there was no significant correlation between age and percentage venodilation (r² = 0.18, P = NS), after which all data were pooled.

With the subject in the supine position in a temperature-controlled laboratory (28°C to 29°C), the arm under investigation was placed on a rigid support at an angle of 30° from the horizontal to allow complete emptying of the superficial hand veins. A sphygmomanometer cuff placed on the upper arm was then inflated to 45 mm Hg. A suitable large superficial vein with no apparent tributaries in the immediate area of examination was chosen, and a 23-gauge butterfly needle was inserted into the vein. The lightweight (0.2-g) probe of the LVDT was placed over the summit of the chosen vein 10 mm downstream from the tip of the needle. Under these conditions, dorsal hand vein distensibility is maximal during venous occlusion. When the venous pressure remains constant at 45 mm Hg, changes in venous diameter are proportional to changes in venous tone.

Owing to the low venous tone present under these conditions,17 venodilator effects can be quantified only on veins that have been preconstricted. To examine furosemide-induced venodilation, we used continuous infusion of increasing concentrations of norepinephrine to precontract the veins. Infusion of the norepinephrine concentration that achieved a precontraction of ~30% of maximal vein diameter was sustained throughout the experiment. Previous experiments from our laboratory showed that this method has a good reproducibility: In 15 subjects, the coefficient of variation of the maximal vasoconstrictor response to norepinephrine (before and after an interval of 2 hours) was 9%. In addition, norepinephrine dose-response curves on different days did not differ significantly from each other. Sustained infusion of norepinephrine alone resulted in a stable vasoconstrictor response (70±7% contraction after 10 minutes and 73±6% after 60 minutes, n = 10), indicating the absence of tachyphylaxis to norepinephrine. During the experiment, blood pressure and heart rate were monitored every 5 minutes by a Dinamap 1846 SX attached to the contralateral arm.

Direct Venous Vasoactivity of Furosemide

In a total of 20 subjects, NaCl 0.9% (0.1 mL/min) was replaced by five increasing doses of furosemide (1, 3, 10, 30,
Involvement of Vascular NO Synthesis in the Direct Venous Vasoactivity of Furosemide

In vivo, an increase in the venous capacitance induced by systemically administered furosemide has been reported to be inhibitable by indomethacin. This observation suggests a role for prostaglandins as a mediator of vasoactive effects of furosemide. The source of the prostaglandins involved in this mechanism may be the kidneys, because they may release prostaglandins into the systemic circulation; alternatively, local production in the peripheral vasculature could be involved. To determine the role of the nonrenal prostaglandins in the venous vasoactive effects of furosemide, we examined the effect of locally administered indomethacin (12.5 µg/min, 10 minutes) on the furosemide-induced venous vasoactivity. In 8 subjects, furosemide (100 µg/min) together with a placebo (NaCl 0.9%, 0.1 mL/min) was locally infused into a preconstricted vein for 10 minutes. Venodilation was assessed, after which placebo was replaced by indomethacin for 10 minutes and venodilation was assessed again.

To exclude a possible constrictor response by indomethacin alone, control experiments were performed in 4 subjects to determine the effect of indomethacin (12.5 and 125 µg/min) on baseline venous tone.

Involvement of Vascular NO Synthesis in the Direct Venous Vasoactivity of Furosemide

NO is a potent vasodilator released by vascular endothelial cells. Although the furosemide-induced vascular effects in vitro appear to be independent of the endothelium, a recent study showed that furosemide augmented the NO production of isolated cultured endothelial cells. To study the role of NO in the furosemide-induced venous vasoactivity, we repeated the protocol as described above, now using L-NMMA (60 µg/min) instead of indomethacin to inhibit NO production. Extensive studies have shown that this dose of L-NMMA has no effect on basal venous tone and is able to block the venodilation caused by acetylcholine.

Effect of Systemic Administration of Furosemide on Dorsal Hand Vein Distensibility

All previous reports concerning the effects of furosemide on human vein capacitance used systemic administration. To examine whether furosemide administered systemically in therapeutically dosages exerts a vasodilator activity comparable to that of locally administered furosemide, we administered furosemide (40 mg) intravenously in the contralateral arm in 15 subjects. Venous distensibility of the precontracted hand vein was recorded during the following 8 minutes.

Drugs

Furosemide solutions were freshly prepared from 2-mL ampoules containing 10 mg/mL furosemide as a disodium salt (Lasix, Hoechst Marion Roussel) and were further diluted in physiological saline immediately before each experiment. Norepinephrine (1-ng/mL ampoules, indomethacin (Indocid PDA, Merck Sharp and Dohme, 1 mg/mL), and L-NMMA acetate (Clinalfa) were dissolved in physiological saline immediately before use.

Data Analysis

Data are expressed as mean±SEM unless noted otherwise and were analyzed by Student’s t test or repeated measures ANOVA for paired data if appropriate. If ANOVA showed that a significant difference existed between conditions, it was followed by post hoc t tests (including Bonferroni correction) to determine dose dependency or time dependency. Linear regression analysis was performed on the relation between age and percentage furosemide-induced venodilatation (correlation coefficient according to Pearson). A value of P<.05 was considered to indicate significance.

Direct arterial vasoactivity. To reduce the variability of blood flow data and to correct for systemic changes, the ratio of the FBF measurements in the infused and noninfused arms was calculated for each time point, with the noninfused arm used as a contemporaneous control for the infused arm. The FBF values of the last 3 minutes of each drug infusion were averaged to one value.

Direct venous vasoactivity. The response of norepinephrine-induced constriction was measured, and furosemide-induced effects were expressed as the percentage attenuation of the average control constriction. All results are expressed as a percentage of baseline vein size. The furosemide-induced venodilation was determined during the last 3 minutes of each furosemide infusion.

Results

Systemic Effects

Forearm volume averaged 984±32 mL. During the arterial vasoactivity experiments, blood pressure, heart rate, and FBF in the noninfused arm did not change significantly after intrabrachial infusion of furosemide. During local administration of furosemide in the venous vasoactivity experiments, blood pressure increased over 1 hour from 113±2/62±1 to 116±2/66±2 mm Hg (for systolic and diastolic blood pressures, P=.03 and P=.001, respectively, ANOVA with repeated measures). There was no change in heart rate (61±2 to 61±2 bpm, P=NS).

More relevantly, blood pressure increased within 5 minutes after systemic administration of 40 mg of furosemide from 118±1/68±2 to 121±2/71±2 mm Hg (P=.01 and P<.0001, respectively, Student’s t test). Heart rate remained unchanged (63±2 to 65±1 bpm).

Direct Effects on FBF

Ratios of infused to control FBF and ipsilateral venous plasma concentrations of furosemide are shown in Fig 1 (top). During five increasing dosages of furosemide, there was no significant effect on FBF compared with the placebo infusion. In 6 subjects, we infused furosemide 1000 µg·min⁻¹·D⁻¹ for 6 minutes, leading to local furosemide plasma concentrations of 234±40 µg/mL. In these subjects, furosemide increased FBF in the infused arm significantly, by 23±9.7% (P<.05), but without a significant effect on the FBF ratio of the infused and noninfused arms (P=.08).

Intra-arterial bumetanide infusions led to local plasma concentrations ranging from 39±11 to 1748±327 ng/mL and also failed to alter FBF (data not shown).

Effect of furosemide on norepinephrine-induced vasoconstriction. As shown in Fig 1 (bottom), local infusion of norepinephrine into the brachial artery led to a dose-dependent decrease in FBF (P<.001), with no significant effect on systemic blood pressure. This vasoconstriction was not inhibited by local infusion of furosemide (P=NS).

Direct Effects on Dorsal Hand Vein Distensibility

Vein diameter of the participants was 0.74±0.05 mm. On average, infusion of norepinephrine constricted the vein of investigation to 31±2% of the control size.
Fig 1. Direct arterial vasoactivity of furosemide. Top, Bars represent mean±SEM measured local venous plasma concentrations of furosemide (right axis). Line graph shows mean±SEM FBF ratio (infused/noninfused arm) during intrabrachial infusions of furosemide (left axis) as measured by venous occlusion plethysmography. There was no significant change in FBF ratio. Values are mean±SEM of 8 and 6 experiments. Bottom, Ratio of FBF during intrabrachial norepinephrine administration, both in the presence of placebo (solid circles/solid line) and after and during concomitant infusion of furosemide (10 μg·min⁻¹·dl⁻¹, open circles/dashed line). Constrictor response to norepinephrine (P=.01) was not inhibited by local furosemide administration (P=NS). Values are mean±SEM of 4 experiments. P values refer to statistical differences between conditions for these dose responses as analyzed by ANOVA with repeated measures over complete dose-response curves.

Fig 2 demonstrates that continuous local infusion of furosemide results in a dose-dependent attenuation of the constrictor effect of norepinephrine (P<.001). Post hoc t tests (with Bonferroni correction) revealed a dose-dependent venodilation between doses of 0, 1, 10, and 100 μg/min. This direct venodilating effect of furosemide was rapid in onset. After the last furosemide infusion was replaced with NaCl 0.9% infusion, venodilation waned within a few minutes.

**Involvement of vascular prostaglandin synthesis in the direct venous vasoactivity of furosemide.** In 8 subjects, furosemide-induced venoarterial administration (contralateral antecubital vein) of 40 mg furosemide led to increases in vein diameter of 18±8%, 26±11%, and 45±11% at 2, 4, and 8 minutes, respectively (P<.01). Post hoc tests (Bonferroni) revealed that venodilation was significantly different from baseline at t=4 minutes (P=.028) and 8 minutes (P=.001).

**Discussion**

It is generally accepted that the reduction in venous return as a result of a nondiuretic vascular effect by furosemide is therapeutically important in achieving rapid symptomatic relief for patients with left ventricular heart failure. The mechanism of this action is unclear. Because of the absence of data on the direct vasoactivity of furosemide in vivo, we examined the vascular effects of local furosemide administration on arterial and venous vessels in humans.

**Effect of systemic administration of furosemide on dorsal hand vein distensibility.** As shown in Fig 2 (right), paren-
does have a direct venodilator effect, which is associated with vascular prostaglandin synthesis.

**Direct Effects on FBF**

Our results demonstrate the absence of a direct effect of loop-active diuretics on FBF during intra-arterial infusions, which results in clinically relevant plasma concentrations in the infused forearm. The absence of vasoreactivity in this concentration range is consistent with most previous experiments on isolated arteries. In these in vitro experiments, direct vasodilator properties of furosemide were observed only at concentrations \( >10^{-4}\) mol/L, whereas in our first series of experiments, the furosemide concentration at the highest infusion rate reached 47±10 \( \mu g/mL \), equivalent to \( 1.4\times10^{-4}\) mol/L. To examine the direct arterial effects of furosemide at very high concentrations in vivo, we infused furosemide 1000 \( \mu g\cdot min^{-1}\cdot DL^{-1} \) into the brachial artery, leading to a local concentration of 234±40 \( \mu g/mL \) (0.71±10\(^{-3}\) mol/L). Even at these supratherapeutic concentrations, only a negligible increase in FBF was observed.

In the rat, furosemide did not change baseline mesenteric blood flow, but systemic administration did inhibit the decrease in blood flow produced by angiotensin II and norepinephrine. In contrast, we observed no effect of local furosemide on norepinephrine-induced attenuation of FBF. From our studies, we conclude that furosemide does not exert any direct arterial vasodilator or antivasoconstrictor activity in the human forearm. As such, the previously reported decrease in FBF after systemic administration of furosemide is probably such, the previously reported decrease in FBF after the increase of venous compliance observed after systemic administration of furosemide may be the result of direct effects on the venous circulation. Compared with other substances such as nitroprusside and substance P, which exert venodilatory properties at an infusion rate of nanograms per minute, furosemide is much less potent. However, its effect does have clinical relevance, especially in the first few minutes after parenteral administration.

**Mechanism of Action**

Two hypotheses concerning the direct vascular effects of furosemide emerge from the literature. The first hypothesis focuses on furosemide-induced inhibition of vascular Na-K-2 Cl cotransport, whereas the second is directed to the role of prostaglandins in the vascular activity of furosemide.

**Na-K-2 Cl Cotransport Inhibition**

The presence of Na-K-2 Cl cotransport in endothelial and vascular smooth muscle cells has been established, but its role in the regulation of vascular tone is unclear. In a recent report, furosemide relaxed canine venous but not arterial vessels taken from a variety of vascular beds. In the same vessels, Na-K-2 Cl cotransport distribution was determined, and the magnitude of the vasodilator effect was found to correlate with Na-K-2 Cl cotransport distribution. The correlation between Na-K-2 Cl cotransport distribution and vascular activity suggests a role for this cotransporter in the vascular action of furosemide. However, inhibition of renal Na-K-2 Cl cotransport occurs at \( 10^{-4}\) to \( 10^{-3}\) mol/L furosemide, concentrations 10 to 50 times the local concentration in the hand vein, and the importance of this action of furosemide to its venodilator properties remains uncertain.

**Augmented Prostaglandin Synthesis**

The effect of systemic administration of furosemide on venous capacitance has been compared between healthy subjects and anephric patients. Venous capacitance increased in healthy volunteers but not in anephric patients. This effect could be blocked by pretreatment of the cyclooxygenase inhibitor indomethacin, suggesting a role for renal prostaglandin release in the vascular effects of furosemide. Our results indicate that renal prostaglandin synthesis is not necessarily important for the direct venous vasodilation, because the release of renal prostaglandins cannot have been stimulated after the local furosemide infusions. This does not rule out the possibility that furosemide-induced vasodilation is mediated by activation of vascular PGI2 synthesis. Lundergan et al, using an isolated canine lung lobe perfused with autologous blood at constant flow, demonstrated that furosemide-induced decreases in pulmonary artery perfusion pressure were mediated by prostaglandins because they were abolished by treatment of the lung with indomethacin. Recently, it was shown in cultured bovine aortic endothelial cells that furosemide stimulated the production of prostacyclin and NO at clinically relevant concentrations. In our study, the direct venodilator effect of furosemide on veins was almost totally abolished by local administration of indomethacin, indicating that this direct vascular effect is dependent on local vascular prostaglandin synthesis. It is unclear whether the endothelial or the vascular smooth muscle cell is the source of the prostaglandin production augmented by furosemide. In vivo endothelial stripping with distilled water seems a possibility to address this question, but these experiments are quite invasive, and NSAID treatment will be necessary to prevent blood clotting, which will obscure the interpretation of the furosemide-induced venodilatation. It is unknown whether the furosemide-enhanced vascular PGI2 production is
the consequence of a nonspecific action of furosemide or of inhibition of the vascular Na-K-2 Cl cotransporter. Furthermore, the effect of systemic treatment with indomethacin or other NSAIWs on the furosemide-induced venorelaxation and its clinical implications are unknown.

The venorelaxation persisted after addition of L-NMMA, so it appears that the effect is not mediated by endothelial NO release.

**Conclusions**

The present study provides the first evidence that furosemide at therapeutic concentrations exerts no direct vasodilator or antivasoconstrictor effect on arterial resistance vessels in the human forearm but rather directly dilates veins in humans. The direct venodilation was inhibited by local indomethacin administration but not by blockade of NO synthesis, indicating that the direct vascular venodilation is dependent on local prostaglandin but not on NO production. Hemodynamic changes observed directly after systemic administration of furosemide are probably due to a direct venodilator effect of the drug.

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