



A Pilot Study of Perioperative External Circumferential Cryoablation of Human Renal Arteries for Sympathetic Denervation

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Purpose: Cryoablation, which induces cellular death without extensive tissue damage, has been extensively used to denervate the myocardium. However, periadventitial external circumferential application of cryotherapy to denervate the renal artery sympathetic nerves has, to our knowledge, never been tested in humans. The main aim of this study was to examine the safety and potential effects of cryotherapy on ambulatory blood pressure levels and other outcomes that are indirectly related to sympathetic tone, including pulse-wave velocity, central pulse pressure, and glucose levels.

Materials and Methods: Five patients who underwent the denervation of the renal arteries during open surgery of the abdominal aortic aneurysm and four controls who did not undergo denervation during the surgery were enrolled. An argon-powered cryotherapy catheter (Cardioblade; Medtronic Inc., USA) was applied periadventitially to each renal artery in the five patients and then activated by infusion with liquid nitrogen for 1 minute.

Results: No cryoablation-related complications occurred in the five consecutive patients. Their ambulatory blood pressures 3 to 5 months after surgery did not demonstrate any general blood pressure-reducing effects, but two patients responded favorably with the reduction of antihypertensive medication and decreased 24-hour blood pressure. The patients' mean HbA1C levels decreased from $5.9 \pm 0.78\%$ to $5.6 \pm 0.71\%$ ($P=0.042$).

Conclusion: This pilot study suggests that periadventitial cryoablation during open surgery can be used in the study of renal denervation in humans, as it had no complications in five patients in this study. The effectiveness of cryoablation for treating hypertension should be proven in a phase II clinical trial.

Key Words: Cryosurgery, Adventitia, Essential hypertension, Sympathectomy, Renal artery

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INTRODUCTION

Surgical sympathectomy was historically used as a desperate measure to lower blood pressure before the advent

of effective antihypertensive medical therapies [1]. Although this procedure was seemingly effective in lowering blood pressure, it also carried severe unwanted side effects such as pronounced postural hypotension [1]. Today, many

patients with hypertension can reach ambitious blood pressure goals by taking combinations of several antihypertensive drugs, but therapy-resistant hypertension is still a common condition [2]. Therapy-resistant hypertension is well known to be a result of poor treatment compliance, and this is one reason for the interest in non-pharmacological antihypertensive therapies [3].

The renal sympathetic nerves run close to the adventitia of the renal arteries, and the efferent nerves elevate blood pressure by multiple mechanisms, including the release of renin. Afferent signaling in the renal sympathetic nerves also stimulates the central nervous system to increase the sympathetic tone, which induces vasoconstriction and increases cardiac output. A percutaneous catheter-based cryoablation of renal arteries has been proven to be effective for sympathetic denervation in a sheep model, as it caused a pronounced blood pressure reduction [4]. However, renal sympathetic denervation in humans has mainly relied on the percutaneous application of radiofrequency catheters [3]. Initial reports of prospective trials rendered impressive results on blood pressure levels [5], and the technique became clinically accessible as a treatment option. However, in the Symplicity HTN-3 trial, in which sham operation was compared with catheter-based radiofrequency ablation, the mean effect on systolic blood pressure was a modest -2.4 mmHg [6]. This figure closely matched the value that was initially achieved with the same device in a subset of patients who underwent 24-hour ambulatory blood pressure measurements in the Symplicity HTN-2 trial, a blood pressure measurement technique that is devoid of a placebo effect [7]. The modest blood pressure reduction in the Symplicity HTN-3 trial has been discussed [6], and one potential explanation could be that the catheter-based technique did not achieve complete denervation for different technical reasons [6,8-10]. As the renal sympathetic nerves often lie close to the adventitia [11], the radiofrequency energy from intraluminal application might not penetrate the intima and media sufficiently to fully denervate the arteries [10]. Cryoablation has been extensively used to denervate the myocardium [12], and it induces cellular death without extensive tissue damage. In animal experiments and, recently, in humans, catheter-based cryotherapy has been applied in renal arteries and has reduced blood pressures and sympathetic nervous system activity [4,10]. However, the external circumferential application of cryotherapy in the denervation of the renal artery sympathetic nerves periadventitally has, to our knowledge, never been used in humans. Hence, to potentially achieve a more complete renal sympathetic denervation, we conducted a pilot study in five subjects who underwent a pre-planned protective surgery for an abdominal aortic aneurysm. Dur-

ing the open surgery, a cryotherapy catheter was applied circumferentially to each renal artery for 1 minute and activated with liquid nitrogen. The study was non-randomized, and four subjects who did not receive perioperative cryoablation during their corresponding open aortic surgery were recruited as controls. The main aim of this study was to examine the safety and potential effects of periadventitial cryoablation on ambulatory blood pressure levels and other outcomes that are indirectly related to the sympathetic tone, including pulse-wave velocity, central pulse pressure, and glucose levels.

MATERIALS AND METHODS

1) Patients

Patients with a history of elevated blood pressure in relation to a risk of rupture of a known abdominal aortic aneurysm were eligible for participation in the study. Severe mental disorder, inability to understand the study protocol and procedure, renal insufficiency (glomerular filtration rate, <45 mL/min/1.73 m²), liver dysfunction, and symptomatic hypotension were the exclusion criteria. Both the patients and controls also had to have known abdominal aortic aneurysms planned for surgical treatment with elective open transabdominal surgery to avoid rupture. All the patients underwent preoperative computer tomography with contrast enhancement.

2) Clinical physiological and laboratory investigations

Standard laboratory tests were performed at the Department of Clinical Chemistry, Linköping University Hospital, in accordance with the accredited routine methods before and 3–5 months after surgery. Chromatographic determination of the presence of methoxy-catecholamines in urine was performed at the Department of Clinical Chemistry, Ryhov Hospital in Jönköping, Sweden, after collection of acidified urine for 24 hours.

Ambulatory blood pressure measurement devices (Spacelab 90217; Spacelabs Inc., Redmond, WA, USA) were set to measure blood pressure at 20-minute intervals for 24 hours and before and 3 to 5 months after surgery.

Applanation tonometry was performed with the SphygmoCor system (Model MM3; AtCor Medical, Sydney, Australia) 3 to 5 months after surgery. From the radial pulse waveform recorded with a Millar pressure tonometer, the corresponding ascending aortic pulse waveform was derived using the validated transfer function incorporated in the software (SphygmoCor version 8.0 software; AtCor Medical), which also calculated the central blood and pulse

pressures. The foot pulse arrival to the right carotid and femoral arteries was achieved by measuring the time from the appearance of an R-wave on electrocardiography at the respective site during 10 seconds in two consecutive recordings and subtracted to achieve the time delay. The arterial path length was estimated using straight body surface measurement in accordance with the subtraction method. The mean aortic pulse wave velocity (distance/time) was calculated automatically.

3) Ethical considerations

The study was approved by the Regional Ethics Committee of Linköping (approval number 2014/45-31) and performed in accordance with the Declaration of Helsinki of 1975. The ClinicalTrials.gov registration number was NCT02345603. All the participants provided consent to the extra intervention during surgery after receiving information from the surgeon.

4) Statistical analyses and power calculation

Statistical estimates were calculated using the IBM SPSS Statistics 23.0 software (IBM Corp., Armonk, NY, USA). Comparisons within groups were performed with the Wilcoxon signed-rank test or as stated in the RESULTS section. Mean values and standard deviations are presented. Statistical significance refers to two-sided P-values of ≤ 0.05 . No formal power calculation was performed because this was a pilot study.

RESULTS

From December 2014 to March 2015, seven patients were asked to undergo cryoablation, of whom five accepted the procedure. Four control patients, who did not undergo cryoablation, agreed to perform the additional blood pressure recordings and laboratory investigations according to the protocol, before and after the aortic surgery. Fig. 1 shows a flowchart of the trial. None of the patients or controls had multiple renal arteries or renal artery stenosis. The aneurysm sizes of the participants ranged from 55 to 59 mm in diameter.

1) Surgery

The patients were operated under general and epidural anesthesia. The operation was performed through a midline incision and transperitoneal approach using the standard procedure. Renal arteries were identified and exposed by ligating and dividing the left testicular vein and retraction of the left renal vein. Each renal artery was examined on perioperative duplex ultrasonography, including measurement of the peak systolic flow velocity. Each renal artery was wrapped with an argon-powered Cardioblate Cryo-Flex Surgical Ablation Probe (Medtronic Inc., Minneapolis, MN, USA) and exposed to -120°C for 60 seconds (Fig. 2) after administration of a standard dose of 5,000 units of unfractionated heparin. The artery was then heated up by flushing with a physiological saline solution at room temperature. A new duplex scanning was performed, after which the operation continued with reconstruction of the aortic aneurysm. All the patients underwent an infrarenal reconstruction and clamping below the renal arteries. Three

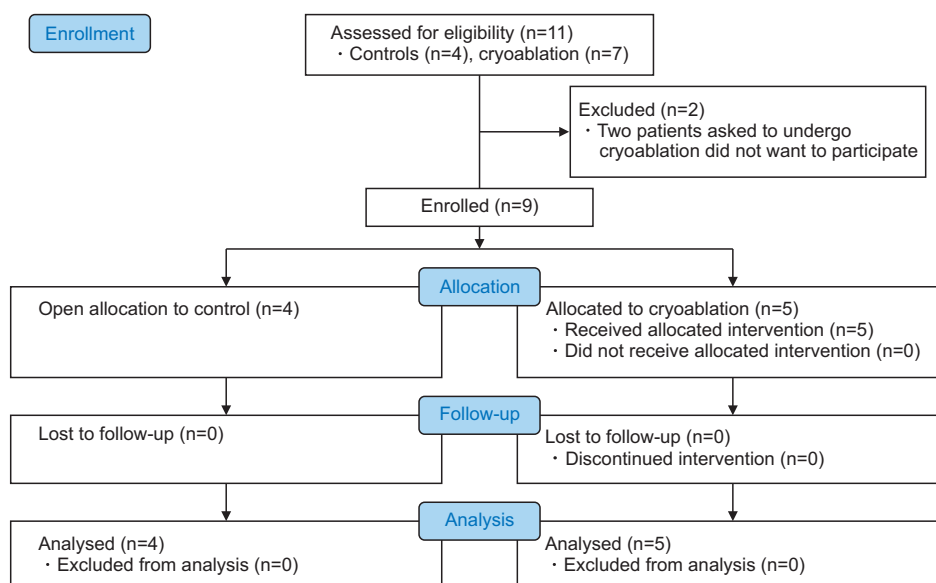


Fig. 1. Flow diagram for pilot study of cryoablation.

patients who underwent cryoablation received aorto-bi-iliac grafts, and the remaining two patients received straight grafts.

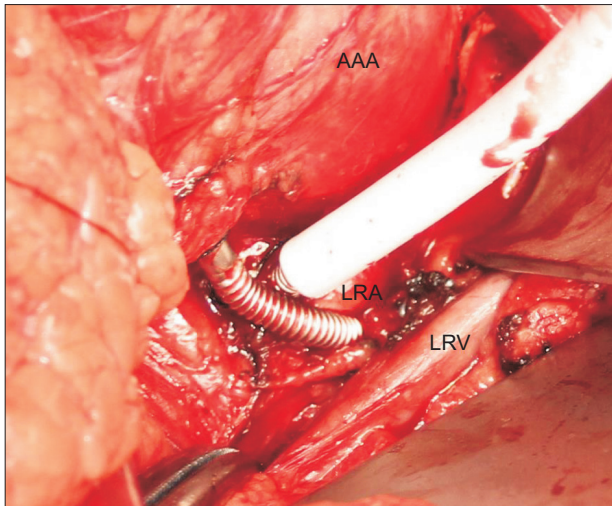


Fig. 2. Perioperative photograph of a cryoprobe wrapped around the left renal artery (LRA). Shown at the bottom is the right left renal vein (LRV) and at the top is the abdominal aortic aneurysm (AAA).

Postoperatively, all the patients were analgesized with continuous epidural infusion for 3 days and discharged uneventfully after 5 to 7 days. No complications occurred in any patients who underwent periaortic cryoablation.

Antihypertensive medications were unchanged except in one case in which three drugs were reduced to one because of low blood pressure combined with orthostatic symptoms. Individual baseline data (before surgery) and results on ambulatory blood pressure and laboratory analyses 3 to 5 months after surgery are displayed in Table 1 (cases) and Table 2 (controls). None of the patients who underwent cryoablation showed statistically significant changes in ambulatory blood pressure levels, pulse wave velocity, or central pulse pressure as compared with the control group (all $P > 0.22$). However, the controls exhibited an increase in pulse wave velocity that bordered on statistical significance (from 11.0 ± 1.2 m/s before to 12.2 ± 1.3 m/s after surgery, $P = 0.066$). The patients showed a decrease in mean glucose levels, measured as glycated hemoglobin A1c (HbA1c) levels, from $5.9\% \pm 0.78\%$ to $5.6\% \pm 0.71\%$, $P = 0.042$). No statistically significant change in creatinine level (from 0.936 ± 0.15 mg/dL before to 0.954 ± 0.081 mg/dL after surgery, $P = 0.50$) was observed in the patients. No marked individual increas-

Table 1. Individual changes in five patients who underwent cryoablation

Variable	Time	Cryo 1	Cryo 2	Cryo 3	Cryo 4	Cryo 5
Age (y)		65	66	69	70	71
Weight (kg)	Pre	110	78.5	86	95	82.5
	Post	106	74.5	81	91.5	80.5
S-Creatinine (mg/dL)	Pre	0.78	0.93	0.95	1.16	0.86
	Post	0.84	1.02	0.98	1.03	0.90
U-MNE (mg/24 h)	Pre	^a	0.25	0.30	0.46	0.35
	Post	0.29	0.29	0.29	0.39	0.37
U-ME (mg/24 h)	Pre	^a	0.14	0.14	0.14	0.10
	Post	0.08	0.12	0.12	0.12	0.12
HbA1c (%)	Pre	6.9	5.6	6.0	5.6	5.5
	Post	6.5	5.5	5.7	5.2	5.4
24-h ABPM (mmHg)	Pre	128/72	120/73	130/76	123/83	118/81
	Post	134/70	147/89	112/68	124/81	130/83
PWVcf (m/s)	Pre	14.2	10.8	8.2	10.8	9.3
	Post	12.3	14.8	7.5	10.0	9.2
Central PP (mmHg)	Pre	46	37	41	32	28
	Post	36	41	32	33	22
AHT (no. of drugs)	Pre	1	3	2	1	0
	Post	1	1	2	1	0

All the parameters were measured before (pre) and 3 to 5 months after surgery (post).

Cryo, cryoablation; U-MNE, methoxy-norepinephrine in urine; U-ME, methoxy-epinephrine in urine; HbA1c, glycated hemoglobin A1c; ABPM, ambulatory blood pressure monitoring; PWVcf, carotid-femoral pulse wave velocity; PP, pulse pressure; AHT, antihypertensive treatment.

^aBaseline levels were analyzed by mistake as epinephrine and norepinephrine levels in urine that were within the normal limits.

Table 2. Individual changes in controls

Variable	Time	Control 1	Control 2	Control 3	Control 4
Age (y)		63	65	70	77
Weight (kg)	Pre	110	97	82	86
	Post	110	97	78	85
HbA1c (%)	Pre		5.5		5.2
	Post	5.0	5.6		5.4
24 h ABPM (mmHg)	Pre	108/69	126/79	122/80	114/66
	Post	108/65	127/76	121/79	118/68
PWVcf (m/s)	Pre	9.2	12.1	11.2	11.3
	Post	10.4	13.3	11.8	13.1
Central PP (mmHg)	Pre	26	38	39	42
	Post	50	32	53	56
AHT (no. of drugs)	Pre	1	1	1	1
	Post	1	1	1	1

All the parameters were measured before (pre) and 3 to 5 months after surgery (post).

HbA1c, glycosylated hemoglobin A1c; ABPM, ambulatory blood pressure monitoring; PWVcf, carotid-femoral pulse wave velocity; PP, pulse pressure; AHT, antihypertensive treatment.

es in serum creatinine level were observed in any of the five patients who underwent cryoablation. All the patients who underwent cryoablation had renal arterial patency as determined in a perioperative duplex investigation after the ablation. No cases of procedure-related clinical complications such as arterial stenosis, thrombosis, or pseudoaneurysms occurred. Data on this were also extended to regular clinical follow-up for around a year, as the study was performed between the years 2014 to 2015.

Further scrutiny of the individual participants revealed that Patient 3 showed a significant decrease in ambulatory blood pressure level while receiving unchanged medications (Table 1). In Patient 2, on the other hand, the number of antihypertensive medications was reduced from 3 to 1 while the mean 24-hour blood pressure level increased (Table 1).

DISCUSSION

In this pilot trial, we showed that five patients could safely be subjected to perioperative external circumferential cryoablation of the renal arteries. Perioperative ultrasonographic investigations revealed patent renal arteries without thrombosis, dissection, and pseudoaneurysm after the ablation, and serum creatinine levels did not increase significantly. The numerical increase in creatinine level was 0.014 ± 0.17 mg/dL in the controls and 0.018 ± 0.088 mg/dL in the patients. An increase in creatinine level is common after open aortic surgery in general [13]. We found no clinical signs of damaged renal arteries after cryoablation. However, this cohort of patients with severe aortic disease who

required open aortic surgery likely had extensive arterial calcifications in general. Indeed, 6 of the 9 patients in the total cohort had a pulse wave velocity of >10 m/s, which is indicative of clinically significant arterial calcification [14]. We also acknowledge the limitation of the modest study sample of only five patients. We used ambulatory blood pressure recordings to discern the potential blood pressure lowering effect of cryoablation. No apparent general blood pressure decrease was found. However, the recruited participants did not have classic resistant hypertension at the baseline investigation, and the blood pressure-reducing effect of antihypertensive drugs is well known to be related to baseline blood pressure levels such that it is weaker in patients with near-normal blood pressures. Hence, full doses of antihypertensive drugs can be prescribed to normotensive patients [15-17]. Indeed, in most earlier trials, renal denervation was applied in patients with hypertension deemed to be resistant to medical treatment [8,18]. As all the patients in our trial had severe aortic disease, we were motivated, and so were the ethics review board, to test a new method to potentially achieve better blood pressure control non-pharmacologically. In Sweden, the clinical routine treatment for severe aortic disease is aimed at complete normalization of blood pressure as much as possible to avoid aortic rupture and other cardiovascular complications. We found only small fluctuations in urinary methoxy-catecholamine levels in the patients who underwent cryoablation. As an example, Patient 3 had an 18-mmHg lower systolic 24-hour blood pressure at follow-up without displaying a clinically relevant reduction in urinary

methoxy-catecholamine levels. However, the reliability of the measurement of urinary excretion of catecholamines for quantifying renal sympathetic nerve activities remains controversial [9].

Mean blood glucose levels, measured as HbA1c levels, were reduced significantly in the patients who underwent cryotherapy, despite the presumed increase in insulin resistance due to in part to postoperative immobility and pain [19]. We acknowledge missing data in this respect in the control group. However, in the two controls who had HbA1c levels determined before and after abdominal surgery, the levels did not display any numerical reduction. Renal sympathetic denervation has been hypothesized to increase insulin sensitivity and hence to be an adjunct therapy for type 2 diabetes [20], as a high sympathetic nervous tone hinders insulin release and decreases sensitivity to insulin [21,22].

The effectiveness of cryoablation for the treatment of hypertension was not clearly demonstrated in this trial. Periadventitial cryoablation for 1 minute may not be enough to fully denervate the renal arteries. The optimal duration, frequency, and location of renal cryoablation must be refined in a future phase II trial. This treatment is expected to induce a more complete sympathetic nervous system denervation (and is likely faster than surgical denervation) than intraluminal radiofrequency applications [11], although no established methods are presently available to prove such an effect in humans.

CONCLUSION

In this phase I trial, the renal arteries were safely subjected to periadventitial circumferential cryoablation without complications in five consecutive patients for 1 minute with the activation by liquid nitrogen. After surgery, one

patient had a clinically relevant decrease in blood pressure and another displayed an orthostatic reaction that required a reduction in the number of the antihypertensive medications. Hence, some signs of an antihypertensive effect were observed that could be tested in future trials of the cryoablation procedure.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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AUTHOR CONTRIBUTIONS

Concept and design: CF, NB, FHN. Analysis and interpretation: CF, NB, FHN. Data collection: NB, FHN. Writing the article: FHN. Critical revision of the article: CF, NB, FHN. Final approval of the article: CF, NB, FHN. Statistical analysis: NB, FHN. Obtained funding: FHN. Overall responsibility: FHN.

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