Vasoreactivity and histology of the radial artery: comparison of open versus endoscopic approaches

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Abstract

Objective: Radial artery harvesting using the less invasive endoscopic technique involves dissection in a narrow tunnel and may cause an injury or induce vasospasm to the conduit. To assess this hypothesis, radial artery segments harvested endoscopically or conventionally were studied for reactivity and integrity. **Methods:** Rings of radial arteries from 80 CABG patients who had their radial artery harvested either open ($n = 40$) or endoscopic ($n = 40$), were attached to a force transducer then subjected to norepinephrine (NE, 10^{-6} M), acetylcholine (ACh, 10^{-5} M), followed by sodium nitroprusside (SNP, 10^{-7} to 10^{-5} M) to test endothelial dependant and non-dependant relaxation. Vessels' integrity was assessed by microscopic staining with hematoxylin—eosin for muscle layers, Masson trichrome for collagen content and von Gieson for elastica layers. **Results:** Contraction and relaxation in response to NE, ACh and SNP were similar in both techniques. Arterial layers, collagen content and elastic lamina were preserved in all radial rings. Both techniques were found to be equally efficient in physiological and microscopic tests. **Conclusions:** The similar reactivity and integrity of the radial artery in both techniques should encourage the less invasive endoscopic approach.
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Keywords: CABG; Arterial grafts; Endoscopy; Vascular tone and reactivity

1. Introduction

The radial artery has been used as a conduit for coronary artery bypass grafting (CABG) since the early 1970s by Carpentier et al. [1]. After initial disappointment due to low patency rates the procedure was abandoned. Since the mid 1990s there has been a new interest in this conduit and several articles have documented its patency rates as well as its rate of complications [2—6], which seem to be more prevalent then previously thought [3]. In those studies the radial artery was harvested in an open technique. Recently, the endoscopic radial harvesting technique was introduced and in addition to superior cosmetics results it seems to carry less complications relating to the vessel harvesting [7—9]. The endoscopic technique involves manipulation of the conduit in a limited space, potentially injuring the conduit and inducing vasospasm [10]. Lately the endoscopic versus conventional open radial artery harvest technique was studied using thromboxane analog U46619 and hematoxylin—eosin staining to assess the vasoconstrictor response and radial artery integrity [10]. In view of these clinical implications this work was designed to:

1. Assess the effect of the conventional open and endoscopic harvesting techniques on the vasoreactivity of the radial arteries using normal physiologic pressures [11], and vasoactive drugs that are commonly used in clinical life.
2. Analyze the histological structure of the radial arteries relative to the harvesting technique, using several staining techniques: hematoxylin—eosin for muscle layers, Masson trichrome for collagen content and von Gieson for elastica layers.

2. Materials and methods

Eighty patients undergoing first-time isolated CABG with the use of the radial artery were enrolled in the study. Patients were prospectively assigned into two groups on the basis of radial artery harvest technique: endoscopic (Endo

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group) and conventional open (Open group). Harvesting technique was the choice of the surgeon. Only patients in whom the preoperative plan was to graft the radial artery to either high marginal branch of the circumflex artery, or to the proximal part of the posterior descending artery were included in the study. As a result, the extra length of the radial artery allowed us to give up its proximal part for laboratory analysis.

2.1. Radial artery harvesting

The endoscopic group had their radial artery harvested endoscopically as described by Connolly et al. [8]. In short, via a single wrist incision of 2—3 cm, dissection with the aid of harmonic scalpel was performed. Cardiovations equipment (a division of Johnson & Johnson, Somerville, NJ) was used. Proximal control was achieved via the wrist incision by the application of an endoscopic vascular clip. The open group had their radial artery harvested as described by Reyes et al. [12]; however, the harmonic scalpel was used for dissection as in the endoscopic group. After disconnecting the artery from the arm, a segment between 1 and 1.5 cm long was cut from its proximal (elbow) end, placed in cold (4 °C) Krebs-Henseleit solution, and immediately transferred to the laboratory for studies of vascular function (in less than 15 min). Approval to use discarded radial arteries was granted by the hospital human ethics committee.

2.2. Organ bath technique

Krebs-Henseleit (Krebs') buffer solution contained in mmol/l the following: NaCl, 118.3; KCl, 4.7; CaCl, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.1; and Na_2EDTA, 0.026. Norepinephrine (NE), acetylcholine (ACh), and sodium nitroprusside (SNP) were from Sigma—Aldrich, USA. The vessels were carefully dissected from their surrounding fat tissue and divided into 3 mm long vascular rings. Two wire stirrups were placed through each ring and attached to a force transducer for isometric tension recordings. The upper wire is attached to a grass force transducer while the lower is attached to a micrometer. The rings were lowered into separate organ chambers, filled with 20 ml of Krebs' solution (pH 7.4, 37 °C) bubbled with 95% O_2 + 5% CO_2. Each ring segment is equilibrated unstretched on the wire hooks for 40 min. A standardization procedure was followed thereafter to set a baseline resting tension at which subsequent tension measurements were obtained as follows:

the wires are moved apart in steps every minute while the force (f[g]) and the micrometer reading (ℓ[mm]) are recorded. The Laplace law for thin pressure cylindrical vessels together with an exponential connection between wall tension and artery assumed circumference (L[mm]) are exploited to determine (by solving a nonlinear equation) the theoretical lumen circumference that would have corresponded to a circular vessel distended by a transmural pressure of 100 mmHg [13]. This process is performed online using a computer code so as to make sure we do not overstretch the radial artery. This value is called L100; the lumen circumference equivalent to a transmural pressure of 100 mmHg. The artery is then

relaxed to a circumference equal to 0.9 L100 kept constant throughout the remaining of experiment [11].

2.3. Mechanical properties of vessels

The radial arteries rings were equilibrated in the passive tension for 45 min then NE (10^{-6} M), followed by ACh (10^{-5} M) and SNP (10^{-7} to 10^{-5} M) were added to assess the endothelial integrity and non-endothelial vessel properties [14]. No washout was done after each step. All vessels underwent the same protocol. The contraction was expressed either as contraction force ([g]) or as contraction force normalized by the circumference [11]. This normalization procedure gives the force produced by 1 mm of the circumference of the vessel and let us compare between rings with different diameters.

2.4. Histological structure

Radial segments (open $n = 6$, endoscopic $n = 6$) were used for histological assessment. Studies were performed on vascular strips separated prior to the organ chamber experiments and fixed immediately in formalin 4%. Sections were cut at 5 μm and stained with hematoxylin—eosin (H&E) for morphological evaluation, Masson trichrome (for the examination of fibrosis and smooth muscle) and Verhoeff van Gieson's elastin (to identify elastic layers) stains [15]. The slides were examined by a pathologist blinded to the clinical data (A.T.). Internal elastic layer was evaluated for fragmentation, multilayering, and presence or absence of calcifications. Media was examined for, bleeding, presence of fibrosis, and hypertrophic or hyperplastic changes in the smooth muscle cells.

2.5. Statistical analysis

Unless otherwise specified, data are presented as mean ± standard deviation for continuous variables or as absolute values with percentages for categorical variables. Comparison between groups for clinical data was performed with unpaired t-test for continuous variables and with χ^2 or Fisher's exact test, as appropriate, for categorical variables. Within and between groups comparison for the different doses and agents used was performed with two-way ANOVA with post hoc Bonferroni correction. The final analysis from each vessel was expressed as an average response of multiple rings for the same patient. Contraction induced by NE was considered as 100%. Relaxation is expressed as the percent decline in the maximal NE-evoked contraction. A previous pilot analysis revealed an average evoked force difference between groups by NE, of 0.7 g with standard deviation of 1.5 g. To provide 80% power with α = 0.05, a sample size of 37 pairs was calculated. A p value of less than 0.05 was considered significant.

3. Results

Baseline patient characteristics are presented in Table 1. Baseline patient profile was similar among the groups, except for a trend of being older in the standard group and having

Table 1
Baseline patient characteristics

	Endo (*n* = 40)	Open (*n* = 40)	*p*
Age (years)	62 ± 10	66 ± 10	0.06
Female sex	5 (12.5)	7 (17.5)	0.53
Diabetes	15 (37)	12 (30)	0.6
Hypertension	32 (80)	32 (80)	1
Dyslipidemia	30 (75)	22 (55)	0.06
Smoking history	25 (62.5)	23 (57.5)	0.65
Triple vessel disease	38 (95)	39 (97.5)	0.5
Left main disease >50%	10 (25)	12 (30)	0.62
Severe left ventricular dysfunction	2 (5)	2 (5)	1

Data are presented as mean ± standard deviation for age, or as number of cases (percent) for all other variables.
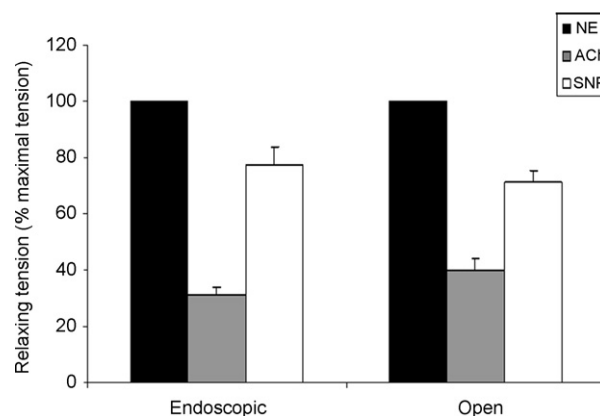

Fig. 1. Maximal relaxing response (% of maximal tension) for human radial artery precontracted with norepinephrine (NE) 10^{-6} M from open and endoscopic harvest procedures and subjected to acetylcholine (ACh) 10^{-5} M and sodium nitroprusside (SNP) 10^{-5} M. Values are expressed as a percentage of the maximal tension developed in the presence of NE and are the means ± S.E.M. Contraction in response to NE is considered as 100% in the first column and the relaxation in response to ACh and SNP in the two other columns.

more dyslipidemia in the open group. There was no perioperative mortality, postoperative myocardial infarction, or stroke in any group. Radial artery harvest-related outcomes were also similar, with no incidence of hand ischemia, motor deficits, or bleeding complications. There was one wound infection in the open group that was treated conservatively.

3.1. Vasoreactivity of the radial artery

The diameter of the rings at a transmural pressure of 100 mmHg was 2.79 ± 1.01 mm for the open group and 2.60 ± 0.68 mm for the endoscopic group, *p* > 0.05. The resting force was 2.63 ± 0.41 g for the open group, and 3.27 ± 0.42 g for the endoscopic group, *p* > 0.05. NE-evoked 8.68 ± 1.73 g force in the open group, and 9.68 ± 1.35 g in the endoscopic group, *p* > 0.05. Normalizing these values to the radial diameter revealed similar values: 0.73 ± 0.163 g/mm force in the open group, and 0.84 ± 0.66 g/mm in the endoscopic group, *p* > 0.05.

3.2. Relaxation

Endothelial dependant relaxation in response to ACh 10^{-5} M, and endothelial independent relaxation in response to increasing concentration of SNP were found to be similar in both groups (Fig. 1). Significant differences in force of relaxation were found in response to incremental elevation of SNP concentration starting from 21 ± 5.2% versus 17 ± 3.5% with SNP 10^{-7} M, reaching maximal relaxation at 10^{-5} M, 71 ± 4.5% versus 77 ± 4.4%, for the open and endoscopic radial arteries, respectively (*p* < 0.001).

3.3. Histological examination

Routine H&E, Masson trichrome, and Verhoeff van Gieson's stains revealed that all the arterial layers were preserved in all segments harvested by both surgical approaches (Fig. 2). By H&E, a mild degree of arteriosclerosis was seen in most of the arteries. Some cases showed small calcifications. The internal elastic layer was fragmented mildly in some cases but without differences between both procedures. In the media a mild degree of fibrosis is present but it is not accompanied by changes in the smooth muscle cells. Inflammatory cells were not present in the sections examined, and no signs of bleeding were identified. In summary, no signs of acute injury were found in either group.

4. Discussion

The radial artery turned out to be a popular conduit during CABG due to favorable data regarding its patency rate. Calafiore et al. identified nearly perfect patency after 3 years when the radial artery was used to revascularize the lateral wall [16]. Caputo et al. reported even better midterm clinical results of the radial artery over the right internal thoracic artery [17]. Tatoulis showed in a long-term angiographic follow-up the superiority of the radial artery over vein grafts especially when used in high-grade coronary lesions [6]. However, not all results are encouraging. In patients predominantly presenting with signs and symptoms of myocardial ischemia after CABG, radial artery grafts showed lower patency rates than left internal mammary artery and saphenous vein grafts [18]. The less invasive endoscopic radial artery harvest technique was recently introduced to reduce the morbidity associated with conventional radial artery harvest and to improve cosmetics [8]. Although reported short-term clinical outcomes of the endoscopic technique are excellent [7–9], the endoscopic technique involves manipulation of the conduit from a distance in a two-dimensional limited space, and dissection of the pedicle with control of side branches by using harmonic shears in close proximity to the conduit, exposing the vessel to a potential mechanical or thermal injury. Normal structure and function of the vascular endothelium is essential to the function of the artery [19], and mechanical trauma to the conduit during harvest has been identified as a major factor responsible for endothelial dysfunction [20], and may affect graft patency. The production of endothelial nitric oxide (NO) is essential for the normal function of arterial vessels. It inhibits platelet aggregation, leukocyte adhesion, and smooth muscle proliferation and migration, and as such is believed to have strong antithrombotic and antiatherosclerotic effects [19,21]. In the present study we have demonstrated that structural integrity and vasoreactivity of radial arteries harvested endoscopically or in open technique remained intact. We have used different microscopic
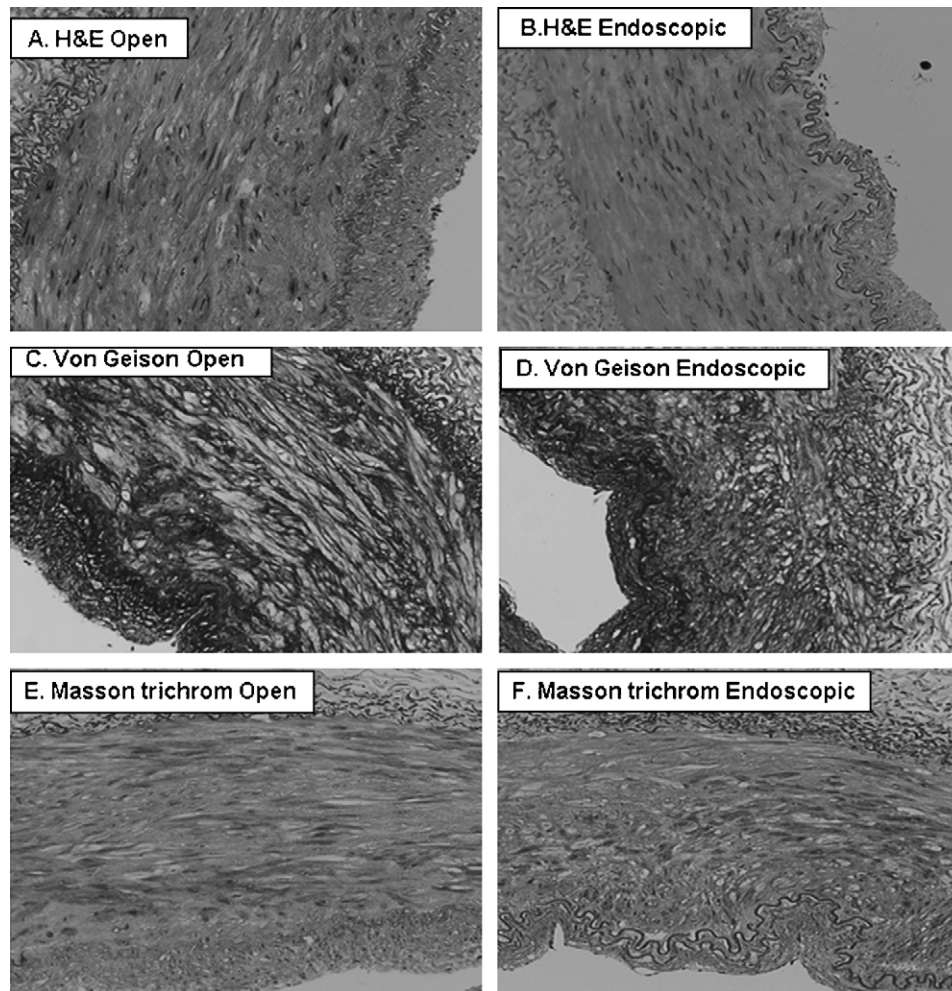
Fig. 2. Examples of radial arteries from open and endoscopic harvest procedures. (A and B) Arterial wall showing mild thickening of the intima. The elastic layer appeared intact. There is no thickening of the smooth muscle cells layer (magnification ×100, H&E stain). (C and D) Elastic stain showing minimal fragmentation of the elastic layer (magnification ×100, von Gieson stain). (E and F) Very mild fibrosis of the media is present (magnification ×100, Masson trichrome stain).

techniques for the evaluation of the integrity of the different arterial layers, smooth muscle cells, collagen fibers and elastic layer that take part in the contraction and relaxation of the vessels. The microscopic examination showed no differences between the groups. Under both methods, the arterial wall showed mild arteriosclerosis with scattered calcifications, mild degree of fibrosis in the media. Except for mild elastic layer fragmentation in some cases that may be signs of acute injury, no other signs of acute injuries like bleeding, the presence of inflammatory cells, or endothelial damage could be identified in the specimens studied.

The results of this study are in accordance with previous study of Shapira et al. who showed that there are no differences between the less invasive endoscopic and the conventional open radial artery harvest techniques [10]. They used segments of radial artery that were initially stored in papaverine, a potent vasodilator that may affect the response of the segments studied to other vasoactive agents. We did not expose the radial artery to any vasoactive agent prior to the analysis to overcome this concern. In their chamber methodology, they used rings resting tension that produced a maximal response of 80 mmol/l KCl. We used a different approach in order to mimic the physiological condition as much as possible by normalizing each vessel to a resting tension calculated according to its own length tension curve as suggested by He and Yang [11]. We thereafter assessed the function of the vessels using vasoactive and relaxing drugs commonly used in the operating room and postoperative intensive care unit. Studies have demonstrated that the radial artery has a higher response to NE than the internal thoracic artery [22]. The stimulation by catecholamines of α-adrenoceptors may be the cause of radial artery spasm. The human radial artery is an α-adrenoceptor-dominant artery with little β-adrenoceptor function [23]. Therefore we studied the response of the different radial artery segments to NE, which is a $\alpha 1$ and $\alpha 2$ agonist and is commonly used in clinical situations. Shapira et al. used thromboxane analogue as a vasoconstrictor agent [10]. In our organ chamber method, we showed that endothelial-dependent, NO-mediated vascular relaxation (ACh), and endothelial-independent vascular relaxation (SNP) were similar. These results of functional tests support the results obtained in microscopy where no injury to vessels was observed using both harvesting techniques.

4.1. Study limitations

Although this is a prospective study it is not a random one, and as such carries the risks of bias. We studied the proximal segments of the radial artery; while there is a concern that the biological behavior of different segments of the artery is not similar [24,25], it is unlikely that there will be different responses between groups along the arteries.

5. Conclusion

Endoscopic harvesting of the radial artery does not seem to alter radial artery vasoreactivity or endothelial integrity compared with the open harvest technique, in physiologic like conditions. The superior cosmetics and fewer complications should encourage the less invasive endoscopic approach and further long-term comparison studies.

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