Biseko® colloidal solution diminishes the vasoreactivity of human isolated radial arteries

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Abstract

Objective: Radial arteries are increasingly used as grafts in coronary artery bypass surgery. The surgical preparation and intraoperative management of this conduit artery may affect its early and long-term patencies. We investigated the effects of the colloidal Biseko® and 5% albumin solutions as well as the crystalloid physiological saline (0.9% NaCl) and Bretschneider solutions on the contractile and relaxing capacities of isolated human radial artery grafts. Method: Radial artery segments were harvested using the technique with an ultrasonic scalpel, and 2.5—3 mm rings were obtained from the proximal part of the artery. Arterial rings were stored in Biseko® or 5% albumin solutions and in 0.9% NaCl or Bretschneider solutions for 45 min. Isometric tensions of radial arteries obtained from 26 patients were measured in isolated organ baths. Contractions were induced by 0.31 μmol L−1 5-hydroxytryptamine and 10 μmol L−1 noradrenaline. Endothelium-dependent relaxations were induced by 10 μmol L−1 acetylcholine and 1 μmol L−1 bradykinin as well as the endothelium-independent relaxations by 10 μmol L−1 glyceryl trinitrate and 100 μmol/L papaverine. Results: Contractions of radial arteries induced by 5-hydroxytryptamine were significantly lower following storage in Biseko® solution (12.6 ± 4.4 mN) than in 5% albumin (37.9 ± 13.0 mN, p = 0.03) or in 0.9% NaCl solution (35.9 ± 11.9 mN, p = 0.04). Noradrenaline-induced contractions of the arteries were also diminished in Biseko® solution compared to those stored in 5% albumin (32.9 ± 6.2 mN vs 49.2 ± 6.4 mN, p = 0.01). No significant differences in relaxations were obtained between the two crystalloid and the two colloidal solutions using endothelium-dependent and independent vasorelaxants. Conclusion: Our results suggest that storage of radial artery in Biseko® colloidal solution before coronary artery bypass grafting decreases the sensitivity of the graft to vasoconstriction, thereby decreasing the risk of intra/perioperative graft failure.

Keywords: Radial artery; Biseko® solution; Graft storage; Vasoconstriction

1. Introduction

The use of radial artery for coronary bypass surgery has increased in the past years due to its excellent early and long-term patency [1,2]. Improved outcomes have been achieved with novel techniques regarding the graft preparation and the prevention of perioperative vasospasm. The radial artery grafts are usually stored in crystalloid or colloidal solutions for 30—45 min before the surgical implantation of the graft. These solutions are used to maintain the integrity and function of the radial artery known to frequently develop spasm in the intra/perioperative period of coronary artery bypass grafting (CABG) [3]. None of the currently used solutions provides an ideal graft function. The most convenient storage solution, in addition to its non-thrombotic property, would be expected to be inert and even to minimize the occurrence of vasospasm. This can be achieved by preserving the vascular endothelial function and/or preventing the enhanced contractile capacity of the arterial smooth muscle. Although graft segments stored in heparinized whole blood had greater endothelium-dependent relaxation to acetylcholine [4], blood-stored radial artery grafts revealed markedly increased smooth muscle contractions [5]. Papaverine, a widely used smooth muscle relaxant in storage solutions, impairs endothelial function [6].

The colloidal Biseko® solution is a cold sterilized liquid in which coagulation factors and bacterial toxins are absent or minimal. The immunoglobulin concentrations and activities are equivalent to those in normal serum. Biseko® solution in patients with autoimmune diseases decreases the risk of
infections and adverse drug reactions in comparison to fresh frozen plasma [7]. In addition, a preliminary observation has suggested that this colloidal solution might protect vascular tissues by decreasing the permeability of the endothelium (unpublished observation).

In the present study, we compared the effect of the colloidal Biseko® as a storage solution, to 5% albumin as a control colloidal solution as well as to two crystalloid, physiological saline (0.9% NaCl) and the cardioplegic Bretschneider solutions, on the contractile and relaxing capacities of isolated human radial artery grafts.

2. Patients and methods

2.1. Patients

This investigation received the approval of the local institutional review board (Human Investigation Review Board, University of Szeged, Hungary, No. 164/2002 and No. 161/2004). Each patient gave informed consent to accept the aim and protocol of investigation. At our institution, between November 2005 and November 2007, 87 coronary artery bypass graft operations were performed on the beating heart using radial artery from the non-dominant forearm. Modified Allen test and measurement of the radial artery diameter (>2 mm) were used to select the patients for operation. The positive Allen test provided evidence for an acceptable blood flow from the ulnar artery to the palmar circulation after a short-term compression of the radial artery. The patients with negative results were excluded. The radial artery was used as aortocoronary bypass graft to the right coronary artery or to the obtuse marginal branch of the circumflex coronary artery. Before operation, the diameter of radial artery had been measured with ultrasonic technique (General Electric Logiq 7, linear head, USA). Twenty-six patients were divided into group I, in that radial arteries were incubated in crystalline solutions, and group II, in that radial arteries were incubated in colloidal solutions. Two vascular preparations from each patient were studied in parallel; group I patients: 0.9% NaCl, Bretschneider solution (group II patients: 5% albumin as a control colloidal solution as well as to two crystalloid, physiological saline (0.9% NaCl) and the cardioplegic Bretschneider solutions, on the contractile and relaxing capacities of isolated human radial artery grafts.

Table 1 Characteristics of patients undergoing coronary revascularization.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group I: Radial artery incubated in crystallloid solutions</th>
<th>Group II: Radial artery incubated in colloidal solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Sex</td>
<td>9 males, 3 females</td>
<td>11 males, 3 females</td>
</tr>
<tr>
<td>Age (year)</td>
<td>59.0 ± 2.4</td>
<td>60.1 ± 2.2</td>
</tr>
<tr>
<td>Diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>12 (100%)</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>11 (92%)</td>
<td>13 (93%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (17%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta blockers</td>
<td>11 (92%)</td>
<td>13 (93%)</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>10 (83%)</td>
<td>13 (93%)</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>1 (8%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Antihyperlipidemics</td>
<td>11 (92%)</td>
<td>13 (93%)</td>
</tr>
</tbody>
</table>

% represents the percent occurrence of the diseases or treatments within the group.

a Either 0.9% NaCl or Bretschneider solution.
b Either 5% human albumin or Biseko® solution.
c ACE: angiotensin converting enzyme.

2.2. Preparation of radial artery segments for in vitro testing

Arterial samples were prepared atraumatically with the Ultracision Harmonic Scalpel (Ethicon Endo-Surgery, USA). Once harvesting of the arterial tissue samples had been started, low dose nifedipine (0.2–0.4 mg h⁻¹) was given in intravenous infusion to prevent early vasospasm. We prepared a 5–6 mm long segment of the radial artery (RA) at the origin of the brachial artery (proximal part). The RA was then carefully dissected and cleaned from the surrounding connective tissue. The segment of the artery was cut into two 2.5–3 mm long rings and submerged to 0.9 % NaCl and Bretschneider solutions (group I patients) or 5% human albumin and Biseko® solutions (group II patients) for 45 min. Biseko® is a 5% human serum protein solution produced from a plasma pool of at least 1000 healthy donors. Each ml contains 50 mg human serum protein (approximately 31 mg human albumin and approximately 10 mg human immunoglobulin). Lipoproteins are removed by adsorption to achieve a good storage stability and the preparation does not contain coagulation factors. The solution is free of hemolysins and can therefore be administered regardless of blood group.

2.3. Isometric tension measurement and protocol of investigation

Two rings of RA grafts were mounted in parallel on stainless-steel hooks and placed into organ chambers containing 2 ml Krebs–Henseleit solution maintained at 37 °C. The solution was continuously aerated with a gas mixture of 95% O₂ and 5% CO₂ at pH 7.4. One of the hooks was anchored and the other one was connected to a force–displacement transducer (Hugo Sachs Elektronik, Type F30, Germany) to measure changes in isometric tension as described previously [8]. Vessel rings were subjected to 20 mN tension and equilibrated for 45 min. During this period the tension was continuously readjusted to the above value of stretch and the medium was changed every 15 min. The assessment of the optimized value for resting (basal) tone has been presented formerly [9].

Following the equilibration period the rings were precontracted with 80 mmol L⁻¹ KCl. After washing the arterial rings contractions were repeated with 0.3 μmol L⁻¹ 5-hydroxytryptamine (5-HT). Endothelium-dependent relaxations were induced by acetylcholine (10 μmol L⁻¹) and bradykinin (1 μmol L⁻¹). Endothelium-independent relaxations were produced by glyceryl trinitrate (10 μmol L⁻¹) and papaverine (100 μmol/L). In a separate series of experiments RA rings obtained from group II patients were contracted with noradrenaline (10 μmol L⁻¹).

2.4. Statistical analysis

Contractions and relaxations were expressed in milli-Newton (mN). Data are presented as mean ± standard
deviation (mean ± SD). For comparisons of the data one-way analysis of variance followed by Newman–Keuls multiple range test was used. In the case of bradykinin relaxations were also analyzed with the non-parametric Mann–Whitney test. Statistical significance between two groups was tested with Student’s t-test and p values less then 0.05 were considered as significant.

2.5. Drugs and solutions

5-Hydroxytryptamine (5-HT: serotonin creatinine sulfate complex), noradrenaline (norepinephrine hydrochloride), acetylcholine (acetylcholine chloride), bradykinin (bradykinin acetate) and papaverine (papaverine hydrochloride) were obtained from Sigma–Aldrich (St Louis, MO, USA). Glyceryl trinitrate was purchased from Pohl Bosphat (Hohenlockstedt, Germany). The solutions used and their compositions: 5% human albumin (50 g L⁻¹ human albumin, 4 mmol L⁻¹ caprylate, 4 mmol L⁻¹ acetyltryptophan, 145 mmol L⁻¹ sodium, 2 mmol L⁻¹ potassium) and Biseko® solution (50 g L⁻¹ human serum protein (31 g L⁻¹ albumin, 10 g L⁻¹ human immunoglobulin), 154.85 mmol L⁻¹ sodium, 4.09 mmol L⁻¹ potassium, 1.99 mmol L⁻¹ calcium, 0.82 mmol L⁻¹ magnesium, 100.56 mmol L⁻¹ chloride) were obtained from Biotest (Hungaria Kft., Törökbalint, Hungary); Bretschneider solution (15 mmol L⁻¹ NaCl, 10 mmol L⁻¹ KCl, 4 mmol L⁻¹ MgCl₂, 180 mmol L⁻¹ histidine, 2 mmol L⁻¹ tryptophane, 30 mmol L⁻¹ mannitol, 1 mmol L⁻¹ potassium dihydrogen oxoglutarate) and Krebs–Henseleit solution (120 mmol L⁻¹ NaCl, 4.2 mmol L⁻¹ KCl, 1.5 mmol L⁻¹ CaCl₂, 20 mmol L⁻¹ NaHCO₃, 1.2 mmol L⁻¹ MgCl₂, 1.1 mmol L⁻¹ KH₂PO₄, 11 mmol L⁻¹ glucose and 0.27 mmol L⁻¹ EGTA) and 0.9 % NaCl were purchased from Reanal (Budapest, Hungary).

3. Results

3.1. Contractions of human isolated radial arteries

3.1.1. Contractile tensions induced by potassium chloride after incubation of the grafts in crystalline and colloidal solutions

Maximum tensions of radial artery grafts were obtained by using 80 mmol L⁻¹ potassium chloride (KCl) as contractile agent. No significant differences were found between the reactivity of the arteries in group I patients (in 0.9 % NaCl = 38.4 ± 7.5 mN and in Bretschneider = 41.1 ± 6.6 mN, n = 12 and 12) or in group II patients (in 5% albumin = 36.5 ± 6.4 mN and in Biseko® = 34.6 ± 6.8 mN, n = 14 and 14).

3.1.2. Contractile tensions induced by 5-hydroxytryptamine after incubation of the grafts in crystalline and colloidal solutions

Contractions of the radial arteries induced by 5-hydroxytryptamine (5-HT, 0.31 µmol L⁻¹) are depicted in Fig. 1. It can be seen that, following incubation of the radial artery segments in crystalline solutions, no considerable difference was found in their reactivity in 0.9 % NaCl (35.9 ± 11.9 mN, n = 12) and in Bretschneider (26.7 ± 8.3 mN, n = 12) solutions. Incubation of the radial arteries in the colloidal

Biseko® solution revealed significantly smaller contractions to 5-HT than those obtained after incubation of the graft samples in 5% albumin (Biseko® = 12.6 ± 4.4 mN vs 5% albumin = 37.9 ± 13.9 mN, n = 14 and 14, p = 0.03) or in 0.9 % NaCl (35.9 ± 11.9, p = 0.04 compared to the values in Biseko® solution).

3.1.3. Contractile tensions induced by noradrenaline after incubation of the grafts in crystalline and colloidal solutions

In order to exclude a specific serotonergic mechanism of Biseko® solution, we performed experiments in new arterial rings obtained from group II patients. 10 µmol L⁻¹ noradrenaline-induced contractions were significantly less in the radial artery rings incubated in Biseko® (32.9 ± 6.4 mN, n = 14) than that incubated in the control colloidal solution, 5% albumin (49.2 ± 6.4 mN, n = 14, p = 0.01 vs Biseko®).

3.2. Relaxations of human isolated radial arteries

3.2.1. Endothelium-dependent relaxations after incubation of the grafts in crystalline and colloidal solutions

Endothelium-dependent vasorelaxants, acetylcholine (Ach, 10 µmol L⁻¹) or bradykinin (BK, 1 µmol L⁻¹) were administered in 0.31 µmol L⁻¹ 5-HT-precontracted radial artery rings (Fig. 2). No significant differences in relaxations were obtained among the two crystalloid and the two colloidal solutions. In all the four groups, small endothelium-dependent relaxations and large individual variabilities could be detected. BK did not relax the radial artery in Bretschneider solution (compared to hypothetical zero values (n = 12), not significant with Mann–Whitney non-parametric test).

3.2.2. Endothelium-independent relaxations after incubation of the grafts in crystalline and colloidal solutions

Fig. 2 (right side) demonstrates that the endothelium-independent vasorelaxant, glyceryl trinitrate (GTN,
or minimal in Biseko as thrombin and tissue factor were found to cause direct contractions evoked by 5-HT in the isolated radial arteries. The endothelial stimulators, acetylcholine and bradykinin, as well as the endothelium-independent dilator, glyceril trinitrate (GTN, 10 μmol L⁻¹), were applied to 5-hydroxytryptamine (0.31 μmol L⁻¹) precontracted arterial rings. Values are shown as mean ± standard deviation obtained from 12 (crystalline, group I) or 14 (colloidal, group II) individuals.

10 μmol L⁻¹) exerted similar relaxations against 5-HT-induced contractions in crystallloid and colloidal solutions. Papaverine (100 μmol L⁻¹) almost completely relaxed the contractions evoked by 5-HT in the isolated radial arteries. The magnitudes of the decrease of arterial tensions were as follows: in 0.9% NaCl = −48.8 ± 10.7 mN, n = 12; in Bretschneider solution = −43.8 ± 9.0 mN, n = 12; in 5% albumin = −48.3 ± 7.5 mN, n = 14; in Biseko® = −37.2 ± 4.6 mN, n = 14).

4. Discussion

Human isolated radial arteries incubated in the colloidal Biseko® solution produced diminished contractions to 5-hydroxytryptamine as well as to noradrenaline as compared to the colloidal albumin or physiologic saline solutions. The maximum vasoconstrictive and vasodilating capacities of the isolated radial artery, measured with potassium chloride and papaverine, respectively, did not differ in the four storage solutions. Vasodilating functions of the arteries obtained with the endothelial stimulators, acetylcholine and bradykinin, as well as with the direct relaxant of the smooth muscle, glyceryl trinitrate, also revealed no significant differences.

5-Hydroxytryptamine (serotonin) is considered to be an important factor for inducing vasospasm and may cause intra/perioperative failure of bypass conduits [10]. In the present study storage of the radial artery preparations in Biseko® solution 45 min before the measurement of tensions in vitro, contractions to 5-hydroxytryptamine and noradrenaline were considerably decreased compared to those in arteries submerged into 5% albumin. Although pure colloids are hypocoagulable by their own, the standard colloidal albumins are usually contaminated with coagulants and also with bacterial toxins, viruses and prions [11,12] being absent or minimal in Biseko® solution. Some coagulation factors such as thrombin and tissue factor were found to cause direct vasoconstriction and protein C enhanced the α-adrenergic receptor mediated contractile responses of arteries [13,14].

A bacterial toxin, e.g. Escherichia coli hemolysin, is able to release thromboxane, a potent vasoconstrictor of the radial artery with damaged endothelium [15,16]. The ultimate presence of one or more of these contractile factors may render the standard albumin preparations unsuitable for the storage of radial artery before coronary artery bypass graft surgery. Because the whole blood also contains some of the above-mentioned mediators, this partly explains why blood-stored radial artery grafts revealed augmented smooth muscle tensons [5]. Arteries stored in Biseko® solution also responded with significantly less contractions to 5-hydroxytryptamine compared to those stored in physiological saline and albumin-stored arterial responses did not differ from saline-stored ones. These results exclude a non-specific effect of N-acetyl-L-tryptophan and caprylate, stabilizers of pharmaceutical-grade albumins, on the tone of the radial arteries [17]. We assume that the decreased endothelial permeability, evoked by the colloidal Biseko® solution, is partly responsible for the diminished transport of the hydrophylic contractile amines through the endothelial pores to the underlying smooth muscle cells. In our present investigation, the endothelium-dependent relaxation of the radial arteries did not exceed an average of about 20% compared to that evoked with papaverine. In Bretschneider solution, even no endothelial relaxation was detected by bradykinin that can be explained by the presence of high potassium (10 mmol L⁻¹) in this storage solution known to further impair the endothelial function [18]. The endothelial damage of vascular tissues during the preparation could be excluded because the blood vessels were prepared with an atraumatic ultrasound technique. In order to avoid further damages the inflation of the arteries was also avoided before the measurement of tension as it was widely used for exploring small holes in the artery before implantation. The poor endothelial function rather represents a more severe arterial disease of patients included in this investigation compared to those undergoing radial artery bypass graft in another center of cardiac surgery (55 ± 6% acetylcholine-induced relaxation) [19]. The radial artery is known to have a high prevalence of atherosclerosis [20,21]. Indeed, in our study, more than 91% of patients had hypercholesterolemia and 100% had hypertension indicating advanced cardiovascular diseases. This represents remarkably more severely diseased patients than those of the same ages undergoing CABG, elsewhere [22]. The radial artery is used as the third or fourth choice among conduit grafts following the left and right internal mammary artery and sometimes the gastroepiploic artery [23]. Our patients who required multi-vessel CABG or repeated surgical interventions should have severely damaged endothelial vasodilating function, a condition known to be predictive factor for arterial vasospasm. This assumption is in line with our previous observation that proximal radial artery showed larger contraction than the left internal mammary artery [7]. The routine use of an ‘antispasmodenic’ solution such as the Biseko® solution for the storage of radial artery grafts appears to be essential.

The results suggest that storage of radial artery in Biseko® colloidal solution before the implantation during CABG decreases the sensitivity of the graft to vasoconstriction. The superiority over human serum albumin, physiologic saline and Bretschneider solutions may make Biseko®
solution useful for decreasing the risk of intra/perioperative spasm of radial artery bypass grafts and for preventing subsequent graft failure.

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References


