Catheter-Based Intramural Delivery of Red Blood Cells in an Animal Model of Atherosclerosis

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ABSTRACT

This report demonstrates intramural red blood cell (RBC) delivery in an atherosclerotic rabbit aorta model and validates the ability of fluoroscopy and computed tomography to verify RBC deposition. A microinfusion catheter with a 35-gauge needle delivered RBCs mixed with iodinated contrast agent to the aorta wall. Six rabbits were sacrificed after injection to confirm RBC delivery. Iron deposition was examined in four additional rabbits 3–7 weeks after injection. Imaging demonstrated 86% sensitivity and 100% specificity for the detection of RBC deposition (n = 25 injection attempts). Iron deposits were found in all intraplaque injection sites 3–7 weeks after injection.

ABBREVIATIONS

H&E = hematoxylin-eosin, IPH = intraplaque hemorrhage, RBC = red blood cell

Although vessel stenosis in atherosclerotic arteries is a well-established risk factor for downstream cardiovascular and cerebrovascular events, plaque composition plays a key role in determining future events. Intraplaque hemorrhage (IPH), an important feature of advanced plaques, is a risk factor for clinical events (1–3). Deposited red blood cells (RBCs) contribute key ingredients that may promote plaque instability. RBC membranes are rich in free cholesterol, which is associated with disrupted plaques (4). Hemoglobin-derived iron moieties can oxidize plaque lipids, creating oxidation products that provoke inflammatory cell activity (5).

Testing novel interventional strategies targeted toward mitigating the effects of IPH necessitates an in vivo model that mimics the effects of IPH on plaque progression. The most direct approach is the injection of RBCs within arterial walls, previously accomplished in the rabbit aorta via laparotomy and a fine needle from the adventitial surface (6,7). However, laparotomy is an invasive procedure with access to a limited portion of the vasculature. We present a catheter-based approach for injecting RBCs into plaques combined with fluoroscopy and computed tomography (CT) localization. We hypothesize that noninvasive imaging can identify vessel portions with intramural RBC deposition.

MATERIALS AND METHODS

Experiments were carried out in New Zealand White male rabbits initially weighing 3.0–3.5 kg (Charles River, Saint-Constant, Canada) in accordance with institutional animal care committee guidelines. Two groups of rabbits were studied. Animals in group 1 were euthanized immediately after intramural RBC injection to validate noninvasive imaging for the depiction of RBC deposition sites, and animals in group 2 were euthanized weeks after intramural injection to examine persistent plaque changes secondary to RBC deposition.

For group 1, rabbits (n = 6) were fed a high-cholesterol diet containing 2% cholesterol and 6% peanut oil (Harlan Laboratories, Inc, Madison, Wisconsin) for 3–4 weeks. Endothelial denudation by balloon injury was performed 2 weeks after diet initiation (3 weeks if total fasting serum cholesterol was < 20 mmol/L after 2 weeks). Rabbits were transitioned to a 0.15% cholesterol and 6% peanut oil diet 1 week after denudation to prevent diet-related illness. Intramural injection of RBCs and CT imaging were performed 10–18 weeks after denudation, followed by euthanization.

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For group 2, 4 additional rabbits were fed a 2% cholesterol diet for 3 weeks and switched to normal chow 1 week after denudation. Intramural injection was performed 5 weeks after denudation. Rabbits were euthanized 3 weeks (n = 1), 5 weeks (n = 2), and 7 weeks (n = 1) after intramural injections.

For endothelial denudation, anesthesia was induced with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) and maintained using inhaled isoflurane (0.5%–5%). A 3-F arterial embolectomy balloon catheter (Edwards Lifesciences Corp, Irvine, California) was advanced under fluoroscopic guidance (OEC 9800; GE Healthcare, Waukesha, Wisconsin, or BV Pulsera; Philips Healthcare, Inc, Andover, Massachusetts) through a 4-F right femoral sheath (Cordis Corp, Miami Lakes, Florida) to the level of the renal branches of the aorta. The balloon was inflated and pulled back to the right iliac artery three times. For pain management and prevention of infection, animals received buprenorphine (0.02–0.05 mg/kg subcutaneously twice a day for 3 d) and a combination of procaine penicillin G (150,000 IU intramuscularly) and benzathine penicillin (150,000 IU intramuscularly).

Intramural injections were done under fluoroscopic guidance with the same drug regimens as for the endothelial denudation procedure. After obtaining an angiogram, a 5-F microinfusion catheter with a 0.4-mm long 35-gauge microneedle (modified Bullfrog; Mercator MedSystems, Inc, San Leandro, California) was advanced through a left femoral 5-F sheath over a 0.014-inch guide wire (WIZDOM; Cordis Corp). The microinfusion catheter is a modified version featuring a shorter needle length than the clinical version, which was designed for perivascular and adventitial delivery. The microneedle resides in a deflated balloon and is pushed into the vessel wall during inflation. About 100–200 μL of injectate is required to prime the line, and infusion is done manually with a 1-mL syringe. Autologous RBCs collected immediately before surgery were washed with saline and mixed with saline and iodinated contrast agent in a 70:15:15 ratio of RBCs, saline, and contrast agent. About 70–100 μL of mixture was injected into each site (50–70 μL of RBCs), with multiple sites spanning each aorta from the iliac bifurcation to the renal ostia. In each rabbit in group 1, some locations were also injected with a control mixture containing saline instead of RBCs. See Table 1 for site distribution in group 1 rabbits.

Fluoroscopy during injection showed injectate exiting the needle in either a steady plume that remained on balloon deflation or in a flickering intraluminal plume that disappeared on deflation. A steady plume expanding parallel to the long axis in one direction away from the needle was interpreted as plaque injection owing to resistance from dense plaque (Fig 1a–d). A plume expanding in both directions was interpreted as adventitial injection (Fig 2a–c).

Table 1. Distribution of RBC and Saline Injection Attempts in Rabbits in Group 1

	Rabbit No.						
	1	2	3	4	5	6	Total
RBC injection attempts	4	4	4	10	5	4	31
Saline injection attempts	5	1	1	3	4	2	16
RBC sites analyzed	2	4	4	7	4	4	25

RBC = red blood cell.

Rabbits were scanned in a 320-slice CT system (Aquilion ONE; Toshiba Medical Systems Corp, Otawara, Japan) at 80 kVp 10–90 minutes after intramural injection while still under anesthesia. CT images were acquired before and after intravenous injection of 2 mL iodixanol contrast agent (Visipaque 320; GE Healthcare) and reviewed using Vitrea (Toshiba Medical Systems Corp) or Aquarius iNtuition 4.4 (TeraRecon, Inc, San Mateo, California) to determine the extent of the injection plume and whether injection was mainly in the plaque (bright signal encroaching on lumen) or the adventitia (bright signal outside of lumen).

Aortas from the iliac bifurcation up to and including the renal ostia were excised and stretched to in vivo length as measured on imaging. Vessels were pinned every 10 mm while stretched before being cut into blocks of 5 mm (group 1) or 3.3 mm (group 2) length, fixed in formalin, and embedded in paraffin. Sections of 4 μm thickness were cut for hematoxylin-eosin (H&E) and Perls' iron staining. Section location was determined by relative distances to the iliac bifurcation and left renal branch. For validation of noninvasive imaging, the presence or absence of extraluminal and intramural (combined intima, media, and adventitia) RBCs was recorded for each H&E section.

RESULTS

In group 1, 47 injections were attempted, 31 with RBCs and 16 with saline (Table 1). Injection sites completely overlapping with adjacent sites such that H&E sections unique to those sites were unavailable were excluded from analysis (n = 6 RBC injections). Adjacent sites spanning additional, separate H&E sections were included in the analysis. In group 1, 25 RBC injections and 16 saline injections were analyzed. Table 2 summarizes the ability of imaging to detect RBC injection sites confirmed by H&E. Of the 25 analyzed RBC injections, 18 were detected by imaging with corresponding RBC deposition on H&E sections, 4 were not detected on imaging or H&E, and 3 were detected on H&E but not on imaging. With histology as the gold standard, the results show 86% sensitivity and 100% specificity for the detection of intramurally injected RBCs by noninvasive imaging.