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BACKGROUND AND PURPOSE: **Endovascular treatment of aneurysms with coils, a less invasive alternative to surgery, is too often associated with recurrences. In a canine model, recanalization after coil embolization can be inhibited by in situ beta radiation.**

METHODS: **Radioactive platinum coils were produced by immersion in a ^{32}P-oligodeoxynucleotide solution. In vitro and in vivo ^{32}P-oligodeoxynucleotide elution profiles were assessed after incubation or arterial implantation for 14 days or less. Activities within arteries, thrombi, and coils were measured by scintillation counting. Angiographic and pathologic results no more than 12 weeks after standard platinum and radioactive coil embolization of canine maxillary, cervical, and vertebral arteries were compared among 17 animals.**

RESULTS: **Exposure to ^{32}P-oligodeoxynucleotide solution at 65°C yielded coils with an average activity of 0.3 μCi/cm. Elution profiles in vitro and in vivo showed that 50% of total activities eluted from coils within 24 hours at first, but coil activities then paralleled the natural decay of ^{32}P. Radioactivity was present in the thrombi and arterial wall throughout the 14-day observation period. Arteries that were embolized with standard coils recanalized at 2 weeks. Implantation of ^{32}P-oligodeoxynucleotide-coated coils produced total occlusions in 78.6% of arteries throughout the 12-week observation period. Most arteries that were implanted with radioactive coils were filled with fibrous tissue at 3 months.**

CONCLUSION: **Radioactive coils can be produced by using the binding properties of a ^{32}P-oligodeoxynucleotide to platinum. Use of these coils in an animal model was effective in preventing recanalization. This method could be performed on site to provide coils tailored to each intervention.**

Endovascular treatment can improve the outcome of patients treated acutely after aneurysmal rupture (6). The main drawback of coil embolization is the risk of recanalization and recurrences, necessitating long-term angiographic follow-up and subsequent treatments (1, 2, 5, 8, 9).

In situ, beta radiation was found to prevent recanalization after coil occlusion of arteries or aneurysms, a phenomenon that could be exploited to improve long-term results of endovascular treatment (7). Coils have previously been rendered radioactive by ion implantation, a procedure that can be performed only in specialized centers. Ion-implanted coils have to be prepared in advance and may not be appropriate to a specific case (4). Because of the half-life of ^{32}P (14.3 days), management of a radioactive coil inventory may become complex at a large scale. Therefore, a new technique was developed to allow preparation of radioactive coils on site, immediately before the procedure. This method uses the platinum-binding properties of a 15-mer oligodeoxynucleotide in which a ^{32}P atom has been incorporated (3). With this study, we showed in vitro and in vivo that the ^{32}P-oligodeoxynucleotide binds tightly to the platinum coil, and that ^{32}P 15-mer oligonucleotide coils led to permanent occlusion of arteries by fibrous tissue.

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Methods

^{32}P-Oligodeoxynucleotide Binding to Platinum Coils

The ^{32}P-oligodeoxynucleotide was synthesized as described previously, and was purified by using a Waters HPLC system (St-Laurent, Canada). To produce radioactive coils of approximately 0.3 μCi/cm, platinum coils (GDC-18 Soft, 3 mm in diameter, 8 cm in length; Boston Scientific/Target Therapeutics, Fremont, CA) were incubated for 15 minutes at 65°C, 42°C, or 22°C in a sterile solution of 150 μL of the ^{32}P-

oligondeoxyucleotide at a final concentration of 0.8 μCi/μL. Control experiments were performed by exposing coils to an equimolar amount of nonradioactive oligodeoxynucleotide solution or to distilled water at 65°C for 15 minutes. Coils were then washed for 5 minutes in a vial containing 25 mL of phosphate-buffered saline and a magnetic stirrer. Coil activity was then measured by using a Bioscan QC-2000 counter (Bioscan Inc., Washington, DC).

In Vitro ^{32}P Elution from Coils

Radioactive coils prepared as described were incubated in a biologic medium composed of Dulbecco's modified Eagle's medium in the presence of 20% fetal bovine serum (Gibco; Life Technologies, Inc., Gaithersburg, MD) at 37°C with constant agitation. At indicated times (4 hours and 1, 2, 4, 6, and 8 days), the coils were counted "dry," without scintillation liquid, in a scintillation counter (Packard, Montreal, Canada) and placed in fresh media for the next time point.

Animal Experiments

Protocols for animal experimentation were approved by the institutional animal committee in accordance with guidelines of the Canadian Council on Animal Care. Experiments were performed by using 17 beagle dogs weighing 15 to 20 kg each. Dogs were sedated with an intramuscular injection of acepromazine (0.1 mg/kg), glycopyrrolate (0.01 mg/kg), and butorphanol (0.1 mg/kg) and were anesthetized with IV administered thiopental (15 mg/kg). Animals were ventilated artificially and maintained under surgical anesthesia with 2% isoflurane. A percutaneous femoral puncture was used to reach the aorta and bilateral maxillary, cervical, and vertebral arteries with 2F microcatheters (Excelsior, Target Therapeutics) introduced coaxially through 5F catheters.

In vivo elution of ^{32}P 15-mer oligonucleotide from coils. In five animals, six coils were implanted into arteries while two coils were exposed to the aortic blood flow and then retrieved to measure remaining activities 5 and 60 minutes later. Arteries were harvested 1, 3, 7, 10, and 14 days after embolization. Coils, thrombus, and arteries were dissected, and activities remaining on coils were assessed directly by scintillation counting while arterial segments and luminal thrombus were dissolved in triethylamine hydroxide and then submitted to scintillation counting.

Inhibition of recanalization with ^{32}P 15-mer oligonucleotide coils. In 12 animals, ^{32}P-oligodeoxynucleotide-coated coils (n = 14) were compared with uncoated (n = 14) and cold oligodeoxynucleotide-coated (n = 4) coils in a blind fashion. Two coils (3 mm × 8 cm) were implanted into each artery. Follow-up angiography was performed 1, 2, 4, and 12 weeks after embolization. Angiographic observations were interpreted in a blind fashion and arterial blood flow scored as occluded, subocclucded, or patent. Occlusion was defined as the absence of antegrade blood flow through the coiled artery. Subocclusion was described when minimal flow persisted through the coiled artery. The arteries containing coils were excised after 3 months for macroscopic photography and pathology.

Pathology

After the animals were killed, arteries were excised and sectioned in the axial plane. After fixation, cut sections of arteries were photographed by using a stereomicroscope. For microscopic observations, pathologic specimens were studied after formalin fixation, coil removal, axial sectioning, and staining, as previously described (12). Images were captured by using a Labophot-2 microscope (Nikon, Tokyo, Japan). The fibrous tissue composing the occluded area within the arterial lumen was quantified by the Clemex Vision 3.0 software (Clemex Technologies Inc., Longueuil, Canada).

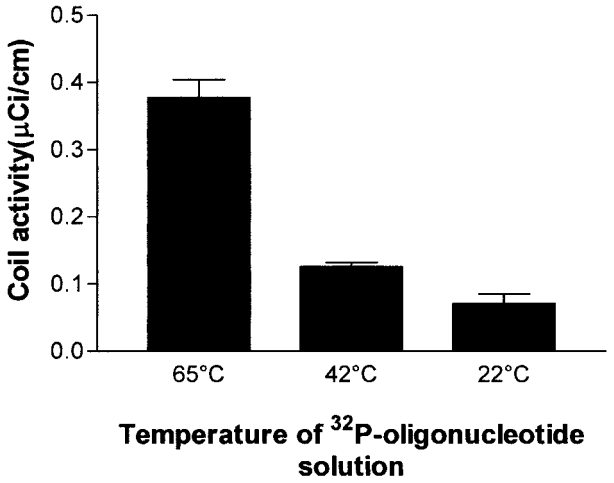

FIG 1. Effect of ^{32}P-oligodeoxynucleotide solution temperature on the activities of coils. Fragments of 1 cm of GDC-18 Soft (3 mm × 8 cm) coils were exposed to the ^{32}P-oligodeoxynucleotide solution (0.8 μCi/μL) at various temperatures for 15 minutes. Coils were then washed and radioactivity levels were assessed. Values are means ± SEM of three experiments.

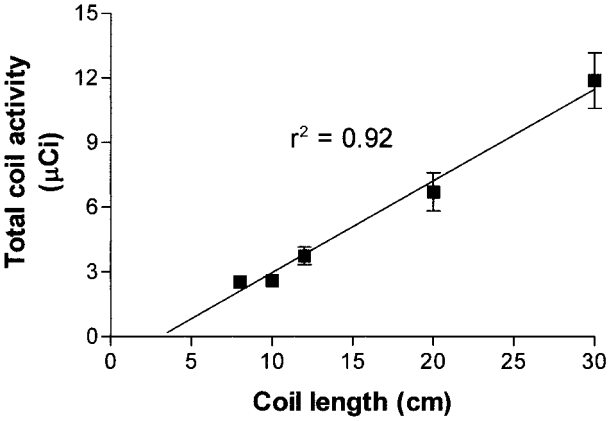

FIG 2. Total radioactivity on coils as a function of length after immobilization with ^{32}P-oligodeoxynucleotide solution. Entire GDCs were exposed to ^{32}P-oligodeoxynucleotide solution (0.8 μCi/μL) heated at 65°C for 15 minutes. Coils were then washed and radioactivity levels were assessed. Values are means ± SEM of at least five experiments.

Results

^{32}P-Oligodeoxynucleotide Binding to Platinum Coils and Elution Profiles

Binding of the ^{32}P-oligodeoxynucleotide was increased when the temperature of the radioactive solution was heated to 65°C, compared with 22°C and 42°C (Fig 1). Total coil activities increased as a function of the length of the coils (Fig 2). The mean activity per centimeter of coil using 65°C solutions averaged 0.34 ± 0.02 μCi (n = 44), with a correlation coefficient (r^2) of 0.92.

Leaching of ^{32}P-oligodeoxynucleotide from coils was evaluated in vitro and in vivo, as illustrated in Figures 3 and 4A, respectively. In both circumstances, an initial rapid loss of radiation occurred within the

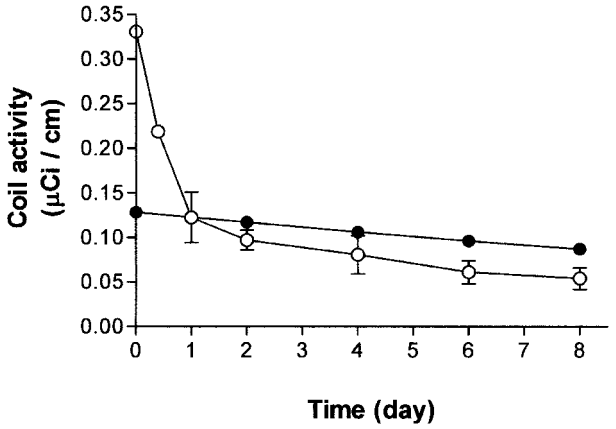

FIG 3. In vitro retention profile of ^{32}P-oligodeoxynucleotide. The remaining radioactivity (*white circles*) was compared with the natural decay for the ^{32}P atom (*black circles*). Values are means ± SEM of duplicate experiments.

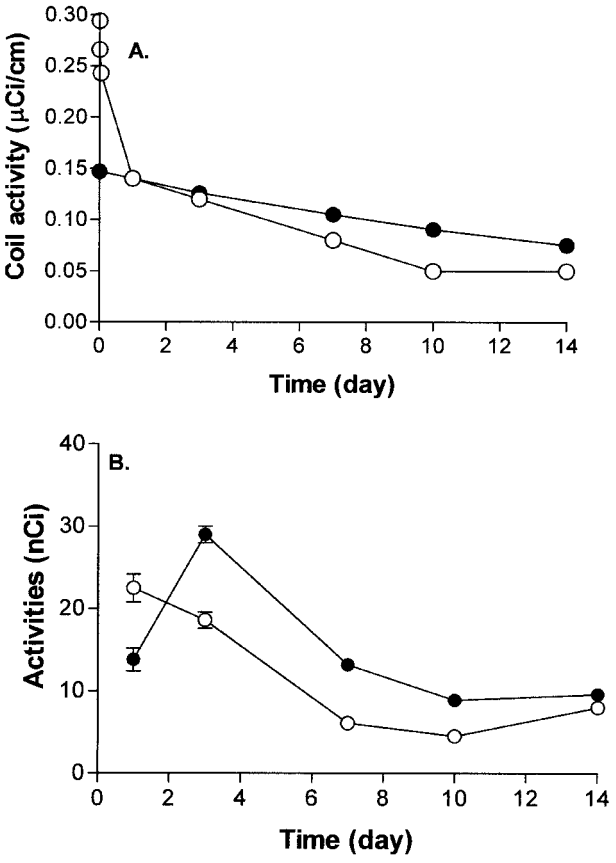

FIG 4. In vivo retention profile of ^{32}P-oligodeoxynucleotide.
A, Remaining radioactivity as a function of time of ^{32}P-oligodeoxynucleotide-coated coils (*white circles*) compared with the natural decay of ^{32}P (*black circles*).
B, Incorporation of ^{32}P-oligodeoxynucleotide into thrombus (*white circles*) and arterial wall (*black circles*) after coil implantation. Values are means ± SEM of six experiments.

first 24 hours and a curve that paralleled the natural decay of ^{32}P was then noted. A fraction of the radioactive material released from the coils was recovered from the thrombus and the arterial wall (Fig 4B).

TABLE Angiographic results after coil occlusion

	Standard Platinum Coil	Radioactive Coil	
	n (%)		*P* Value*
Week 1			.48
Patent	0 (0)	0 (0)	
Subocclusion	0 (0)	1 (7.1)	
Occluded	14 (100)	13 (92.9)	
Week 2			<.0001
Patent	13 (92.9)	0 (0)	
Subocclusion	0 (0)	1 (7.1)	
Occluded	1 (7.1)	13 (92.9)	
Week 4			<.0001
Patent	13 (92.9)	0 (0)	
Subocclusion	0 (0)	2 (14.3)	
Occluded	1 (7.1)	12 (85.7)	
Week 12			<.0001
Patent	14 (100)	0 (0)	
Subocclusion	0 (0)	3 (21.4)	
Occluded	0 (0)	11 (78.6)	

* Pearson's χ^2 test.

^{32}P-Oligodeoxynucleotide-coated Coils Inhibit Recanalization after Occlusion of Arteries

The Table summarizes results after implantation of two standard platinum or radioactive GDC-18 Soft 3 mm × 8 cm coils in each artery. After implantation of coils, total occlusion of the artery occurred within 1 hour in all cases. Treated arteries were scored as patent, subocclusion, or occluded based on follow-up angiography at 1, 2, 4, and 12 weeks (Table). The angiographic profiles of patent arteries showed that contrast media were quickly washed out of the distal artery (Fig 5A). Subocclusion arteries were characterized by some opacification of the distal artery from the coil when exposed to contrast media (Fig 5B). Occluded arteries were angiographically impermeable to contrast media (Fig 5C). Arteries treated with standard platinum coils almost always (13 of 14 cases) recanalized 7 to 14 days after embolization and remained patent at 4 and 12 weeks (Table). Arteries treated with radioactive coils were either subocclusion or totally occluded at all time points (14 of 14 cases). Statistical differences between arteries treated with standard platinum coils and those treated with radioactive coils were significant at 2, 4, and 12 weeks ($P <$ .0001).

Pathologic studies showed that standard coils in recanalized arteries were covered by a film of neointima, leaving large channels in between coils or between the coils and the vascular wall (Fig 5D and G). In contrast, the lumen of arteries treated with radioactive coils was filled with fibrous tissue consisting of fibroblasts in a dense collagenous matrix (Fig 5E, F, H, and I). Subocclusion arteries were characterized by small channels within the lumen that were delimited by coils and fibrous tissue (Fig 5E and F). Oc-

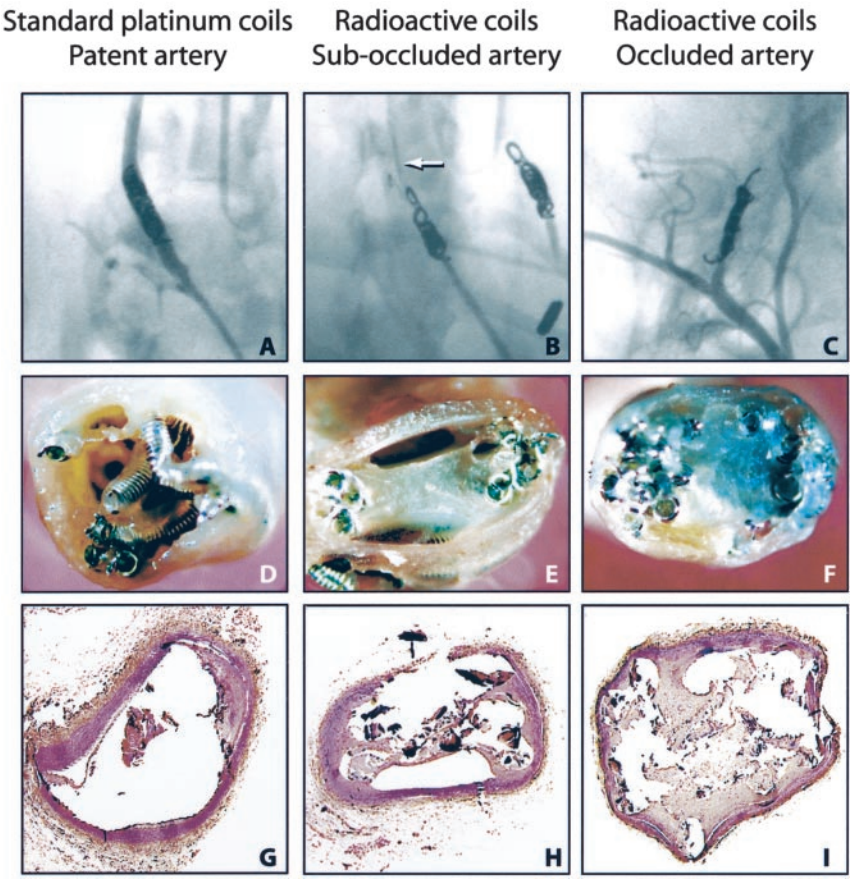

FIG 5. Angiographic and pathologic findings after coil occlusion. Selected views from angiographic (*A*, *B*, and *C*), macroscopic (*D*, *E*, and *F*), and microscopic (*G*, *H*, and *I*) studies performed 12 weeks after standard platinum (*A*, *D*, and *G*) and radioactive coil (*B*, *C*, *E*, *F*, *H*, and *I*) implantation. Arteries treated with standard platinum coils were recanalized (*D* and *G*). Arteries treated with radioactive coils exhibited either subocclusions or total occlusions. Suboccluded arteries treated with radioactive coils (*B*, *E*, and *H*) had increased fibrous tissue content while permitting restricted blood flow (*B*, *arrow*). Occluded arteries treated with radioactive coils (*C*, *F*, and *I*) showed uniform fibrous filling of the lumen. Original magnification in *D* through *F*, ×5; original magnification in *G* through *I*, ×20.

cluded arteries were shown to have fibrin deposition throughout the lumen (Fig 5F and I). The area occupied by fibrous tissue within the lumen of the arteries was significantly increased from 18.12 ± 3.12% for standard platinum coils to 54.62 ± 5.04% for ^{32}P-oligodeoxynucleotide coils (Student *t* test, $P < .01$).

Control experiments were performed to assess whether radiation was the sole factor for preventing arterial recanalization. Arteries implanted with coils exposed to an equimolar amount of nonradioactive oligodeoxynucleotide solution recanalized in all cases (n = 8, results not shown).

Discussion

We present a novel alternative for rapidly producing radioactive coils that have proved effective to prevent recanalization after embolization. Oligodeoxynucleotides can be used as carriers for beta-particle delivery (3). ^{32}P atoms can be incorporated within the backbone of a 15-mer c-*myc* sense phosphorothioate oligodeoxynucleotide. We discovered that platinum coils could be rendered radioactive when dipped in a ^{32}P-oligodeoxynucleotide solution. An important parameter to increase binding efficiency of ^{32}P-oligodeoxynucleotide is temperature. When coils were exposed to solutions at temperatures below 65°C, binding efficiency was suboptimal. These results are reminiscent of Southern hybridization experimentation, whereas optimal temperatures in the 50°C to 70°C range are frequently used for detection of the gene of interest (11). Elevated temperatures allow the ^{32}P-oligodeoxynucleotide strands to become linear and to detach from one another, enabling adsorption onto the platinum surface of the coil.

The elution profiles of the ^{32}P-oligodeoxynucleotide suggest that its absorption occurs in several layers. The radiolabeled coil loses approximately 50% of its activity within 24 hours in vitro and in vivo. After the first 24 hours of elution, the loss of radioactivity is consistent with the natural decay of ^{32}P radioisotope. This incurs the outer layers of ^{32}P-oligodeoxynucleotide to be washed away from the coil within the first 24 hours of exposure while the layers of ^{32}P-oligodeoxynucleotide bound to the platinum surface remain attached for the observation period of the experiment. This profile provides a dual mechanism for the local delivery of radiation to the target tissues. The first mechanism is emission of radiation from the embolic device itself. The second mechanism involves leaching of ^{32}P-oligodeoxynucleotide into the surrounding thrombus and arterial wall. This could be advantageous because leaching of ^{32}P may reach tissues at some distance from the coil surface.

Another advantage of this method is its flexibility. Coils of various lengths and diameters can be radiolabeled rapidly with only minor adjustments of the dipping volume. Therefore, this method could be performed on site to provide radioactive coils tailored to

the needs of each intervention, reducing difficulties intrinsic to managing an inventory of radioactive coils produced by alternative means, such as ion implantation.

Potential disadvantages are the uncertainty of the resulting dosimetry and variable leaching according to coil positioning (submitted to blood flow at the neck versus embedded into clot). Leaching with diffusion at a distance from the coil surface may also increase concerns for radiation damage to surrounding tissues, although activities recovered outside the coil comprised a very small fraction of coil activities (1000-fold fewer).

Platinum coils led to thrombotic occlusion of arteries by inducing the formation of thrombus. Recanalization of thrombus is usually associated with endothelial invasion that precedes α-actin-positive mesenchymal cells and restores a patent lumen (10). It is known that beta-radiation inhibits endothelial cell proliferation (3). Therefore, inhibition of endothelial invasion of the thrombus may be the main mechanism responsible for inhibition of arterial recanalization. Thrombus is transformed into a fibrous tissue mass 12 weeks after implantation of ^{32}P-oligodeoxyoligonucleotide-coated coils, which led to permanent occlusion of arteries in our animal model.

Conclusion

A novel, flexible, and rapid method to render GDC coils radioactive has been developed. This method could be performed on site to provide radioactive coils tailored to the needs of each intervention. ^{32}P-oligodeoxynucleotide-coated coils can prevent arterial recanalization after embolization. This strategy may be useful to improve long-term results of endovascular treatment of aneurysms.

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