A successful experimental model for intimal hyperplasia prevention using a resveratrol-delivering balloon

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Objective: **Restenosis due to intimal hyperplasia is a major clinical problem that compromises the success of angioplasty and endovascular surgery. Resveratrol (RSV) has demonstrated a beneficial effect on restenosis from angioplasty. Unfortunately, the physicochemical characteristics of RSV reduce the practicality of its immediate clinical application. This work proposes an experimental model aiming to setup an intravessel, elutable, RSV-containing compound.**
Methods: **A 140 μg/mL RSV sterile injectable solution with a suitable viscosity for intravascular administration by drug-delivery catheter (RSV-c) was prepared. This solution was locally administered in the common iliac artery of adult male New Zealand White rabbits using a dedicated device (Genie; Acrostak, Geneva, Switzerland) after the induction of intimal hyperplasia by traumatic angioplasty. The RSV concentrations in the wall artery were determined, and the thickness of the harvested iliac arteries was measured over a 1-month period.**
Results: **The Genie catheter was applied in rabbit vessels, and the local delivery resulted in an effective reduction in restenosis after plain angioplasty. Notably, RSV-c forced into the artery wall by balloon expansion might accumulate in the interstitial areas or within cells, avoiding the washout of solutions. Magnification micrographs showed intimal proliferation was significantly inhibited when RSV-c was applied. Moreover, no adverse events were documented in in vitro or in vivo studies.**
Conclusions: **RSV can be advantageously administered in the arterial walls by a drug-delivery catheter to reduce the risk of restenosis. (J Vasc Surg 2014;■:1-7.)**

Clinical Relevance: **The incidence of intimal hyperplasia varies in different risk populations (eg, diabetic patients) up to 35% after bare-metal stent implantation and is reduced, but still exists and is problematic, after implantation of drug-eluting stents. Scouting experiences have shown that treatment with antioxidants improves endothelial cell coverage, decreases intimal hyperplasia, and reduces oxidative stress, thus promoting the patency of stents and grafts. In our experimental model, we observed that resveratrol locally administrated in the artery by a drug-eluting balloon has the potentialities to reduce the intimal hyperplasia thanks to a local anti-inflammatory response.**

Restenosis due to intimal hyperplasia is a major clinical problem that compromises the success of angioplasty and endovascular surgery.[1,2] The pathogenesis of restenosis is multifactorial, involving such events as endothelial injury, inflammation, platelet activation, and hyperplasia of the intima, primarily due to vascular smooth muscle cell (VSMC) replication.[3] The incidence of intimal hyperplasia varies in different risk populations (eg, diabetic patients), up to 35% of whom require bare-metal stent implantation. Clinical evidence has shown that intimal hyperplasia is reduced but continues to cause problems after the implantation of drug-eluting stents.[4] Overall, however, despite many years of clinical experience with drug-eluting balloons, the number of large, high-quality, randomized clinical trials is low, and further data are urgently needed across the spectrum of clinical indications.

Taxol (Bristol-Myers Squib, Princeton, NJ) and other cytostatic drugs destroy a cell's ability to use its cytoskeleton in a flexible manner, and considering the clinical results, further research on a more physiologic mechanism of action should be pursued. Antioxidants are currently under investigation due to their protective activity within the vessels. Rosenbaum et al[5] showed that the endothelialization of prosthetic grafts was significantly reduced and anastomotic hyperplasia was significantly increased in rabbits fed a high-cholesterol diet. Treatment with an antioxidant improves endothelial cell coverage, decreases intimal hyperplasia, and reduces oxidative stress, promoting the patency of prosthetic grafts.

Resveratrol (RSV) is a polyphenolic phytoalexinic antioxidant that is produced by grapes and other plants in response to injurious infections. There are several pioneering reports on RSV, including studies on the inhibition of the

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arachidonate metabolism by interactions with the 5-lipoxygenase and cyclooxygenase pathways in leukocytes.[6-12] However, RSV attracted little interest until 1979, when the "French Paradox"[13] reported the positive benefit of a diet containing RSV, in particular the moderate consumption of red wine, for coronary heart disease.

The molecular structure of RSV, unfortunately, reduces its immediate clinical application for three main reasons: (1) its status as a highly lipophilic molecule; (2) its fast drifting from the *trans*-phase to *cis*-phase, representing an oxidized and inactive state, respectively; and (3) its low circadian bioavailability for rapid hepatic metabolism. As consequence of these features, the oral bioavailability of RSV is negligible because it is rapidly conjugated to improve the solubility of the compound.

The disposition of ^{14}C-labeled RSV, as orally and intravenously administered in healthy volunteers, was evaluated to estimate the extent of the oral dose absorbed, the bioavailability of the unchanged drug, and the drug's metabolic phase. RSV demonstrated high oral absorption but rapid and extensive metabolism, resulting in only trace amounts of unchanged RSV remaining until systemic circulation.[14] Five major metabolites were detected in the urine samples,[14] plasma, and colorectal cancer tissues,[15] although all were only measured qualitatively due to a lack of available reference materials. Metabolite (M) 1 was a RSV monoglucuronide. M2, an isomeric RSV monoglucuronide, was found in greater abundance. M3 was a dihydroresveratrol monoglucuronide, whereas M4 (resveratrol monosulfate) and M5 (dihydroresveratrol sulfate) were two poorly resolved RSV sulfates. Although results on the efficacy of RSV reported in the literature are controversial, very recent data obtained in colon cancer cells have supported the notion that RSV, despite its low bioavailability, is able to act through its metabolites, mainly the sulfoconjugate but also the combination of sulfate/glucuronide derivatives.[15,16]

Despite the wide literature on RSV, only few preclinical studies have demonstrated the efficacy of RSV in an animal model or investigated the possibility of locally administering this antioxidant by drug-eluting stents.[6,7] On the basis of our experience, we decided to setup a sterile, injectable, and hydrophilic RSV-containing compound (RSV-c). This solution was locally administered in the common iliac artery of adult male New Zealand White rabbits using a dedicated device.

METHODS

All experiments in this study were conducted in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals and in accordance with the Università degli Studi di Milano Ethical Committee guidelines. The Italian Ministero della Salute approved the research protocol.

In vitro study

Cell seeding. Human coronary artery smooth muscle cells (HSMCs; C-017-5C, Gibco-Invitrogen, Carlsbad, Calif) were seeded, according to the kit's instructions (2.5×10^3 cells/cm^2 to reach 80% confluence in 7-9 days) and maintained in culture at a lower density in six-well plates to determine the proliferation rate. Experiments were conducted on cells between passages 4 and 6.

Cell proliferation study. HSMCs were divided in three experimental groups:

1. Control: medium (Gibco-Invitrogen Medium 231 with Supplement; n = 8).
2. RSV (Biotivia Italia, Verona, Italy): 25 mM dilution in ethanol, final concentration 100 μM in medium (n = 12).
3. Vehicle (VEH): ethanol 0.4 μL/mL in medium (n = 12).

All groups were incubated for 48 h at 37°C. The medium and treatments were changed every 24 h to prevent RSV degradation. On day 2, the cells were washed twice with phosphate-buffered saline (PBS), harvested by trypsinization, and counted with a Z2 Coulter counter (Beckman Coulter Inc, Fullerton, Calif).

Preparation of the RSV-c

To obtain a sterile, injectable solution, we optimized the solubility of RSV and its viscosity. RSV was dissolved in a 0.40% w/v tamarind seed polysaccharide and 2.50% w/v Kolliphor HS15 (BASF, Florham Park, NJ) solution that had been previously sterilized by vapor steam under pressure. Afterwards, the solution was filtered by disposable, sterile, and pyrogen-free polyether sulfone filters at the nominal porosity of 0.22 μm into a sterile amber type I glass container at a laminar airflow workbench. A media fill program assured the validation of the aseptic process. The containers were stored at room temperature until use.

A placebo formulation was prepared according to the procedure reported above.

To avoid the fast removal of RSV by the blood stream after intimal administration, a high-viscosity solution was prepared. Tamarind seed polysaccharide, a well-known biocompatible polymer, was used to confer a suitable cinematic viscosity to the vehicle for administration by drug-eluting balloon. According to our previous experience,[12] the viscosity of the preparation was based on the nonionic contrast medium iomeprol (Iomeron; Bracco, Milan, Italy), with kinematic viscosity at 37°C of 5.617 ± 0.034 mm^2/s. The final kinematic viscosity of the RSV-c was very close to that of the reference solution (6.097 ± 0.0379 mm^2/s). Kolliphor HS15 is a surfactant used for parenteral preparations and was added to the formulation to dissolve 140 μg/mL RVS.

In vivo study

Animals. Thirty-six male New Zealand White rabbits, weighing from 2.8 to 3.6 kg, were assigned randomly and in equal numbers to different study subgroups (Table). The animals were housed in a dedicated facility and fed with standard diet with free access to water.

Angioplasty and delivery procedures. Preoperative color Doppler ultrasound (Titan; SonoSite Inc, Bothell,

Table. In vivo study design[a]

	Group			
Design	*PK*	*Sham*	*Carrier*	*RSV-c*
Subgroup[b]	2, 6, 24	3, 30	3, 30	3, 30
Time	2, 6, and 24 h	3 and 30 d	3 and 30 d	3 and 30 d
Compound	RSV-c	None	Carrier	RSV-c
Procedure	DDC	Simple PTA	PTA+DDC	PTA+DDC

DDC, Drug-delivery catheter; *PK,* pharmacokinetic; *PTA,* percutaneous transluminal angioplasty; *RSV-c,* resveratrol compound.
[a]Administration of 20 mL carrier or RSV-c by DDC.
[b]n = 4 for each subgroup.

Wash) was used to scan all animals to measure their mean arterial size and femoral artery peak systolic velocity (PSV) and end-diastolic velocity (EDV) to obtain preoperative morphologic and velocimetric data. The mean right iliac artery diameter was 3 ± 0.6 mm. The right femoral artery PSV was 90 cm/s and EDV was 60 cm/s.

The animals were treated with an anesthetic protocol to ensure the full unconsciousness during the surgical procedures and an excellent level of perioperative analgesia. Rabbits were premedicated with a subcutaneous injection of dexmedetomidine (80 μg/kg; Dexdomitor, Orion Corp, Milan, Italy), ketamine (25 mg/kg; Ketavet, Intervet Productions SRL, Latina, Italy), and buprenorphine (20 μg/kg; Temgesic, Schering Plough Spa, Milan, Italy). After the induction, a steady depth of anesthesia was maintained during the experimental protocol by the continuous infusion of a dilute solution of propofol (1-3 mg/kg/h; Fresenius Kabi, Isola della Scala, Italy), into the auricular vein. All animals were heparinized with 80 IU/kg heparin sulfate (Phararepa; PharmaTex, Milan, Italy) 2 minutes before the introducer sheath was inserted.

After the surgical incision, the superficial fascia and muscles were separated bluntly, layer-by-layer, until the right common femoral artery was exposed. Proximal and distal vascular controls were assured with two 2-mm silicon ligatures to minimize bleeding. Using the modified Seldinger technique, we directly inserted a 4F Cook Micropuncture sheath (William Cook Europe ApS, Bjaeverskov, Denmark). To induce and establish intimal hyperplasia in the rabbit's iliac artery, we performed a traumatic angioplasty with a Bantam Alfa balloon catheter (diameter: 3.0 mm, length: 2 cm; ClearStream Technologies Ltd, Enniscorthy, Ireland) with Doppler ultrasound monitoring. Injury was created by inflating the balloon to 8 atm with a manometer syringe for 3 min. Afterwards, the catheter was removed, and the Genie drug-delivery catheter (Acrostak, Geneva, Switzerland) was introduced into the aorta-iliac bifurcation by color Doppler ultrasound monitoring to deliver the 20 mL carrier or RSV-c (Table).

The Genie balloon catheter is designed to dilate and treat arteries by the local delivery of the proposed solution (RSV-c) and reference compounds (vehicle), ensuring a fully controlled release to the vessel wall. Clinical experiences with Genie suggested delivery of RSV in 2 minutes, maintaining a mean inflating pressure of 6 atm.[17] At the end of the procedure, the right common femoral artery was sutured with 7-0 polypropylene interrupted stitches. The blood supply of the leg was not affected by this surgical procedure.

Pain control and antibiotic coverage were achieved through the subcutaneous administration of buprenorphine (15 mg/kg; Temgesic) plus meloxicam (0.2 mg/kg; Mobic, Boehringer Ingelheim, Milan, Italy) plus enrofloxacin (10 mg/kg, Baytril; Bayer SpA, Rome, Italy). At the time points indicated from the experimental protocol (Table), the balloon-treated aorta-iliac bifurcation was surgically explanted, and the animals were euthanized with an anesthetic overdose.

Tissue and serum measurements of RSV-c. Before, immediately after the delivery procedure, and at predetermined times ranging from 15 min to 90 min, blood samples (2 mL) were collected from the auricular vein. Samples were immediately centrifuged at 3500 rpm for 15 min at 4°C, and the serum was frozen and stored at −80°C until further processing. The serum was thawed, 250 μL was added to 1 mL methanol, vortexed 1 minute, and centrifuged at 5000 rpm for 15 minutes at 15°C. The supernatant was analyzed by the high-performance liquid chromatography (HPLC) method reported below.

The animals were euthanized at 2, 6, and 24 h after administration (Table). Segments of the iliac artery were removed and washed with a physiologic solution, and any visible blood coagula or residual fat tissues were carefully removed. The tissue was cut into a small specimen, placed in a vial containing 0.2 mL methanol, and sonicated by a Microson ultrasonic cell disruptor (Qsonica, Newton, Conn) in an ice bath for 30 minutes.

The concentration of RSV was assayed using the following HPLC method:

RSV was analyzed by HPLC (ChemStation HP 1100, Agilent, Santa Clara, Calif), as previously described.[14] Briefly, an ODS Hypersil analytical column (Thermo Scientific, Waltham, Mass) was used as the stationary phase (4.6 × 100 mm; 3 μm particle size), and a combination of MilliQ (Millipore Corp, Billerica, Mass) water/methanol/trifluoroacetic acid (65%/35%/0.3%, v/v/v) was used as the mobile phase. The flow rate was controlled at 0.9 mL/min. The effluent was monitored at 304 and 286 nm for the determination of *trans*-RSV and *cis*-RSV (or isomeric RSV), respectively. The injection volume was 10 μL, and the analysis was performed at 30°C. Because authentic RSV metabolites were not available as reference materials, the amounts of the metabolites were calculated as "RSV equivalents," using the assumption that the recovered characteristics and relationship between peak area ratio and concentration were the same as for the parent RSV.

Histologic measurements

The harvested iliac arteries of sham, carrier, and RSV-c groups were fixed in 10% buffered formalin; then, cross-sections were cut and embedded in paraffin. A morphologic evaluation of the vessel wall thickness and intimal

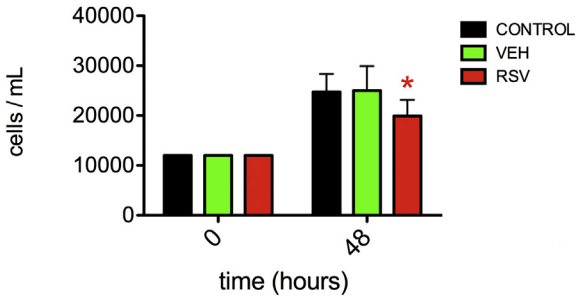

Fig 1. In the human coronary artery smooth muscle cells (HSMCs) proliferation study, resveratrol (*RSV*) slowed cell growth in the absence of pathologic stimuli after 48 hours of treatment. Vehicle (*VEH*) did not alter proliferation compared with the control. **P* < .01 RSV vs VEH and control.

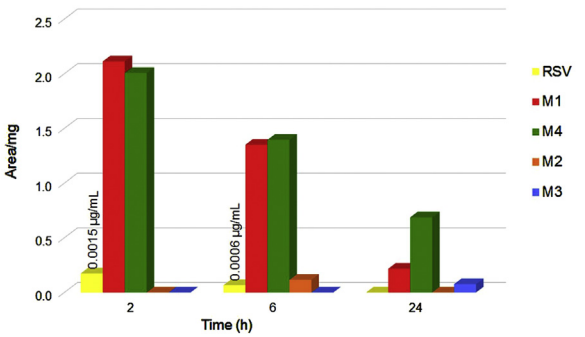

Fig 2. Recovery of resveratrol (*RSV*) and its metabolites in artery tissue after the administration of RVS solution (10 mL) by a drug-eluting balloon. Four major metabolites were identified as RSV monoglucuronide (*M1*), isomeric RSV monoglucuronide (*M2*), dihydroresveratrol monoglucuronide (*M3*), RSV monosulfate (*M4*). Because standards are not commercially available, RSV metabolites are expressed as the peak area at 286 nm corrected for the conversion factor 1.5 normalized for the artery sample weight. The correction factor was calculated as the ratio of peak area of RSV and photodegraded RSV at 304 and 286 nm.

hyperplasia was performed on each tissue block, cutting 4-µm sections. Hematoxylin and eosin staining clearly show the internal elastic lamina, the external elastic lamina, the intimal thickness, and the cells in the vessel wall. Each histologic section was scanned, the intimal and external and internal elastic lamina were manually identified. Intimal layer thickness (ie, distance between the lumen and internal elastic lamina), medial layer thickness (ie, distance between internal and external elastic lamina), lumen area, intimal layer area, and medial layer area were measured using ImageJ image analysis software (National Institutes of Health, Bethesda, Md; http://imagej.nih.gov/ij/, 1997-2014).

Thickness was measured at four points (ie, cardinal points: north, east, south, west), and the mean value was taken. Then, to compare different treatment groups, the intimal/medial thickness ratio, the intimal/medial area ratio, and the intimal proliferation index (ratio of intimal area to [intimal + medial] area) were used for statistical analysis. For group RSV-c30 and sham30, proliferating cells in the intimal layer were identified by immunohistochemistry, using antibodies against Ki-67 protein (Clone MIB-1; DAKO, Glostrup, Denmark). Results of Ki-67-positive cells count were normalized for Ki67/μm^2

All histological analysis were performed in a blinded fashion.

Statistical analysis

Data are reported as the means ± standard deviation. The data from the proliferation studies were analyzed by taking the means of three counts for each well and then considering each of the independent wells as a separate data point. Comparisons among the groups were performed by analysis of variance with Bonferroni correction. $P < .05$ was considered significant.

RESULTS

In vitro evaluation. The number of cells was expressed as cells/mL. Cell proliferation in the presence of the vehicle was similar to that in the control group (25 ± 5 cells/mL in VEH group vs 25 ± 4 cells/mL in control group; $P = .8$, not significant [NS]). RVS significantly inhibited HSMC proliferation (Fig 1) compared with the VEH and control group (20 ± 3 cells/mL in RSV vs 25 ± 5 cells/mL in VEH and 25 ± 4 cells/mL in control, $P < .01$).

Tissue measurements of RSV-c. The RSV was detectable and quantifiable in serum only immediately after administration (<0.2 µg/mL), and none of its metabolites were found.

Only traces of unchanged RSV were detectable in the arterial samples from the iliac vessel until 6 h after the ballooning procedure (Fig 2). However, RSV M1 and M4 metabolites were qualitatively identified ≤2 h and persisted over the considered time period. After prolonged periods of time, the concentrations of these two metabolites decreased and the M2 and M3 metabolites became detectable. From these data, we posit that the RSV-c infused in a solution at the site of the artery by a drug-delivery catheter was retained in the tissue and then underwent an extensive and rapid metabolism.

In vivo evaluation and histologic measurements. No procedure-related deaths were documented during the housing period. Before euthanasia, all rabbits underwent color Doppler ultrasound imaging to test for patency and velocimetric patterns. The RSV-c30 group always showed the patency of their iliac vessels, which was associated with an average value of PSV and EDV of 70 ± 7 and 40 ± 5 cm/s, respectively. In the sham30 and carrier30 groups, we found an average value of PSV and EDV of 30 ± 20 and 20 ± 15 cm/s, respectively. When we analyzed the data together, we found a significant difference ($P < .05$) between the RSV-c30 and the sham30 or carrier30 groups. A literature review showed a lack of comparisons of the

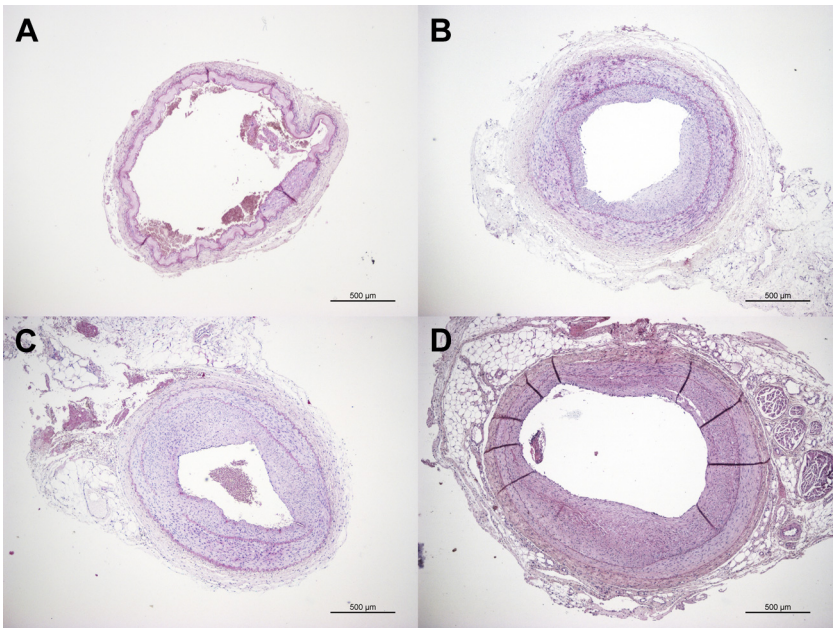

Fig 3. Hematoxylin and eosin-stained histologic cross-sections of rabbit iliac artery after angioplasty (original magnification ×40). **A,** Group RSV-c3 (resveratrol compound at 3 days)—intimal layer denudation with patchy cellularized medial layer and diffuse ialin degeneration of vessel wall. **B,** Group RSV-c30 (resveratrol compound at 30 days)—moderate reduction in lumen size due to slight intimal hyperplasia. **C,** Group carrier30 (group that received carrier at 30 days)—marked reduction in lumen size and asymmetrical thickening of the wall, mainly due to intimal hyperplasia. **D,** Group sham30 (group that received sham treatment at 30 days)—marked intimal hyperplasia, resulting in high intima/media ratio.

data on velocimetry and vessel diameters in the rabbit; nevertheless, we considered the flow reduction (PSV from 90 to 30 cm/s) in the femoral artery in the sham30 and carrier30 groups to be a result of a tight stenosis. Autoptic specimens showed no macroscopic differences among the groups.

Those animals euthanized at the early time point (sham3, carrier3, and RSV-c3) showed hyaline degeneration as a result of the percutaneous transluminal angioplasty (PTA), irrespective of the treatment. However, we noted some peculiar differences in the disposition of ialin degeneration in RSV-c3. Complete circumferential lesions were present in groups sham3 and carrier3, but not in RSV-c3. Despite the presence of ialin lesions in RSV-c3, we did not observe the same homogenous ialin intimal layer as was found in sham3 and carrier3. Micrographs were able to spot some cellularized areas in RSV-c3 (Fig 3). Regardless of whether an empirical evaluation of this early result could be interpreted as a positive effect of RSV-c after PTA, we considered this phenomenon only as a part of the modulatory effects of RSV-c on the local inflammatory response after PTA.

The morphologic data at day 30 are summarized in Fig 4. The intimal hyperplasia was significantly greater in sham30 group compared with the other treated groups ($P < .05$ by one way analysis of variance with Bonferroni correction). The comparison between the carrier30 and

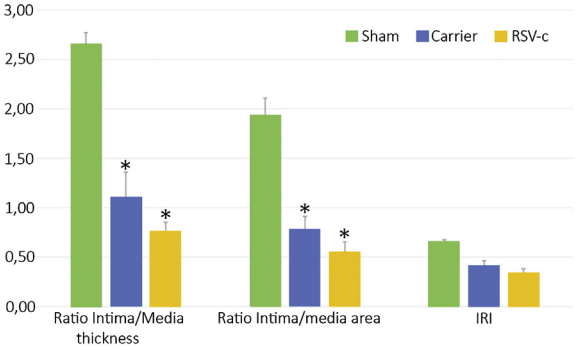

Fig 4. Parameters calculated from histologic measurements: comparison of sham30 (sham group at 30 days), carrier30 (carrier group at 30 days), and RSV-c30 (resveratrol compound at 30 days). Data are shown as mean ± standard error. *IRI*, Intimal proliferation index (ratio of intimal area to [intimal + medial] area). *$P < .05$ compared with sham30 group.

RSV-c30 groups evidenced the beneficial effect of RSV. Indeed, the mean intimal proliferation index value obtained with the RSV-c was ~25% lower than that obtained with the carrier. Nevertheless, this difference did not result significance according to our statistical evaluations.

Differences between RSV-c30 and sham30 were statistically significant ($P < .05$) when counting Ki-67-positive

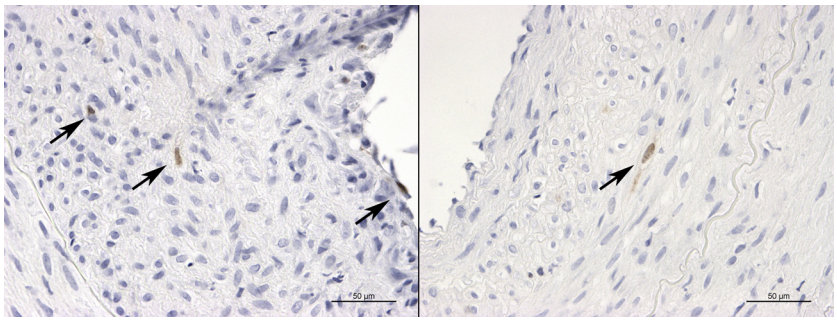

Fig 5. High magnification photomicrograph of rabbit iliac arteries without any treatment after angioplasty (group sham30). Immunohistochemistry cross-sections show nuclear positivityfor Ki-67 protein (*arrows*) in proliferating cells of the intimal layer (immunohistochemistry,original magnification ×400).

cells in the whole vessel wall ($2.42 \pm 0.73 \times 10^{-6}$ Ki-67/μm^2 vs $8.65 \pm 1.48 \times 10^{-6}$ Ki-67/μm^2). When the intimal layer was considered, divergence was still noteworthy ($9.20 \pm 3.20 \times 10^{-6}$ Ki-67/μm^2 vs $11.78 \pm 2.20 \times 10^{-6}$ Ki-67/μm^2) but was not significant according to our statistical evaluations (Fig 5).

DISCUSSION

Many articles have reported good results of RSV for treating intimal hyperplasia. According to the literature,[6-8] RSV has actions that extend beyond its known anti-inflammatory, antioxidative stress, antitumor, and anti-diabetic effects. It has been indicated that inhibition of neointimal hyperplasia by RSV may be dependent on endothelial nitric oxide synthase, but the role of RSV in neointimal prevention/reduction after arterial injury is not yet clear. In a recent study, Khandelwal et al[7] found that RSV inhibited the serum-stimulated increase in VSMC proliferation and migration ability and upregulated markers of the contractile phenotype. These findings imply that RSV blocks the progression of phenotype modulation and promotes reversal of the new synthetic cell type to the differentiated phenotype in vitro. Moreover, they found that RSV could attenuate the phenotypic transformation switching of neointimal VSMCs after balloon injury through a mechanism that involves inhibition of the Notch signaling.

However, despite these positive experiences, we have observed only a fair expectation of RSV in clinical use due to the limitations of its molecular structure and biodynamics. To overcome this boundary, we worked on an experimental model in the hope of establishing an intravessel, elutable RSV-containing compound. The delivery procedure ensured a minimal lack of RSV in the blood. Indeed, <5% of the administered RSV was detected in serum, and in agreement with data in the literature,[16] it was completely eliminated ≤15 minutes.

The rabbit model used in our study allowed us to test the vessels with a mean diameter similar to that of a human coronary artery.[8] Thus, the data obtained could be relevant to intimal VSMC proliferation and hyperplasia after balloon dilation in humans. Furthermore, the lesions generated in the present model encouraged the participation of VSMC proliferation, which, together with VSMC migration and the abnormal production of the extracellular matrix, are considered to be the primary features of the restenotic process in humans. In rabbits, as in humans, ultrasound data offer the possibility of evaluating velocimetric patterns preoperatively and postoperatively to highlight not only the local morphologic effects of RSV but also the relevance of these effects in flow modulation below the lesion. A notable finding was that the segments treated with RSV-c had better velocimetries than in the sham and carrier groups.

We decided to use the Genie catheter[13] because, rather than being restricted to stent struts, catheter-based local antiproliferative therapies offer the advantage of a homogenous drug transfer to the entire vessel wall, thereby allowing for intravessel pharmacotherapies without adding additional layers of other molecules.[17] Moreover, the delivery system of the Genie catheter allowed us to use the compound immediately. In this experimental model, we aimed to solve the problem of restenosis induced by PTA and, at the same time, to control the host reactions induced by a carrier layer.

RSV acted as an antioxidant, inducing a local anti-inflammatory response, and the carrier showed no particular activities on intimal hyperplasia, as observed in sham and carrier groups (Fig 3 and Fig 4), which showed no significant differences in intimal hyperplasia. The Genie catheter was applied in the coronary vessels,[4] and the local delivery resulted in an effective reduction in the rate of restenosis after both plain balloon angioplasty and stenting. As a matter of fact, RSV-c forced into the artery wall by balloon expansion might accumulate in the interstitial areas or within cells, or both, thereby avoiding the washing-out of solutions.

We did not use salicylate to provide complementary data because we considered that the antiplatelet activity of salicylate might act as a bias that could weaken or strengthen the role of RSV-c, either way confounding the final evaluations. Despite the lack of antiplatelet activities, the good results observed in the RSV-c group could encourage further investigations on RSV-c with respect to other treatments against intimal hyperplasia that might necessitate a mandatory double-antiplatelet therapy.

Beside the main goal, the project also provided information of general interest in the fields of optimizing compound production, localizing compounds after their administration within the artery walls by ballooning, and assessing perivascular tissue responses to local, long-acting solutions. To successfully implement the local administration of RSV, many challenges would have to be considered. The localization of RSV after infusion, whether intracellular, intercellular, or both, has not yet been investigated. Moreover, the drug residence time at the injured artery should probably be prolonged.

CONCLUSIONS

Control of intimal hyperplasia and restenosis remain the main goals of postangioplasty therapy. Early and late results have shown that almost 40%[4,17] of the endovascular procedures are associated with recurrences. Even if Taxol and similar treatments helped in controlling such events, the final solution would remain unclear. In our experience, magnification micrographs have shown a significant inhibition of intimal proliferation when RSV-c is applied. Moreover, no adverse events have been documented in in vitro or in vivo studies. We believe that the know-how acquired with this experimental work toward the development of a sterile injectable compound with effective antioxidant properties can be considered a positive scouting experience with future clinical relevance, as well as a strong urging for further investigations.

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AUTHOR CONTRIBUTIONS

Conception and design: VT, SM, FC, PZ
Analysis and interpretation: LC, FS, AM
Data collection: RC, VT, PZ
Writing the article: VT, SM, FC, LC, PZ, AM
Critical revision of the article: RC, SM
Final approval of the article: SM, FC, AM
Statistical analysis: LC, FS, FC
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Overall responsibility: VT

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