

Dimethyl Sulfoxide Inhibits Tissue Factor Expression, Thrombus Formation, and Vascular Smooth Muscle Cell Activation

A Potential Treatment Strategy for Drug-Eluting Stents

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Background—Subacute stent thrombosis is a major clinical concern, and the search for new molecules to cover stents remains important. Dimethyl sulfoxide (DMSO) is used for preservation of hematopoietic progenitor cells and is infused into patients undergoing bone marrow transplantation. Despite its intravenous application, the impact of DMSO on vascular cells has not been assessed.

Methods and Results—In human endothelial cells, monocytes, and vascular smooth muscle cells (VSMC), DMSO inhibited tissue factor (TF) expression and activity in response to tumor necrosis factor- α or thrombin in a concentration-dependent manner. DMSO did not exert any toxic effects as assessed by phase-contrast microscopy, trypan blue exclusion, and lactate dehydrogenase release. Real-time polymerase chain reaction revealed that inhibition of TF expression occurred at the mRNA level. This effect was mediated by reduced activation of the mitogen-activated protein kinases c-Jun terminal NH₂ kinase ($51 \pm 6\%$; $P=0.0005$) and p38 ($50 \pm 3\%$; $P<0.0001$) but not p44/42 ($P=NS$). In contrast to TF, DMSO did not affect expression of TF pathway inhibitor or plasminogen activator inhibitor-1. In vivo, DMSO treatment suppressed TF activity (41% ; $P<0.002$) and prevented thrombotic occlusion in a mouse carotid artery photochemical injury model. DMSO also inhibited VSMC proliferation (70% ; $P=0.005$) and migration (77% ; $P=0.0001$) in a concentration-dependent manner; moreover, it prevented rapamycin and paclitaxel-induced upregulation of TF expression.

Conclusions—DMSO suppresses TF expression and activity, as well as thrombus formation; in addition, it inhibits VSMC proliferation and migration. Given its routine use in modern clinical practice, we propose DMSO as a novel strategy for coating drug-eluting stents and treating acute coronary syndromes.

Key Words: signal transduction ■ stents ■ thrombosis ■ tissue factor ■ thrombus

Dimethyl sulfoxide (DMSO) is used for preservation of hematopoietic progenitor cells and therefore is infused into patients undergoing bone marrow or stem cell transplantation.¹ In this context, peak plasma concentrations reach values as high as 20 mmol/L. DMSO is also used as a solvent for chemotherapeutic drugs and, due to its antiinflammatory properties, has been successfully used in humans for treating rheumatic,² pulmonary,³ gastrointestinal,^{4,5} neurological,^{6,7} urinary,⁸ and dermatological^{9,10} disorders. DMSO further exhibits protective effects in animal models of middle cerebral artery occlusion,¹¹ cerebral hypoperfusion-related neuronal death,¹² mercuric chloride-induced kidney injury,¹³ and

chemical liver injury.¹⁴ A recent report proposed that DMSO reduces ischemic brain damage through both antiinflammatory and free radical scavenging properties.¹¹ Despite all this information, the impact of DMSO on cardiovascular disorders has not been assessed.

Tissue factor (TF) is a key protein in the activation of coagulation.¹⁵ Plaque rupture induces arterial thrombosis and is the main cause of acute coronary syndromes such as unstable angina and acute myocardial infarction. TF plays a major role in determining plaque thrombogenicity,¹⁶ because its expression is

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upregulated in different cell types within the atherosclerotic vessel wall, and it is exposed to the bloodstream after plaque rupture. Many inflammatory mediators are indeed capable of inducing TF expression in monocytes, macrophages, endothelial cells, and vascular smooth muscle cells (VSMCs)¹⁶; not surprisingly, experimental evidence has revealed an important contribution of TF to the pathogenesis of subacute stent thrombosis and restenosis after balloon angioplasty or stent deployment.¹⁶ As a consequence, recent efforts have focused on the therapeutic implications of TF inhibition.¹⁶ Because intravenous delivery of DMSO has become a recognized practice in clinical oncology owing to the increasing use of bone marrow transplants, the present study was designed to investigate the impact of DMSO on TF expression and thrombosis and on activation of VSMCs.

Methods

Cell Culture

Human aortic endothelial cells (HAECs) and VSMCs (Clonetics, Allschwil, Switzerland) were cultured as described previously.¹⁵

Human peripheral blood myocytes (THP)-1 cells (LGC Promocell, Molsheim, France) were cultured according to the supplier's recommendation. Adhering cells were grown to confluence in 3-cm dishes and rendered quiescent for 24 hours before stimulation with 5 ng/mL tumor necrosis factor- α (TNF- α) or 1 U/mL thrombin (both from Sigma, Basel, Switzerland). Cells were pretreated with DMSO (Sigma) for 1 hour before stimulation. To block the mitogen-activated protein (MAP) kinase p38, p44/42 (ERK), or c-Jun terminal NH₂ kinase (JNK), cells were treated with SB203580 (Sigma), PD98059 (Cell Signaling, Danvers, Mass), or SP600125 (Calbiochem, Lucerne, Switzerland) for 60 minutes before stimulation. Rapamycin and paclitaxel (both from Sigma) were added to the cells 1 hour before thrombin (1 U/mL) stimulation. To assess cytotoxicity, a colorimetric assay for detection of lactate dehydrogenase (LDH) was used according to the manufacturer's recommendations (Roche, Basel, Switzerland); in addition, a trypan blue exclusion assay (0.4% solution, Sigma) and morphological examination by phase-contrast microscopy (Leica, Glattbrugg, Switzerland) were performed.

Western Blot Analysis

Protein expression was determined by Western blot analysis as described previously.¹⁷ Cells were lysed in 50 mmol/L Tris buffer; 30- μ g samples

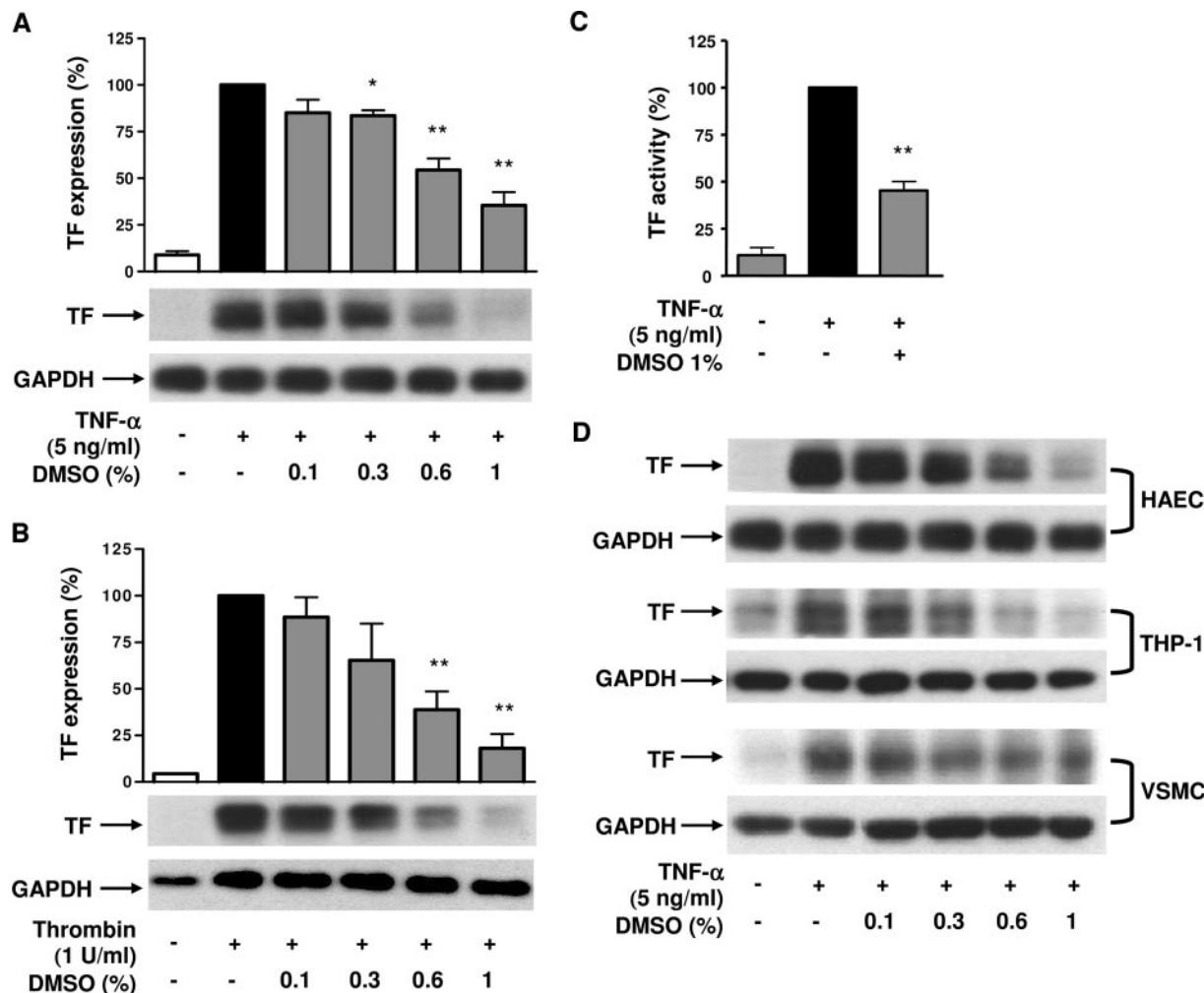


Figure 1. DMSO inhibits TF expression and activity. A, DMSO inhibits TNF- α -induced TF expression of HAECs in a concentration-dependent manner. Values are given as percent of stimulation with TNF- α alone. * $P<0.001$, ** $P<0.0001$ vs TNF- α alone. B, DMSO inhibits thrombin-induced TF expression of HAECs in a concentration-dependent manner. Values are given as percent of stimulation with thrombin alone. * $P<0.001$, ** $P<0.0001$ vs thrombin alone. C, DMSO inhibits TNF- α -induced TF surface activity of HAECs. Values are given as percent of stimulation with TNF- α alone. ** $P<0.0001$ vs TNF- α alone. D, DMSO inhibits TNF- α -induced TF expression in a concentration-dependent manner in HAECs, human monocytic cells (THP-1), and human aortic VSMCs. Values are representative of at least 3 different experiments; all blots are normalized to GAPDH expression.

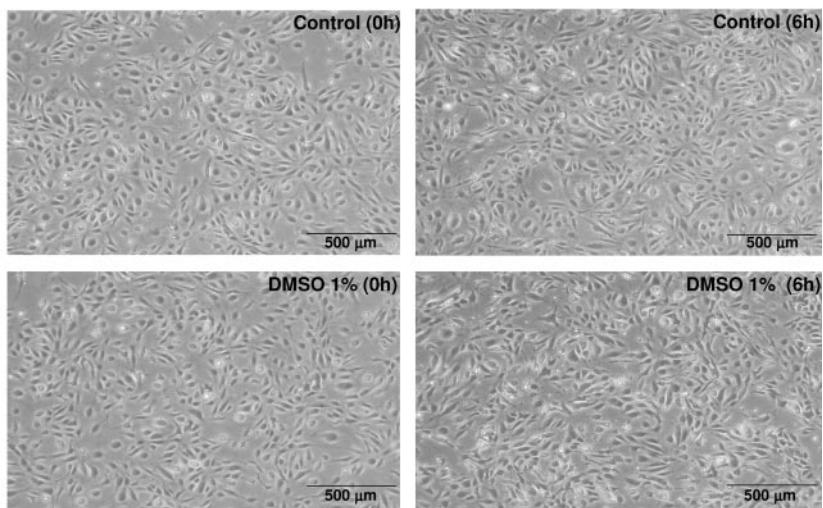
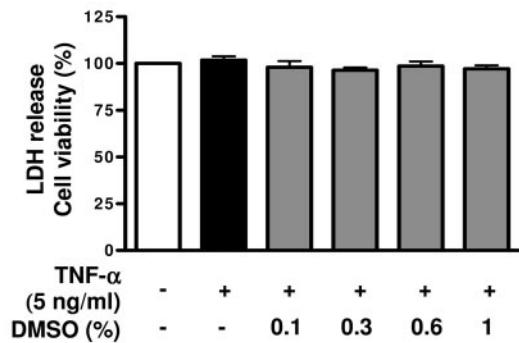
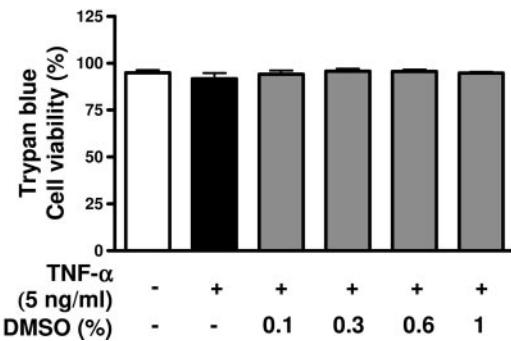
A**B****C**

Figure 2. Lack of DMSO-induced toxicity. A, Incubation with DMSO for 6 hours does not affect cell morphology (magnification $\times 20$). LDH release (B) and the number of trypan blue-positive cells (C) remain unaffected by different DMSO concentrations as well. Values are given as percent of stimulation with TNF- α alone.

were loaded, separated by 10% SDS-PAGE, and transferred to a polyvinylidene fluoride membrane (Millipore, Volketswil, Switzerland) by semidry transfer.¹⁸ Equal loading was confirmed by Ponceau S staining. Antibodies against human TF, TF pathway inhibitor (TFPI; both from American Diagnostica, Stamford, Conn), and plasminogen activator inhibitor-1 (PAI-1; Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) were used at 1:2000 dilution. Antibodies against phosphorylated p38, phosphorylated ERK, and phosphorylated JNK (all from Cell Signaling) were used at 1:1000, 1:5000, and 1:1000 dilution, respectively. Antibodies against total p38, total ERK, and total JNK (all from Cell Signaling) were used at 1:2000, 1:10000, and 1:1000 dilution, respectively. Blots were normalized to GAPDH expression (1:10000 dilution, Sigma). Representative blots are shown; bars represent at least 3 different experiments.

TF Activity

TF cell surface and tissue activity was analyzed in HAEC and mouse carotid artery homogenates, respectively, with a colorimetric assay (Amer-

ican Diagnostica) according to the manufacturer's recommendations with some modifications as described previously.¹⁵ Cells were grown in 6-well plates; after stimulation, cells were washed twice with phosphate-buffered saline (PBS) followed by incubation with human factor VIIa (FVIIa) and factor X (FX) at 37°C, which allowed the formation of TF/FVIIa complex at the cell surface. Right carotid arteries were homogenized in 50 μ L of lysis buffer (50 mmol Tris-HCl, 100 mmol NaCl, 0.1% Triton X-100, pH 7.4; Union Carbide Corp, Danbury, Conn) 2 hours after intraperitoneal application of DMSO or vehicle and left to stand on ice for 30 minutes. TF/FVIIa complex converted human FX to factor Xa, which was measured by its ability to metabolize a chromogenic substrate. A standard curve with lipidated human TF was performed to ensure that measurements were taken in the linear range of detection.

Real-Time Polymerase Chain Reaction Analysis

Real-time polymerase chain reaction (PCR) analysis was performed as described previously.¹⁹ An MX3000P PCR cycler (Stratagene,

Amsterdam, Netherlands) was used according to the manufacturer's instructions. All PCR experiments were performed in triplicate with the SYBR Green JumpStart kit (Sigma). Each reaction (25 μ L) contained 2 μ L of cDNA, 1 pmol of each primer, 0.25 μ L of internal reference dye, and 12.5 μ L of JumpStart Taq ReadyMix (containing buffer, dNTPs, stabilizers, SYBR Green, Taq polymerase, and JumpStart Taq antibody). The following primers were used: TF (F3): sense primer: 5'-TCCCCAGAGTTCACACCTTACC-3' (bases 508 to 529 of F3 cDNA; National Center for Biotechnology Information No. NM 001993), antisense primer: 5'-TGACCAACAAATACCA-CAGCTCC-3' (bases 892 to 913 of F3 cDNA; National Center for Biotechnology Information No. NM 001993); human L28: sense primer: 5'-GCATCTGCAATGGATGGT-3', antisense primer: 5'-TGTTCTTGGCGATCATGTGT-3'. The amplification program consisted of 1 cycle at 95°C for 10 minutes, followed by 40 cycles with a denaturing phase at 95°C for 30 seconds, an annealing phase at 60°C for 1 minute, and an elongation phase at 72°C for 1 minute. PCR products were analyzed on an ethidium bromide-stained 1% agarose gel, and a melting curve analysis was performed after amplification to verify the accuracy of the amplicon. In each real-time PCR run for F3 and L28, a calibration curve was included that was generated from serial dilutions of the respective purified amplicons.

Proliferation and Migration of Human Aortic VSMCs

For proliferation, VSMCs were seeded at a density of 10 000 cells per 35-mm dish and cultured for 24 hours in Dulbecco modified Eagle medium (DMEM) with 10% fetal calf serum (FCS). Subsequently, cells were serum withdrawn in DMEM with 1% FCS for 48 hours. Cells were then maintained in DMEM with 10% FCS in the presence of different DMSO concentrations. Medium and appropriate DMSO concentrations were replaced daily, and cell number was determined after 3 days with a hemocytometer (Assisten, Sondheim, Germany).

For migration, VSMCs were seeded at a density of 250 000 cells per 150-mm dish and cultured for 24 hours in DMEM with 10% FCS. Cells were then harvested and resuspended (500 000 cells/mL) for analysis of migration in response to platelet-derived growth factor 10 ng/mL. Migration was assessed in a modified Boyden chamber system (Neuro Probe Inc, Gaithersburg, Md) as described previously.²⁰ The number of migrated cells was determined by staining (Diff-Quik; Dade Diagnostics Inc, Aguada, Puerto Rico) and counting the cells at 400 \times magnification (Leica) on 4 random microscopic fields per group.

Carotid Artery Thrombosis Model

C57BL/6 mice (6 to 8 weeks old; Charles River Laboratories, Sulzfeld, Germany) weighing an average of 23 ± 2 g were anesthetized with intraperitoneal injection of 2 mg of sodium pentobarbital (Butler, Columbus, Ohio) as described previously.^{21,22} Rose bengal (Fisher Scientific, Fair Lawn, NJ) was diluted to 10 mg/mL in PBS and then injected into the tail vein in a volume of 0.12 mL at a concentration of 50 mg/kg with a 27-gauge Precision Glide needle with a 1-mL latex free syringe (Becton Dickinson, Franklin Lakes, NJ). Mice were secured in a supine position, placed under a dissecting microscope (Olympus C-4040 Zoom; spatial resolution 4.1 megapixels, Olympus Schweiz, AG, Switzerland), and the right common carotid artery was exposed after a midline cervical incision. A Doppler flow probe (model 0.5 VB, Transonic Systems, Ithaca, NY) was then applied and connected to a flowmeter (Transonic, model T106). Six minutes after rose bengal injection, a 1.5-mW green light laser (540 nm; Melles Griot, Carlsbad, Calif) was applied to the desired site of injury at a distance of 6 cm for 60 minutes or until thrombosis occurred. From the onset of injury, blood flow in the vessel was monitored for 120 minutes, at which time the experiment was terminated. Occlusion was defined as a flow of ≤ 0.1 mL/min for at least 1 minute.²³ Mice were divided into 3 groups: DMSO intraperitoneal (1 hour before surgery, 3.8 mL/kg of 40% DMSO in PBS), PBS intraperitoneal (1 hour before surgery, 3.8 mL/kg of

PBS), and an additional control without laser injury. In a separate set of experiments, DMSO or vehicle was applied 5 minutes after onset of laser injury.

Statistical Analysis

Data are presented as mean \pm SEM. Statistical analysis was performed by 2-tailed unpaired Student *t* test or ANOVA as appropriate. A probability value of <0.05 was considered significant.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

DMSO Inhibits TF Protein Expression and Activity

Stimulation of HAECs with TNF- α (5 ng/mL) resulted in a 19-fold increase in TF expression ($n=4$; $P<0.0001$; Figure 1A); similarly, thrombin (1 U/mL) induced a 24-fold increase of TF ($n=4$; $P<0.0001$; Figure 1B). When cells were pretreated with increasing concentrations of DMSO (0.1% to 1.0%), TF induc-

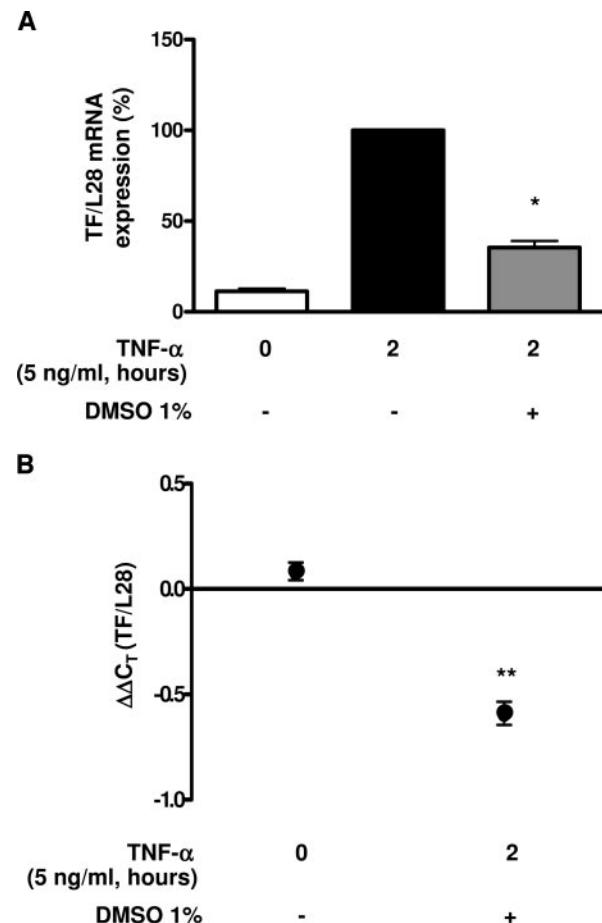


Figure 3. DMSO inhibits TF mRNA expression. A, Real-time PCR demonstrates increased TF mRNA expression after 2 hours of stimulation with TNF- α . DMSO inhibits this effect. Values are given as percent of stimulation with TNF- α alone. * $P<0.001$ vs TNF- α alone. B, $\Delta\Delta C_T$ values (an approximation of transcription based on the change in threshold values for control vs target cells [the cycle number at which fluorescence crosses the threshold of logarithmic increases in cDNA concentration]) confirm that DMSO decreases TNF- α -induced TF mRNA expression. ** $P<0.0001$ vs TNF- α alone. All values are representative of 4 different experiments and are normalized to L28 mRNA.

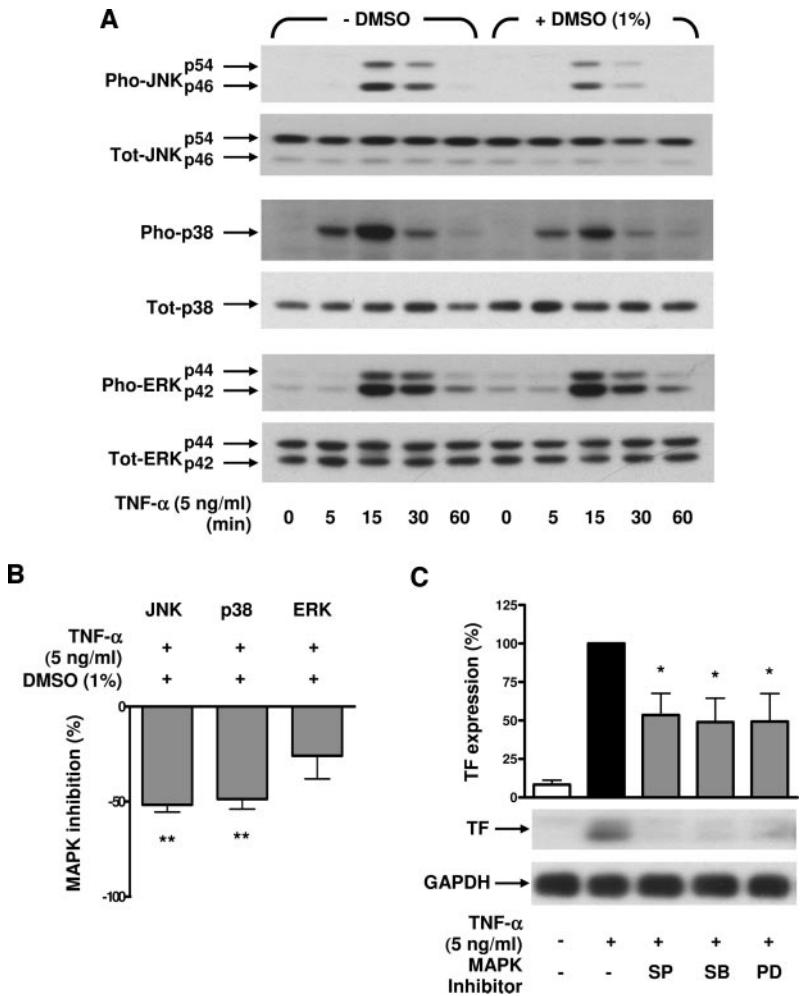


Figure 4. DMSO inhibits MAP kinase activation. **A**, Stimulation with TNF- α leads to transient phosphorylation (Pho) of the MAP kinases JNK, p38, and ERK. DMSO inhibits phosphorylation of JNK and p38 but not ERK. Total (Tot) levels of JNK, p38, and ERK remain unchanged. **B**, DMSO strongly inhibits phosphorylation of JNK and p38 but not ERK. Average values of 4 different experiments. **P<0.001 for JNK and p38 vs TNF- α alone. MAPK indicates MAP kinase. **C**, SP600125 (10^{-6} mol/L), SB203580 (10^{-5} mol/L), and PD98059 (3×10^{-6} mol/L), specific inhibitors of JNK, p38, and ERK, respectively, inhibit TNF- α -induced TF expression. *P<0.001 vs TNF- α alone. Blots are representative of at least 3 different experiments.

tion by both TNF- α and thrombin was inhibited in a concentration-dependent manner (n=4; P<0.0001 for TNF- α or thrombin alone versus 1.0% DMSO; Figures 1A and 1B). This effect was paralleled by a similar inhibition of TF surface activity (n=4; P<0.0001 for TNF- α alone versus 1.0% DMSO; Figure 1C). When DMSO was applied 5 minutes after stimulation with TNF- α , inhibition of TF induction was similar to that observed with DMSO pretreatment (80±6% inhibition versus TNF- α alone; n=4; P<0.0001; data not shown). In THP-1 cells, stimulation with TNF- α (5 ng/mL) induced a 10-fold increase in TF expression (n=4; P<0.0001), which was inhibited by DMSO (n=4; P<0.005 for TNF- α alone versus 1.0% DMSO; Figure 1D). In VSMCs, the 3.5-fold increase in TNF- α -induced TF expression (n=4; P<0.005) was inhibited as well by DMSO (n=4; P<0.05 for TNF- α alone versus 1.0% DMSO; Figure 1D).

DMSO Does Not Exert Toxicity in Human Vascular Cells

To assess potential toxic effects of DMSO, HAECS were incubated with 1.0% DMSO for 6 hours, and a morphological examination was performed. No changes in cell morphology were observed when cells with or without treatment were compared (Figure 2A). In addition, cell death was assessed by both LDH release and trypan blue exclusion in HAECS;

again, no signs of toxicity were detected by either method (n=4; P=NS; Figures 2B and 2C).

DMSO Inhibits TF mRNA Expression

Peak induction of TF mRNA occurs 2 hours after stimulation with TNF- α .¹⁹ Real-time PCR analysis confirmed a 10-fold induction of TF mRNA expression after 2 hours of TNF- α stimulation in HAECS (n=3; P<0.001; Figure 3A). Treatment with 1.0% DMSO inhibited TF mRNA expression by 65±7% (n=3; P<0.0001 for TNF- α alone versus 1.0% DMSO; Figures 3A and 3B).

DMSO Impairs TF Expression via MAP Kinase Inhibition

To assess whether DMSO affects MAP kinase activation, HAECS were examined at different time points after stimulation. JNK, p38, and ERK were transiently activated by TNF- α (Figure 4A). Maximal phosphorylation occurred after 15 minutes and returned to basal levels within 60 minutes (Figure 4A). DMSO inhibited phosphorylation of JNK and p38 by >50% while leaving activation of ERK unaltered (Figure 4A). Indeed, maximal phosphorylation of JNK was decreased by 51±6% (n=4; P=0.0005; Figure 4B) and that of p38 by 50±3% (n=4; P<0.0001; Figure 4B); in contrast, maximal activation of ERK was not reduced significantly

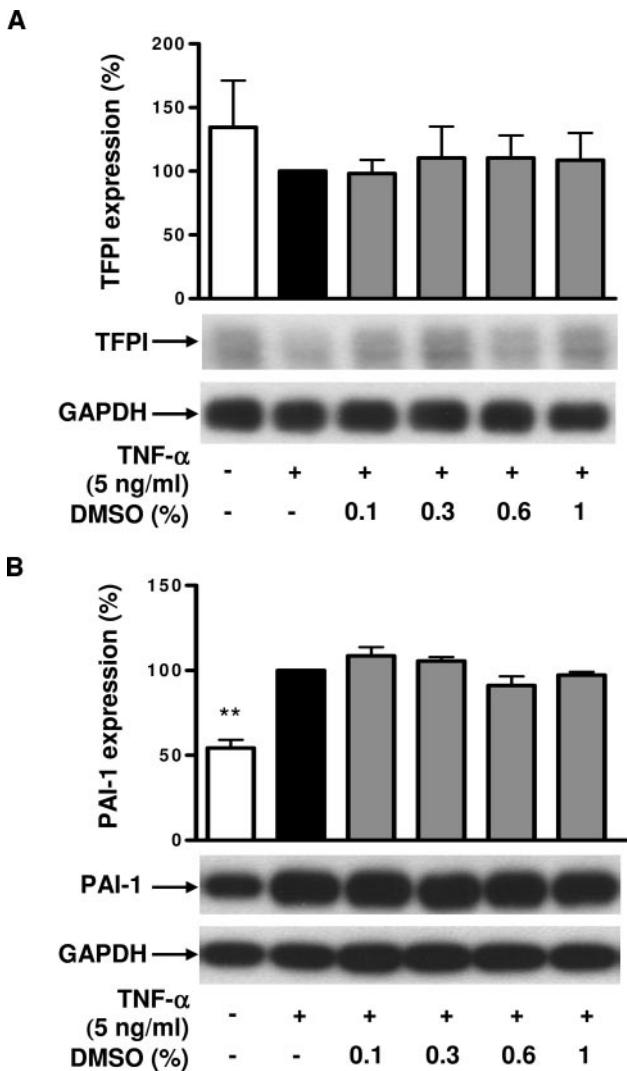


Figure 5. DMSO does not affect TFPI and PAI-1 expression. A, DMSO does not affect TFPI expression. Values are given as percent of TNF- α alone. $P=NS$ vs TNF- α alone. B, DMSO does not affect TNF- α -induced PAI-1 expression. Values are given as percent of TNF- α alone. $P=NS$ vs TNF- α alone. Blots are representative of at least 3 different experiments. ** $P<0.001$.

($n=4$; $P=NS$; Figure 4B). No change in total expression of JNK, p38, or ERK was observed at any time point with or without DMSO. To verify the involvement of MAP kinases in TNF- α -induced TF expression under our experimental conditions, the effect of MAP kinase inhibitors on TF expression was examined in HAECS. SP600125, SB203580, and PD98059, which specifically inhibit JNK, p38, and ERK, respectively, significantly impaired TF induction after TNF- α stimulation ($n=4$; $P<0.001$ for TNF- α alone versus each inhibitor; Figure 4C).

DMSO Does Not Affect TFPI and PAI-1 Expression

The effect of DMSO on the physiological inhibitor of TF, TFPI, and on the antifibrinolytic factor PAI-1 was assessed. Treatment of TNF- α -stimulated HAECS with 1% DMSO did not affect TFPI expression ($n=4$; $P=NS$; Figure 5A). Simi-

larly, treatment of TNF- α -stimulated HAECS with 1.0% DMSO did not alter PAI-1 expression ($n=4$; $P=NS$; Figure 5B).

DMSO Suppresses TF Activity and Prevents Arterial Thrombosis In Vivo

Treatment with DMSO (3.8 mL/kg of 40% DMSO in PBS, corresponding to 80 mg/kg DMSO) reduced TF activity in mouse carotid artery *in vivo* ($n=3$; $P<0.002$ for vehicle [PBS] versus DMSO; Figure 6A). Photochemically induced arterial injury is dependent on TF and is an established model of arterial thrombosis.²¹⁻²⁴ Initial blood flow in vehicle (PBS)-treated mice equaled 1.25 ± 0.15 mL/min ($n=5$); these mice developed thrombotic occlusion within 1 hour after onset of injury (mean occlusion time 63 ± 9 minutes; $n=5$; $P<0.0001$ for initial versus final blood flow; Figure 6B). During injury, it was evident from transient reductions in flow that a dynamic state of thrombus formation and lysis occurred during the short interval before complete occlusion. Cessation of blood flow coincided with the appearance of a faint white occlusive defect that was visible through the microscope in the lumen of the artery. DMSO (3.8 mL/kg of 40% DMSO in PBS, corresponding to 80 mg/kg DMSO)-treated mice exhibited similar initial blood flow (1.14 ± 0.19 mL/min; $n=5$; $P=NS$ for vehicle versus DMSO) but did not develop thrombotic occlusion. Indeed, 120 minutes after onset of injury, blood flow in DMSO-treated mice was still very high (0.75 ± 0.15 mL/min; $n=5$; $P<0.01$ for vehicle versus DMSO). However, DMSO-treated mice exhibited a small but significant decrease in blood flow during the 120-minute observation period compared with control mice that did not undergo photochemical injury ($n=5$; $P<0.05$). Blood flow in the latter did not change significantly during the 120-minute observation period (initial flow 1.12 ± 0.17 mL/min; final flow 0.94 ± 0.09 mL/min; $n=5$; $P=NS$ for initial versus final blood flow). When intraperitoneal application of DMSO or vehicle was performed 5 minutes after the onset of laser injury, DMSO still prevented thrombotic occlusion of the carotid artery in 50% of mice ($n=6$; data not shown).

DMSO Inhibits Proliferation and Migration of Human VSMCs

Treatment with DMSO inhibited VSMC proliferation in response to 20% FCS in a concentration-dependent fashion ($n=4$; $P<0.05$; Figure 7A). Likewise, DMSO inhibited VSMC migration in response to 10 ng/mL platelet-derived growth factor-BB ($n=4$; $P<0.05$; Figure 7B).

Both rapamycin and paclitaxel are known to enhance thrombin-induced TF expression.^{19,25} To determine whether DMSO can prevent this effect, HAECS were incubated with either drug with or without DMSO (Figure 7C). Rapamycin (10^{-7} mol/L) and paclitaxel (10^{-5} mol/L) enhanced thrombin-induced TF expression by $241.9\pm54.61\%$ ($n=4$; $P<0.05$ versus thrombin alone; Figure 7C) and $184\pm35.3\%$ ($n=4$; $P<0.05$ versus thrombin alone; Figure 7C), respectively. DMSO blunted the effect of rapamycin and paclitaxel on TF expression ($n=4$; $P=<0.0001$ versus thrombin plus rapamycin or thrombin plus paclitaxel; Figure 7C).

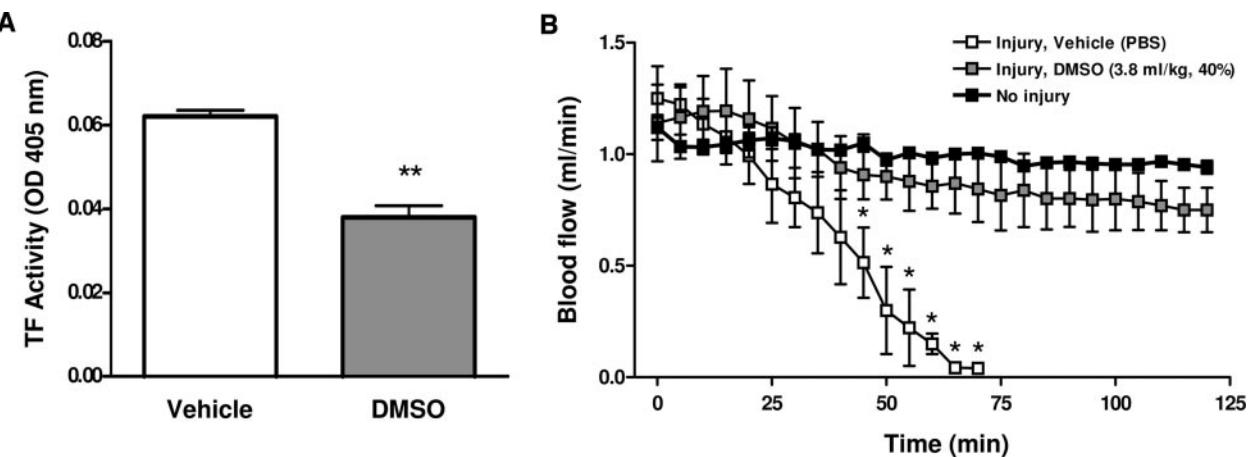


Figure 6. DMSO inhibits carotid artery TF activity and prevents thrombotic occlusion in a mouse model of photochemical injury. A, DMSO inhibits TF activity in mouse carotid artery. Values are given as absorbance at 405 nm. ** $P<0.0001$ vs PBS treated (vehicle). B, Transit-time flow recordings of vehicle-treated (PBS) or DMSO-treated mouse carotid artery after photochemical injury *in vivo*. Laser injury is initiated at time=0 minutes. Uninjured mice are included as an additional negative control. DMSO prevents thrombus formation ($P=0.0090$ for DMSO-treated vs vehicle-treated; $P=0.002$ for DMSO-treated vs negative control). * $P<0.001$.

Discussion

This study demonstrates that DMSO inhibits TF expression and activity via reduced activation of the MAP kinases JNK and p38. Consistent with this observation, DMSO suppresses TF activity and protects against thrombotic occlusion *in vivo*. The study also demonstrates that DMSO inhibits proliferation and migration of VSMCs; furthermore, it prevents TF induction in response to rapamycin and paclitaxel. This profile renders DMSO ideally suitable for application on drug-eluting stents and for the treatment of acute coronary syndromes.

DMSO treatment did not exert any toxic effect even at the highest concentration used (1.0%, equal to 12.6 mmol/L). An extensive study in human endothelial cells also did not demonstrate any toxic effects of DMSO even at concentrations well above those used in the present study.²⁶ Moreover, DMSO has been routinely used to trigger *in vitro* cellular differentiation at concentrations as high as 1.25% (15.7 mmol/L).²⁷ Thus, the DMSO concentrations applied in the present study are clearly below those used to induce cellular differentiation.

Thrombin and TNF- α induced TF expression in HAECS with similar potencies, and incubation with DMSO suppressed this effect irrespective of the stimulus. Furthermore, DMSO reduced TNF- α -induced TF expression not only in HAECS but also in THP-1 cells and VSMCs, which indicates that the effect on TF occurs regardless of the cell type. Therefore, the protective action of DMSO is restricted neither to a specific stimulus nor to a specific vascular cell type.

Activation of MAP kinases mediates TF expression in response to several stimuli.^{15,18,28,29} Indeed, JNK, p38, and ERK were transiently activated in HAECS after stimulation with either TNF- α or thrombin. Treatment with DMSO impaired TF expression by specifically decreasing phosphorylation of JNK and p38 but not ERK; hence, inhibition of these 2 MAP kinases suppressed TF expression in a potent manner. In line with this observation, pharmacological inhi-

bition of the 2 MAP kinases induces a more pronounced reduction of TF expression than inhibition of 1 MAP kinase alone. Consistent with inhibition of MAP kinases, DMSO promoted a strong reduction in TNF- α -induced TF mRNA levels. Thus, the inhibitory action of DMSO is related to a specific inhibition of MAP kinase activation.

TF activity is counterbalanced by its endogenous inhibitor TFPI; the balance of these 2 factors is essential in determining thrombus formation.³⁰ Treatment with DMSO did not affect TFPI expression, which indicates that the antithrombotic action of DMSO on TF expression is not compensated by a concomitant reduction in TFPI expression. PAI-1 is a serpin that suppresses fibrinolysis by inhibiting the activity of plasminogen activator. A recent report demonstrated that DMSO is able to reduce interleukin-1-induced PAI-1 expression in rat microvascular endothelial cells.³¹ In contrast, we did not observe any effect of DMSO on PAI-1 expression in TNF- α -stimulated HAECS; this difference may be related to the different species, the different origin of the cells, or the different stimuli used for induction. Thus, the inhibitory action of DMSO on TF expression does not appear to be modulated by concomitant effects on TFPI or PAI-1.

It is generally accepted that thrombus formation is triggered by TF.³²⁻³⁴ Photochemical injury was selected as a model of thrombosis because it is an established protocol for examining TF-dependent thrombosis *in vivo*²²; moreover, this procedure does not require intra-arterial invasion.^{24,35} We observed that thrombotic occlusion after injury was prevented in DMSO-treated mice, which indicates that treatment with DMSO prevents thrombus formation *in vivo*. Indeed, inhibition of TF activity in the mouse carotid artery within 2 hours of DMSO administration was confirmed and thus is the most likely explanation for the effect of DMSO on thrombus formation after photochemical injury. It cannot be excluded, however, that the effect of DMSO *in vivo* may be attributable in part to its ability to impair platelet adherence and aggregation.³⁶

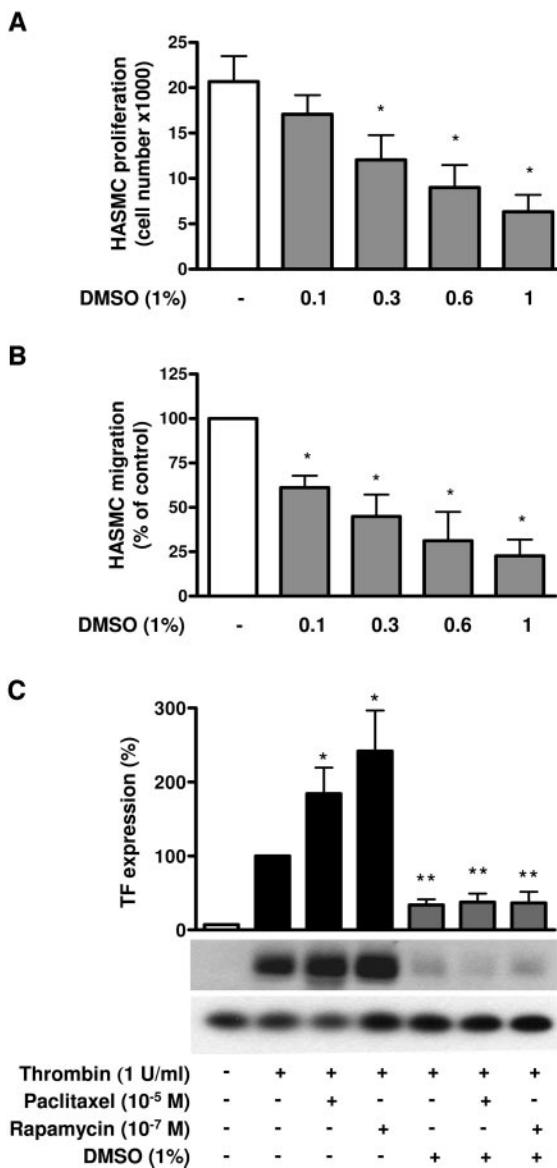


Figure 7. DMSO inhibits proliferation and migration of VSMCs. A, DMSO inhibits VSMC proliferation in response to 10% FCS in a concentration-dependent manner; * $P<0.05$ vs control. HASMC indicates human aortic smooth muscle cells. B, DMSO inhibits VSMC migration in response to 10 ng/mL platelet-derived growth factor in a concentration-dependent manner; * $P<0.05$ vs control. Four different experiments were performed in duplicate for each experimental condition. Values are given as percent of stimulation with mitogen alone. C, Rapamycin (10^{-7} mol/L) and paclitaxel (10^{-5} mol/L) enhance thrombin-induced TF expression in HAECS (* $P<0.05$ for rapamycin or paclitaxel vs thrombin alone). DMSO blunts both thrombin-induced TF expression and the effect of rapamycin and paclitaxel on TF expression. ** $P<0.0001$ vs DMSO, DMSO plus rapamycin, and DMSO plus paclitaxel. Blots are representative of 4 different experiments.

In patients after myeloablative therapy, DMSO is infused intravenously together with hematopoietic progenitor cells; under these conditions, DMSO reaches plasma concentrations of 1.6% (20.0 mmol/L) and only rarely causes adverse effects.¹ The effectiveness of DMSO in inhibiting thrombus formation is underscored by the low dose we applied in the in

vivo experiments (3.8 mL/kg of a 40% DMSO solution). Because <10% of a lipophilic substance is resorbed through the peritoneum within 2 hours,³⁷ the mouse plasma concentration of DMSO is estimated to be 10 times lower than the maximal concentrations observed in humans after stem cell transfusion. Moreover, DMSO was still able to prevent thrombotic occlusion in 50% of mice when applied 5 minutes after the onset of injury, although this greatly reduces resorption time and plasma concentration.

Involvement of TF in acute coronary syndromes was recently underlined by a study that revealed increased levels of TF antigen and activity in atherectomy specimens from patients with unstable angina or myocardial infarction compared with those with stable angina.³⁸ Moreover, in acute coronary syndromes, plasma concentrations of cytokines such as TNF- α and interleukin-6 are increased at the site of coronary artery occlusion to concentrations high enough to induce TF in vascular cells.³⁹ For these reasons, inhibition of TF by DMSO may represent a novel and promising strategy for targeting thrombosis in acute coronary syndromes. In addition, the antiproliferative and antimigratory properties of DMSO, as well as its common use in clinical practice, underscore its suitability for administration to patients with acute coronary syndromes as a coating agent for drug-eluting stents.

Although drug-eluting stents are effective in reducing restenosis rates after percutaneous coronary intervention through inhibition of neointima formation and constrictive remodeling, stent thrombosis remains a concern with these devices. Indeed, the rate of stent thrombosis observed with drug-eluting stents is ≈2% in controlled clinical trials and may be higher in “real world” patients.^{40,41} Moreover, if thrombosis of such stents occurs, it is associated with a high morbidity and mortality.⁴² The present study suggests that DMSO has the potential to inhibit both neointima formation and stent thrombosis if applied on a drug-eluting stent. The known ability of DMSO to inhibit platelet aggregation may be very important in this context as well.³⁶ The ability of DMSO to suppress rapamycin and paclitaxel-induced TF expression is of particular relevance, because this effect of rapamycin and paclitaxel represents a possible cause of stent thrombosis in drug-eluting stents.^{19,25} Indeed, DMSO could be applied on such stents either alone or in combination with rapamycin or paclitaxel. Because this application is technically feasible and appears particularly promising, the potential beneficial effect of DMSO should be confirmed in a large-animal model.

In summary, the present study provides evidence that DMSO specifically suppresses TF expression and activity in addition to preventing TF-dependent thrombosis in vivo; furthermore, DMSO inhibits proliferation and migration of VSMCs and prevents rapamycin- and paclitaxel-induced TF expression. Because the use of DMSO is established in different areas of modern medicine, we propose this drug as a novel strategy for treating acute coronary syndromes; in particular, DMSO appears to represent an attractive compound for application on drug-eluting stents, either alone or in combination with rapamycin or paclitaxel.

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Disclosures

Drs Camici, Lüscher, and Tanner hold a patent on the results reported in the present study and their potential clinical applications. The remaining authors report no conflicts.

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CLINICAL PERSPECTIVE

Subacute stent thrombosis is a major clinical concern. Although drug-eluting stents (DES) are effective in reducing restenosis rates, these stents have not reduced the incidence of stent thrombosis compared with bare-metal stents. Indeed, the rate of stent thrombosis observed with DES is ≈2% in controlled clinical trials and may be higher in “real world” patients. Rapamycin and paclitaxel are used on DES because they inhibit neointima formation; however, these drugs also induce expression of tissue factor (TF), a key protein for initiation of thrombus formation; indeed, this represents a possible cause of stent thrombosis with DES. Therefore, the search for new molecules to cover stents remains important. Dimethyl sulfoxide (DMSO) is used for preservation of hematopoietic progenitor cells and is infused into patients undergoing bone marrow transplantation. We observed that DMSO suppresses TF expression and activity and thrombus formation in a mouse model *in vivo*; in addition, it inhibits vascular smooth muscle cell proliferation and migration. These data suggest that DMSO has the potential to inhibit both neointima formation and stent thrombosis if applied on a DES. Given this profile of action and its routine use in modern clinical practice, we propose DMSO be used to coat DES and treat acute coronary syndromes. Indeed, DMSO could be applied on DES either alone or in combination with rapamycin or paclitaxel. Although this application looks promising and technically feasible, the potential beneficial effects of DMSO must be confirmed in a large-animal model before a clinical trial can be initiated.