Current Concepts and Mechanisms of Drug-Induced Vascular Injury

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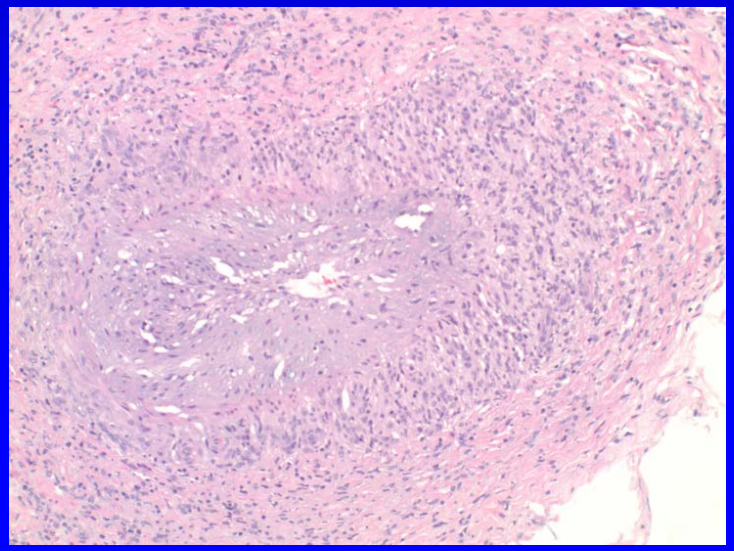
PRESENTATION OUTLINE

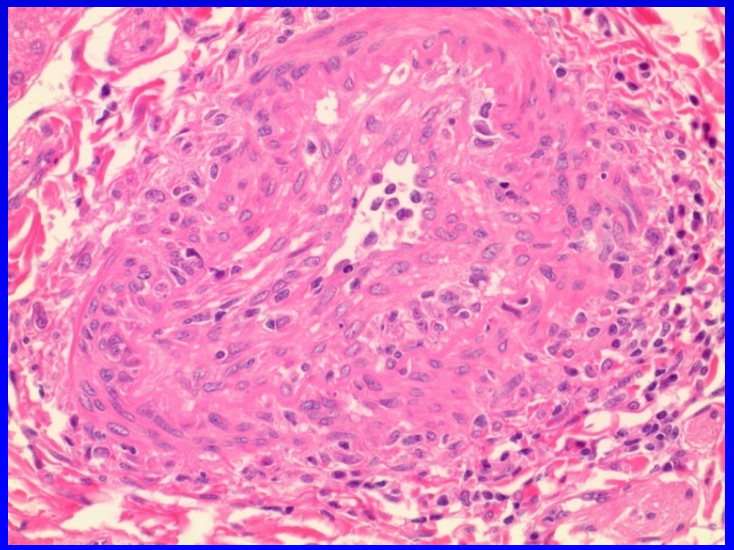
- Terminology : Spontaneous vs drug-induced vascular injury
- Vascular Injury in Toxicology Studies
  - Experimental Manipulations
  - Biotherapeutics
  - Small Molecules
- DIVI: Pharmacologic Diversity Between Small Molecules
- CASE Study: Endothelin Receptor Antagonists hemodynamics
- **MOA Studies: Caveolin-1 and Nitric Oxide Pathway**
  - ✓MOA specific markers
- Assessment of Human Relevance

Natural Disease: Idiopathic polyarteritis of beagles

- ✓ Idiopathic canine polyarteritis (low incidence in mongrels)
- ✓ Juvenile polyarteritis syndrome, polyarteritis, arteritis, periarteritis and panarteritis
- ✓ Necrotizing vasculitis
- ✓ Idiopathic febrile necrotizing arteritis



- ✓ Thickened medial walls
- ✓ Intimal proliferation (occluded lumen with/or without thrombosis
- ✓ Inflammation within the vessel
- ✓ Advential and perivascular inflammation
- ✓ Enlargement of endothelial cell (activation)
- ✓ Variable necrosis/hemorrhage

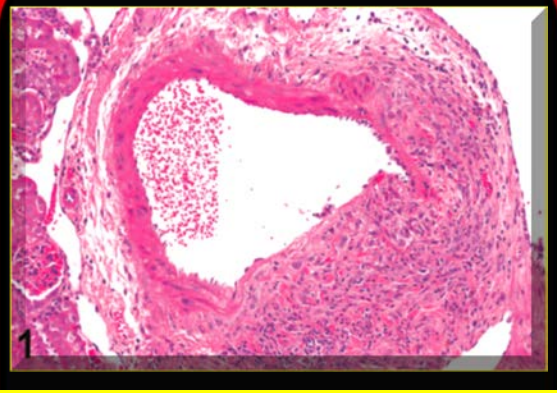


Histopathology Features of Spontaneous Vascular Lesions in the Dogs

- ✓ Thickened media with SMC hypertrophy and hyperplasia
- ✓ Marked intimal proliferation (occluded lumen with/or without thrombosis
- ✓ Inflammation within the medial vessel wall
- ✓ Adventital and perivascular inflammation
- ✓ Enlargement of endothelial cell
- ✓ Variable necrosis/hemorrhage
- ✓ Plexiform lesions

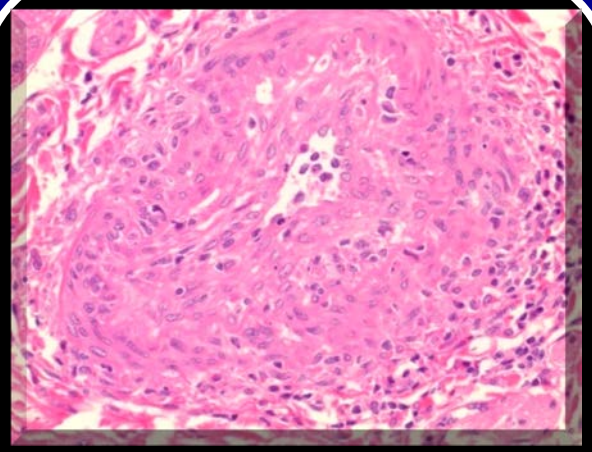
Spontaneous Vascular Lesions in Toxicology Studies

Rat



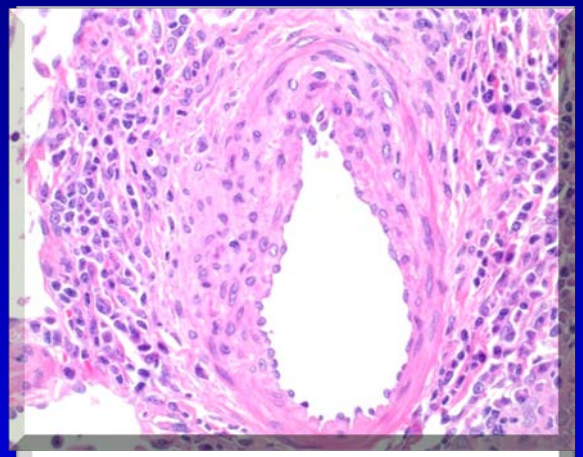
Poly arteritis Nodosa
Polyangitis
Mesentericvasculopathy
Endothelial cell hypertrophy
SMC hypertrophy, thickening
Perivascular inflammation, fibrosis, expansion of adventitia

Dog Lesion



- ✓ **Idiopathic canine polyarteritis (low incidence in mongrels)**
- ✓ **Juvenile polyarteritis syndrome, polyarteritis, arteritis, periarteritis and panarteritis**
- ✓ **Necrotizing vasculitis**
- ✓ **Idiopathic febrile**

Non Human Primate

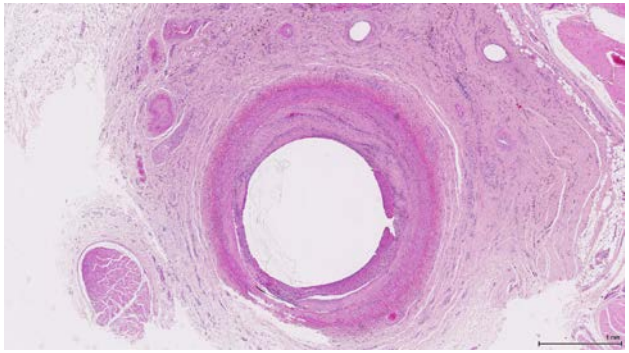


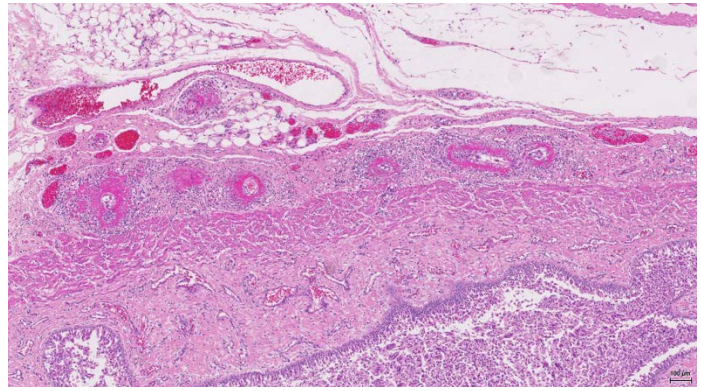
- ✓ Expansion of vascular adventitia
- ✓ Perivascular inflammation
- ✓ Disruption of medial architecture
- ✓ Medial SMC hypertrophy
- ✓ Disruption of Elastic Lamina
- ✓ EC Hypertrophy

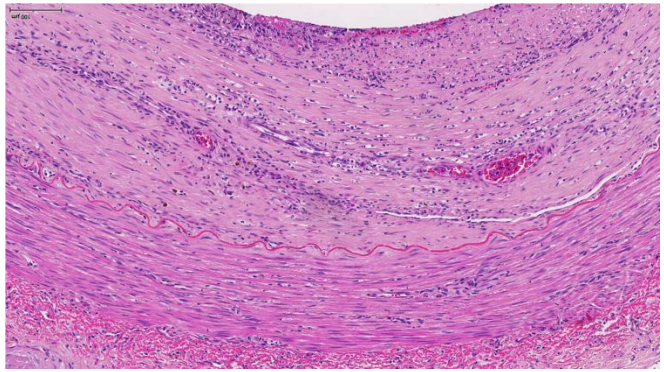
Experimental Manipulations

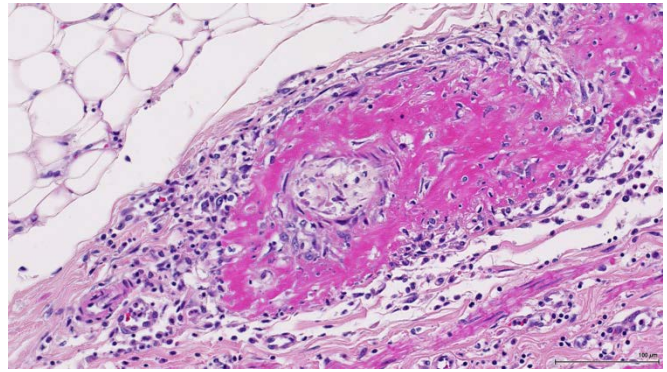
- **Intravenous Drug Administration**
  - **Bolus Intravenous Injection (administration less than 2mins)**
  - **Continuous Intravenous Infusion (30- min to 1 hour)**
- **Long-Term Continuous Intravenous**
  - **In-dwelling catheter for repeated administration**
- **Subcutaneous Administration**

Experimental Manipulation

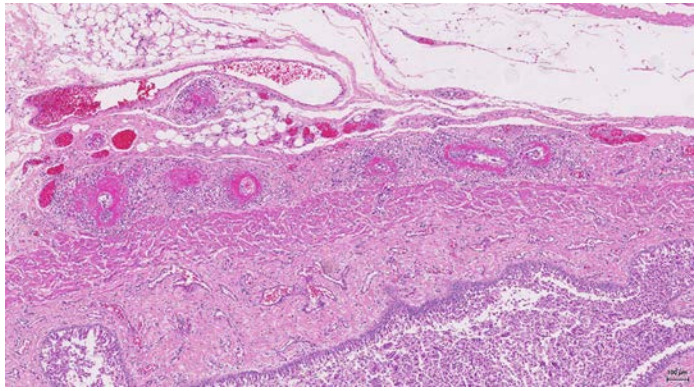


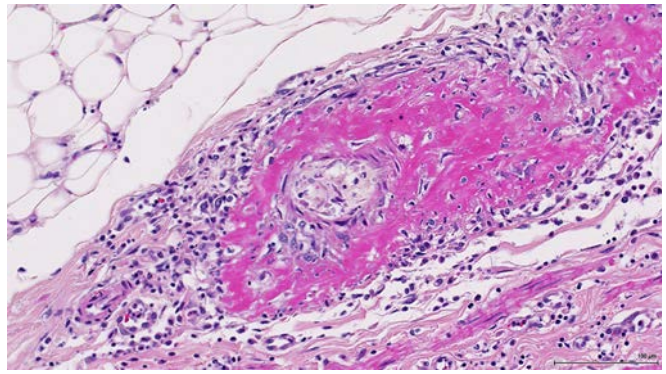


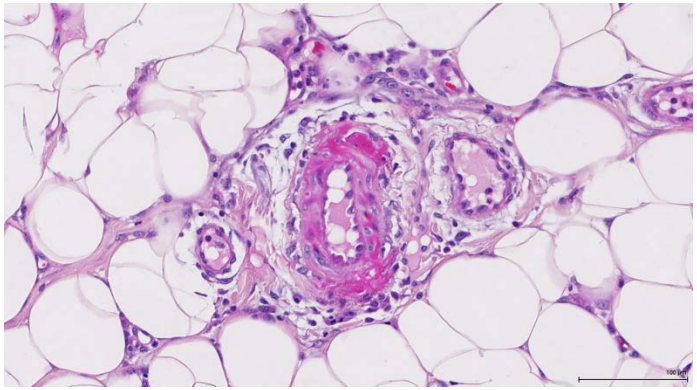


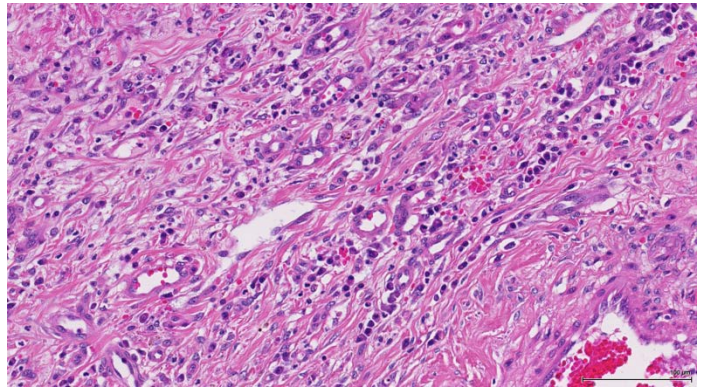


Experimental Manipulation









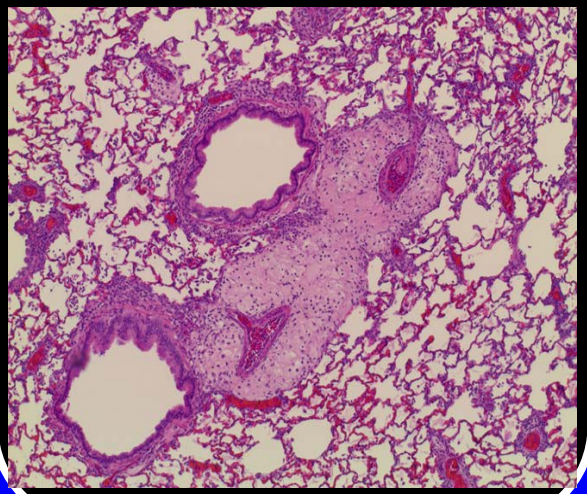
Vascular Lesions in Development of Therapeutics for Human Use

- Small molecules
- Biotherapeutics
  - Large molecules (MAB)
  - Oligonucleotides
  - Vaccines (??)

Vascular Injury and Biotherapeutics

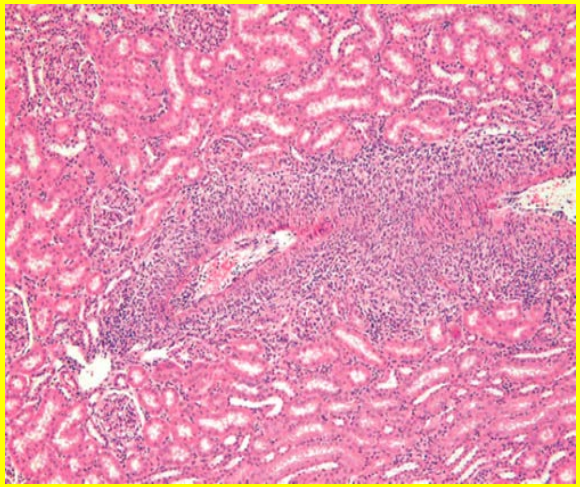
Perivascular edema-vascular leak syndrome

- ✓ RIL-2 administration
- ✓ Pre-clinical to clinical translation
- ✓ Dose-limiting clinically in humans



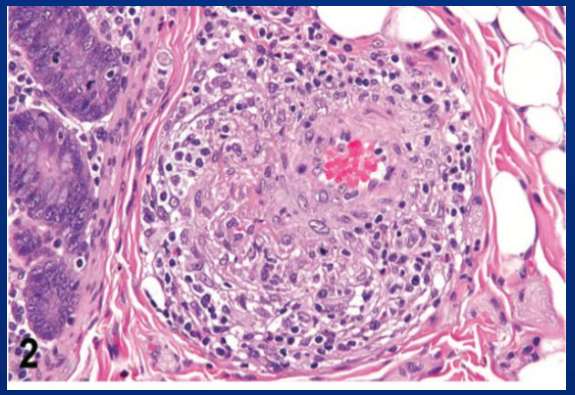
Anti-Sense Oligonucleotide

- ✓ **Systemic perivascular infiltrates of mononuclear cells;**
- ✓ **Well-known feature of ASO toxicity**
- ✓ **Clinical signs often not apparent**
- ✓ **Immune-related (Immunogenicity??)**

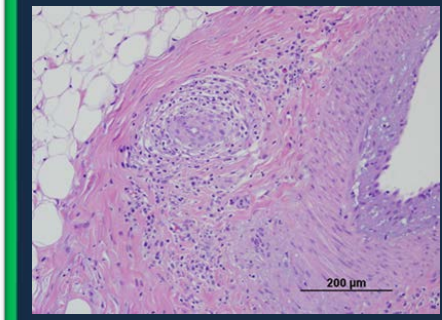


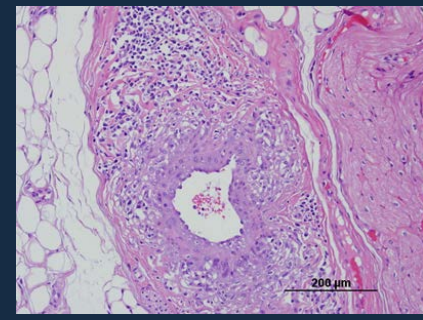
MonoclonalAntibodies

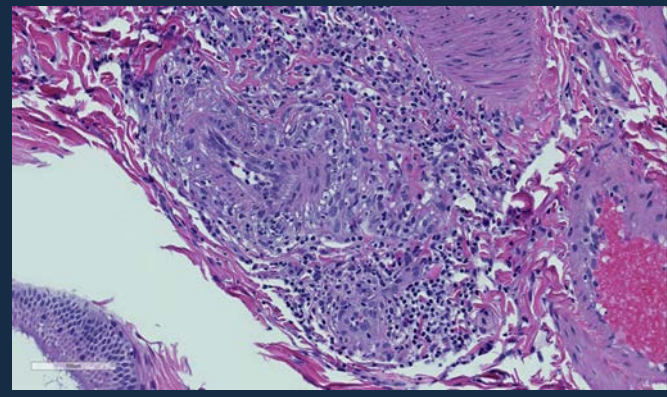
- ✓ **No apparent clinical signs**
- ✓ **Perivascular infiltrates in multiple organs**
- ✓ **Resolution during Recovery Phase**
- ✓ **May or may not be dose-related**



Vascular Toxicity Study in Cynomolgus Monkeys







Toxicology Issue of Concern -DIVI

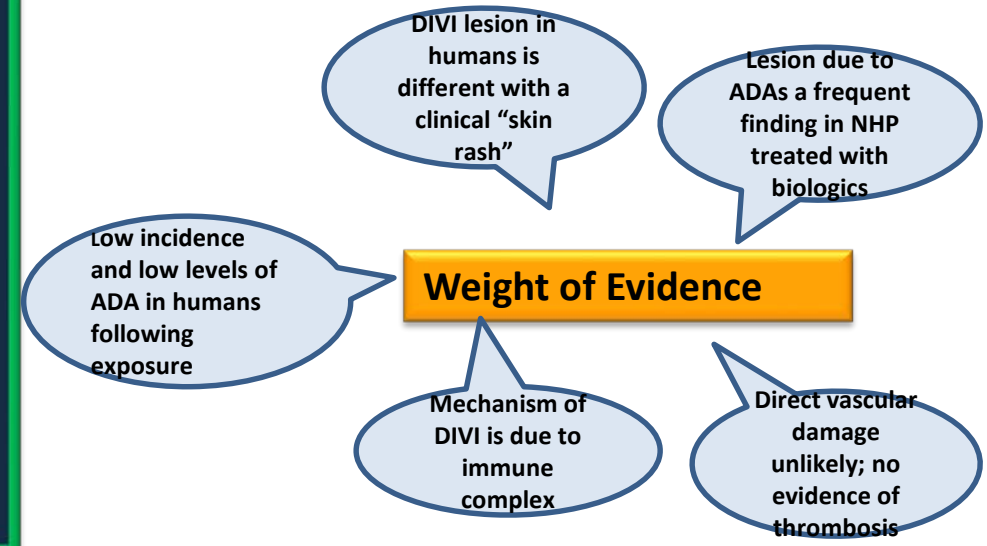
- Systemic perivascular inflammation in multiple organs at all doses; silent "occult" pathology, no biomarkers for clinical monitoring;
- **Finding has regulatory implications with potential impact on clinical development**

Points to consider

- Often related to ADA that show no dose response
- IHC to show immune complex deposition
- Lesions reversible

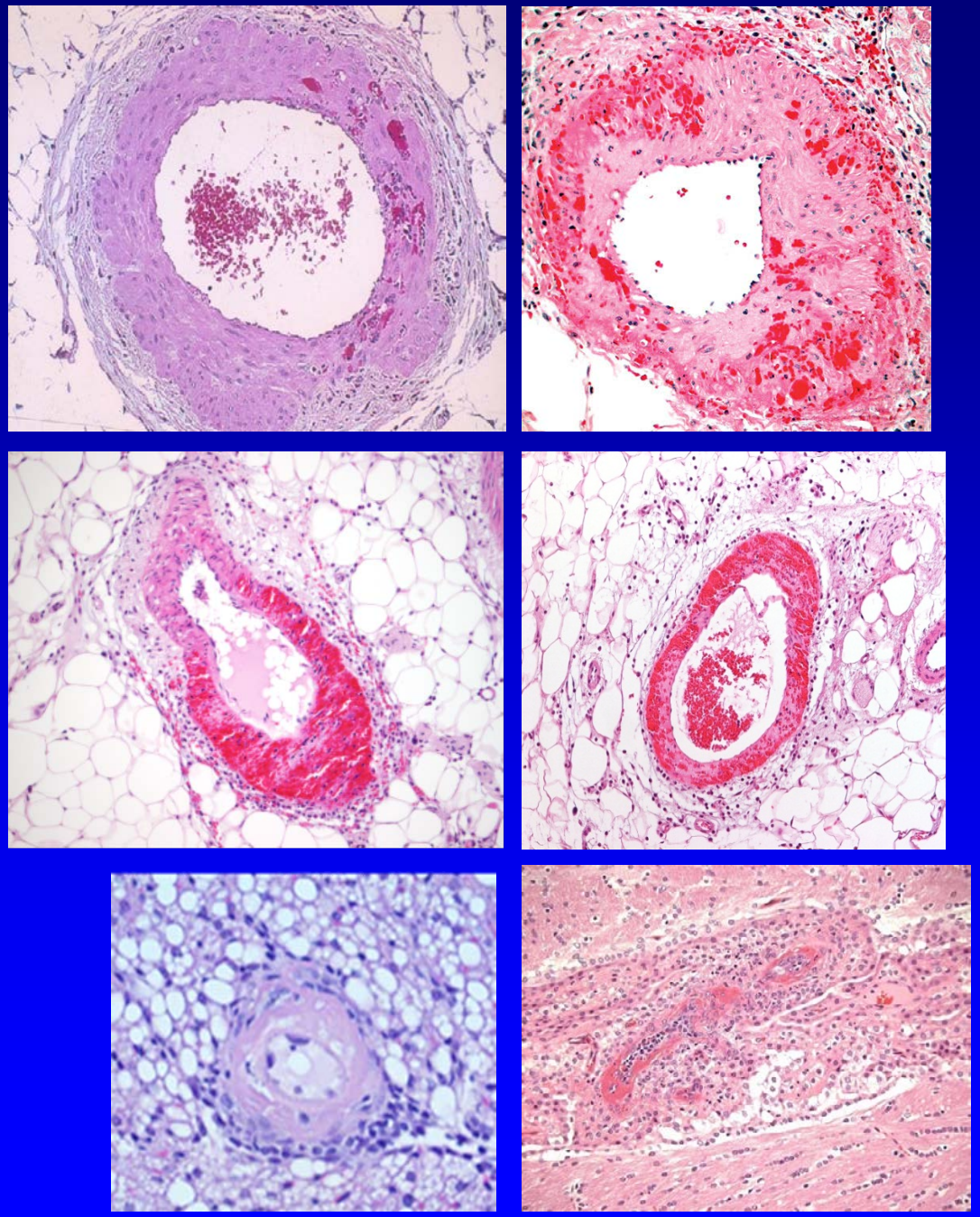
No human relevance

Scientific Justification for Recommendation



Small Molecule: Drug-Induced Vascular Lesions

- ✓ Predilection for the right atrium and coronary arteries in dogs
- ✓ Mesenteric arteries in the rat
- ✓ Mural hemorrhage, segmental, transmural or circumferential), necrosis/edema
- ✓ Branch points; medium sized muscular primarily affected in the rat
- ✓ Often no apparent clinical signs
- ✓ Histologically clear distinction between vasoconstrictor and vasodilator lesions



Drug-Induced Vascular Injury: Pharmacologic Diversity

- **Hemodynamically Active Compounds**
  - ✓ **Vasodilators**
  - ✓ **Vasoconstrictors**
  - ✓ **Regulation of vascular tone**
- **Direct action on EC and/or SMC**
- **Immune System Activation**
- **Direct cytotoxicants**

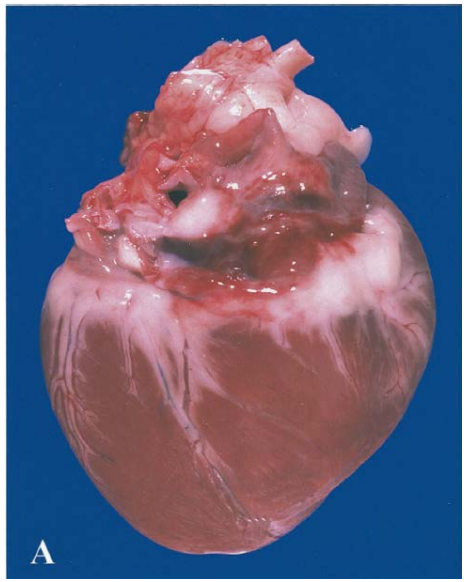
Pharmacologic Class	Compound/Drug Examples	Primary Target
Potassium channel opener	Minoxidil, Hydralazine, Nicorandil, and ZD6169	SMC K+ channels
Phosphodiesterase Inhibitors	SK&F 94418, Milirone, Theobromine, Viagra, Cilomilast	PDE III, IV, & V
Nitric Oxide Synthease (NOS) Pathway	**Sodium Nitroprusside**	**NOS**
Endothelin Receptor Antagonist (ETRA)	**SB 209670; ZD1611; AZD 2574; CI-1020; Bosentan;**	**ETA1 or ETB1 receptors**
Adenosine agonists	CI-947, cyclohexyladenosine	A1 or A2 receptors
Dopamine and Dopaminergic Agonists	Fenoldopam	DA1 & DA2 Receptors
Na, K+ ATPase pump inhibitor	Cardiac Glycosides, Digoxin, Digitalis	Na, K+ ATPase pump
Vitronection receptor antagonist	**SB-273005**	**αVβ3 and αVβ5**

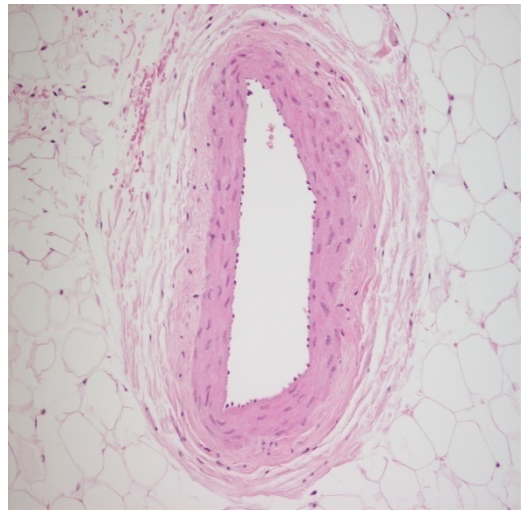
Pharmacologic Class	Compound/Drug Examples	Primary Target
Potassium channel opener	Minoxidil, Hydralazine, Nicorandil	SMC K+ channels
Mixed Channel Openers	Diazoxide	ATP-Sensitive Channel Openers
Phosphodiesterase Inhibitors	PDE 5 (sildenafil, vardenafil, tadalafil avanafil) PDE IV, (Eucrista, Otezla, Ariflo) PDE III (Milirone, Theobromine)	PDE III, IV, & V
Nitric Oxide Synthesase (NOS) Pathway	Sodium Nitroprusside, Nitroglycerin	NOS
Endothelin Receptor Antagonist (ETRA)	4 Approved ETRA and many more tested clinically in humans (SB 209670; ZD1611; AZD 2574; CI-1020)	ETA1 or ETB1 receptors
Adenosine agonists	Regadenoson the adenosine A2A receptor agonist FDA approved	A1 or A2 receptors
Dopamine and Dopaminergic Agonists	Fenoldopam	DA1 & DA2 Receptors
Na, K+ ATPase pump inhibitor	Cardiac Glycosides, Digoxin, Digitalis	Na, K+ ATPase pump

Case Study: Endothelin Receptor Antagonists

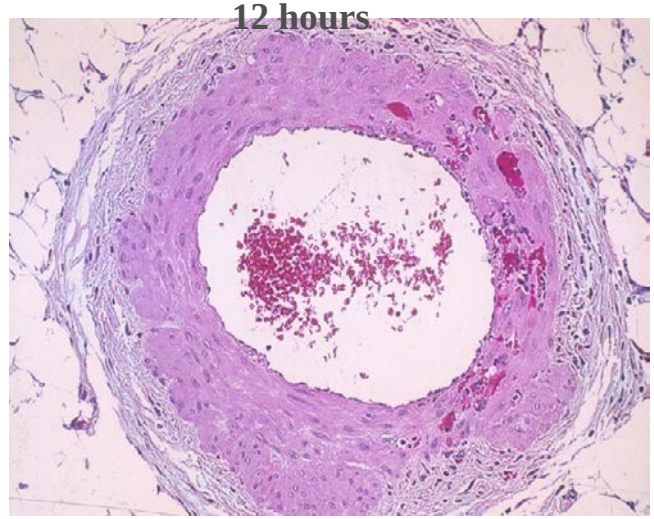
Functional Hemodynamics and MOA Studies:

Drug-Induced Vascular Injury

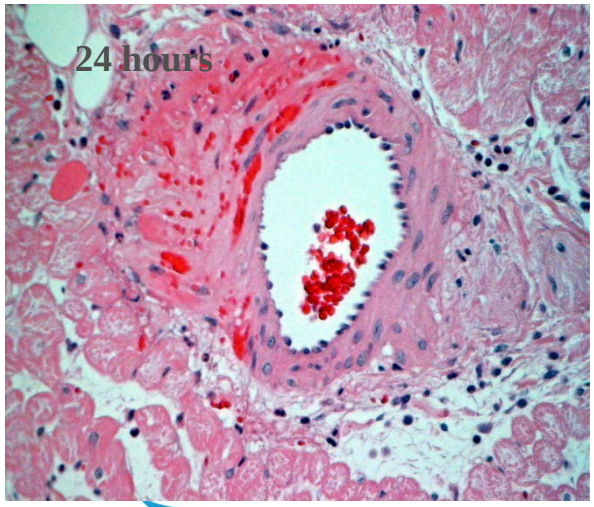




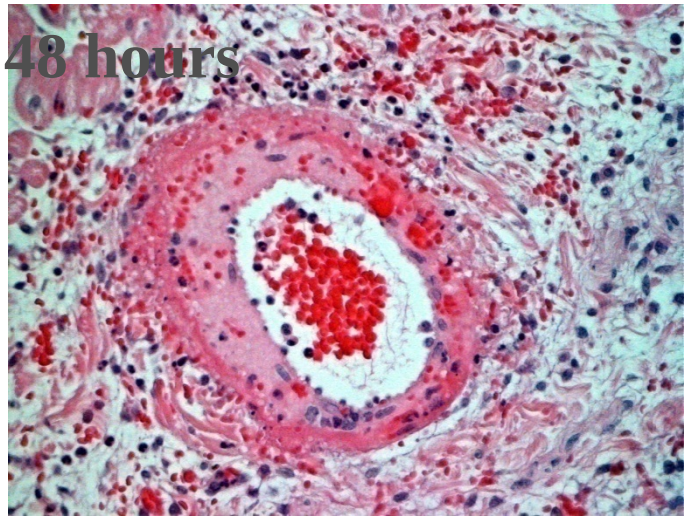
12 hours



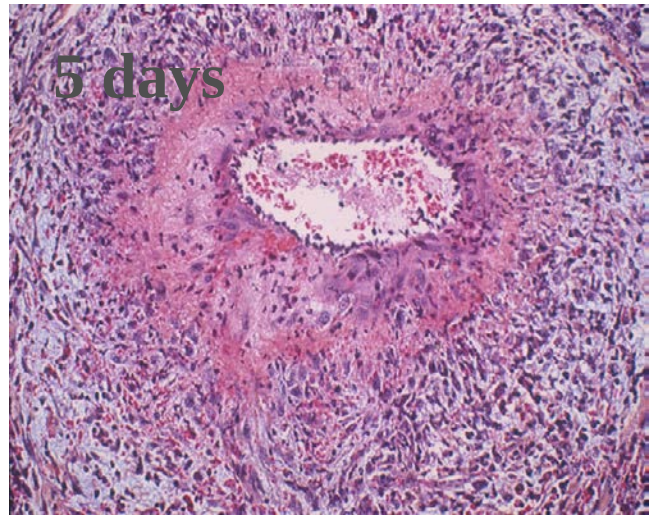
24 hours



48 hours



5 days



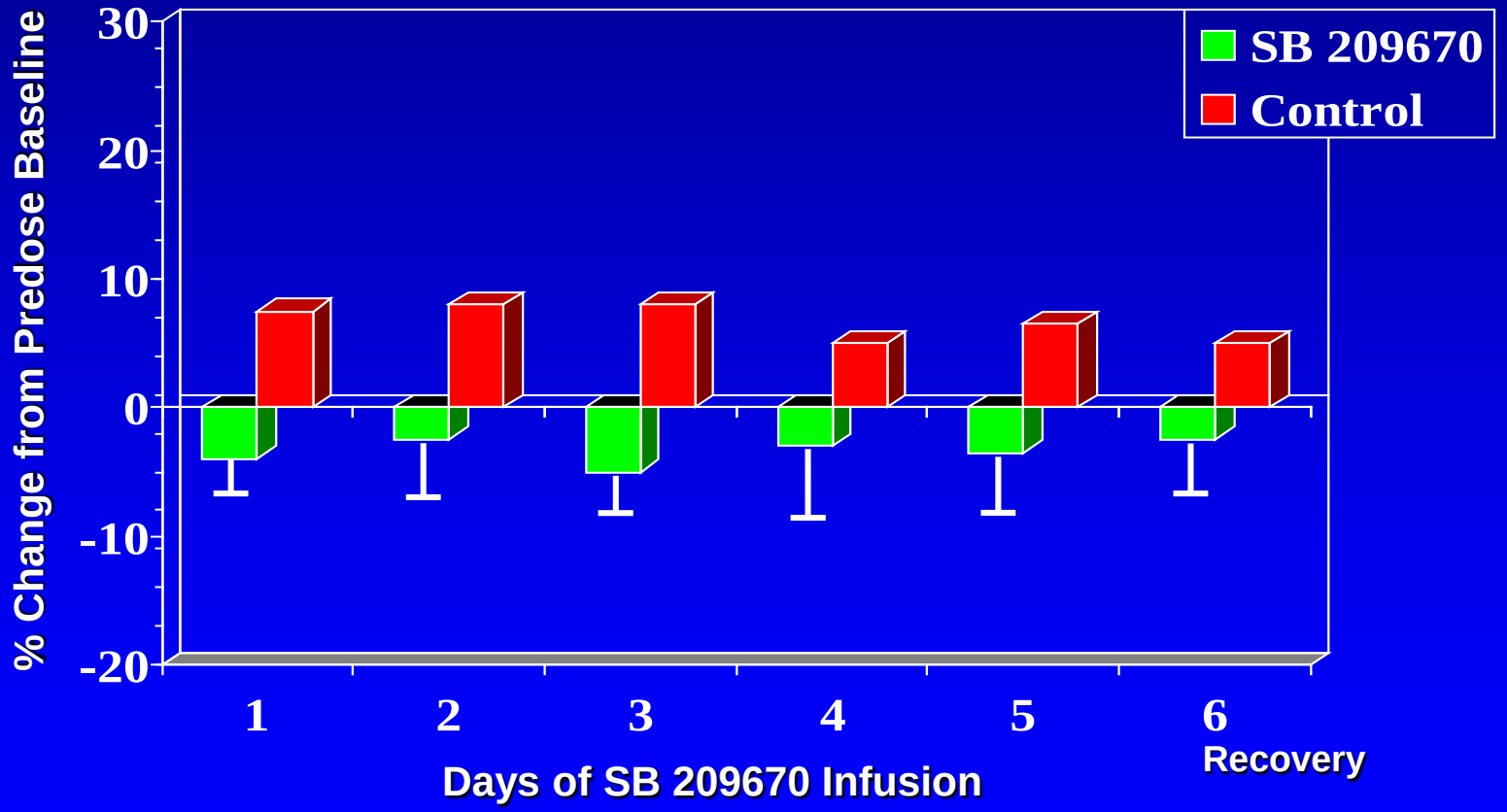
ETRA Induced Vascular Injury: Potential Mode of Action

Endothelin Receptor Subtype Distribution Predisposes Coronary Arterial Damage in the Dog

- Determine the hemodynamic functional consequences in the dog heart during periods of endothelin receptor blockade
- Map endothelin receptors (ET_A and ET_B) distribution and density within specific anatomical regions of the normal dog heart
- Correlate ET receptor density with alterations in regional blood flow and the severity and frequency of vascular damage

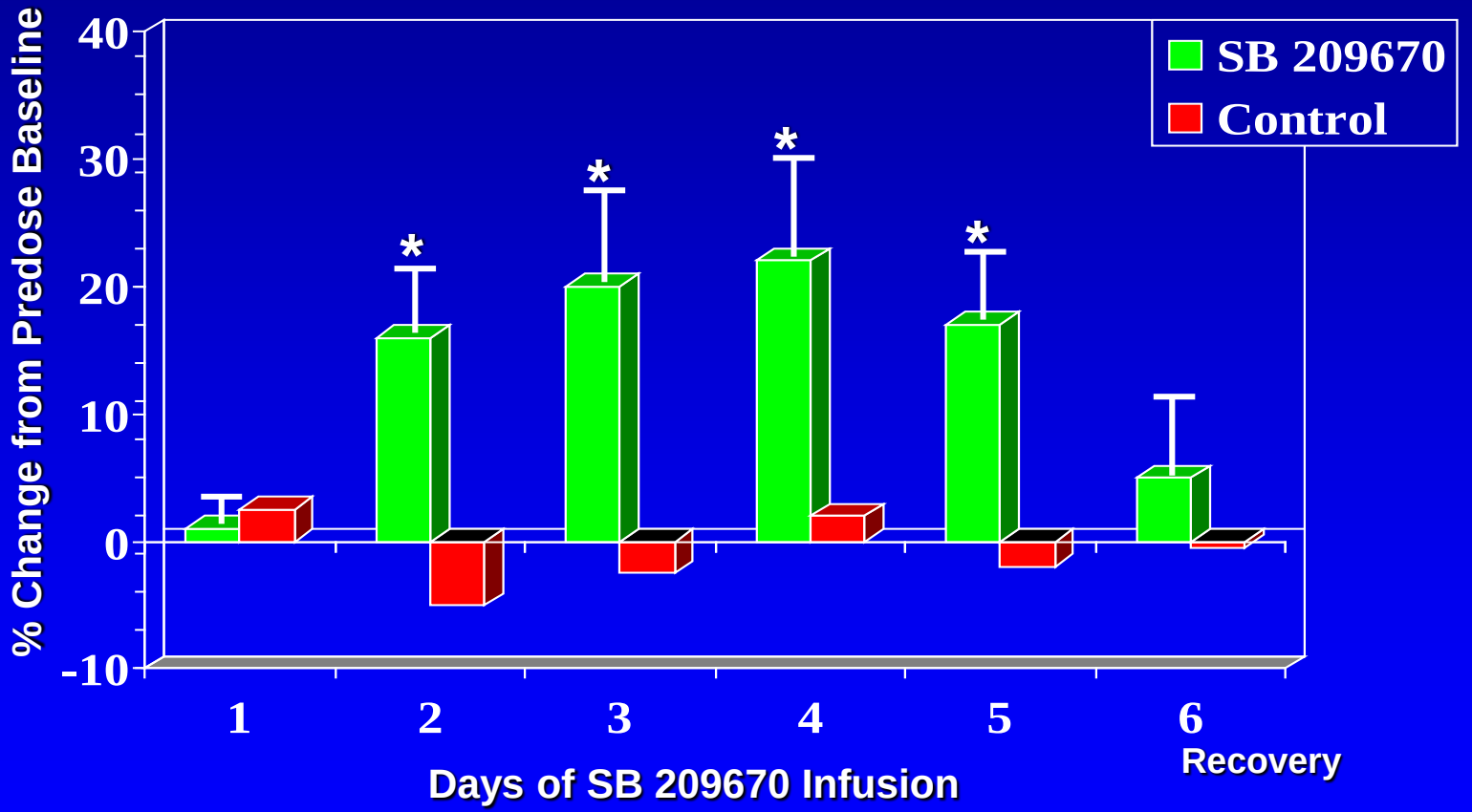
Localize expression of the molecular target to site specific toxicity

Mean Arterial Pressure



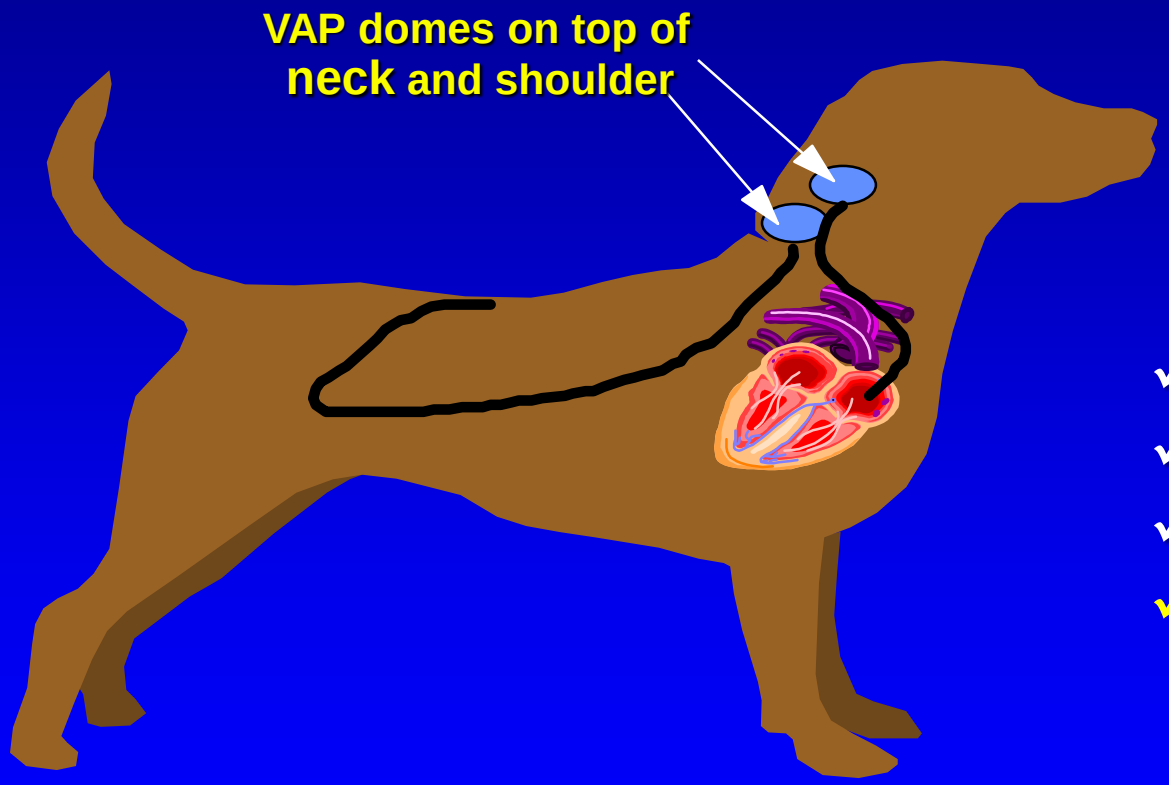
Louden et al., AJP 157: 123-134; 2000

Mean Heart Rate



Louden et al., AJP 157: 123-134; 2000

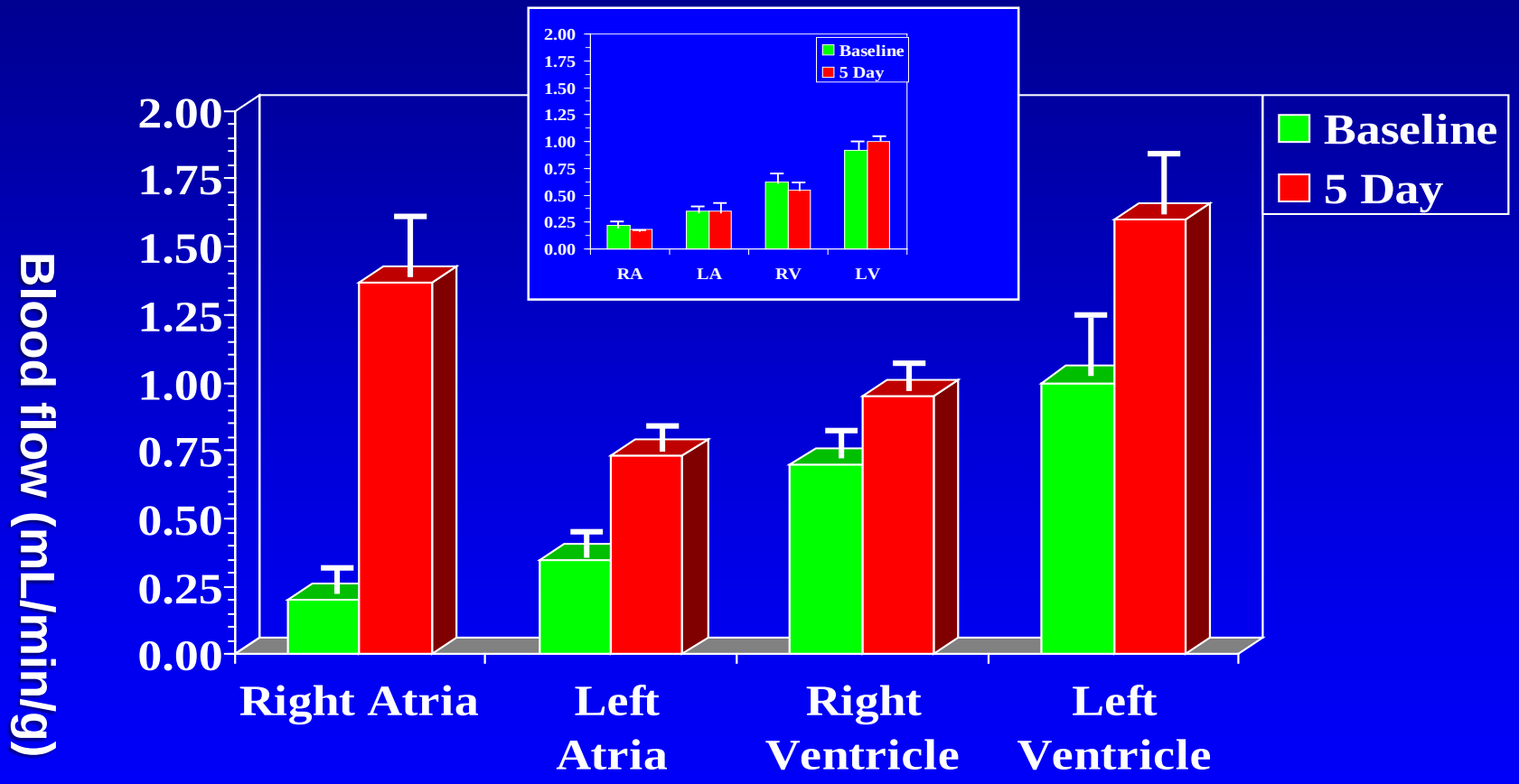
Regional Blood Flow Model



Increases in RBF

- ✓Minoxidil ~6-Fold (dog)
- ✓Minoxidil ~7-Fold (rat)
- ✓Milrinone (PDEI) ~7-Fold (dog)
- ✓**SB 209670 ~6-Fold (dog)**

Regional Myocardial Blood Flow



Louden et. al., Am J Pathol 2000, 157:123-134

Endothelin Receptors

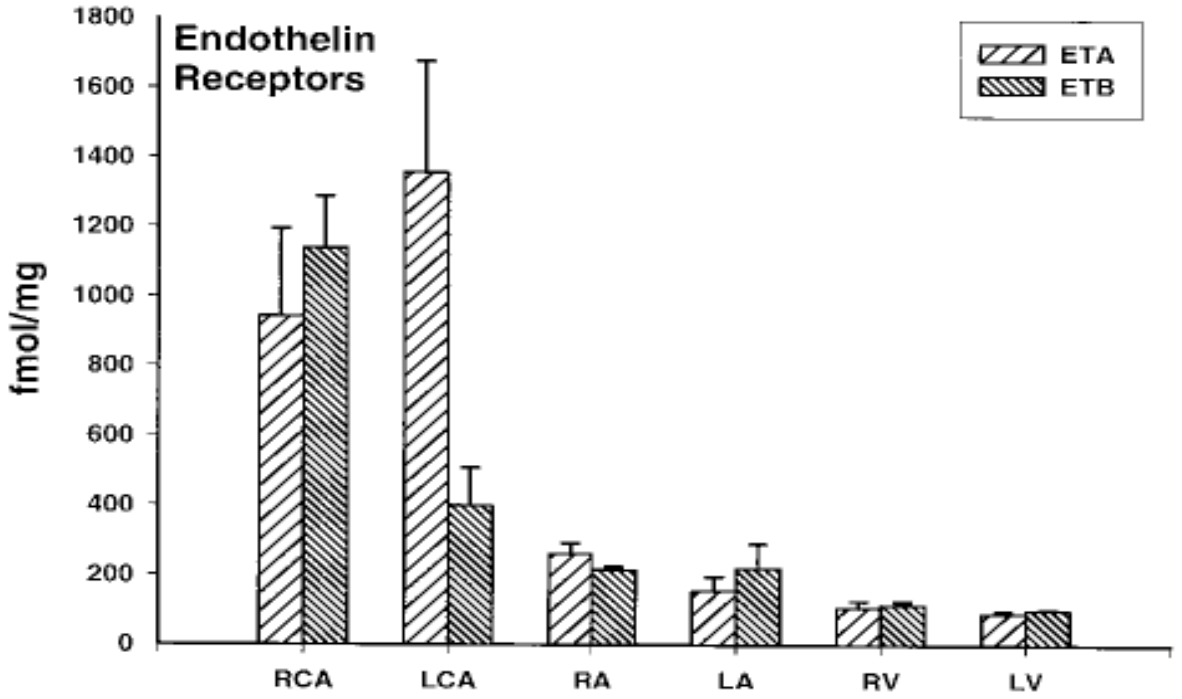


Figure 5. ET_A and ET_B receptor density in different regions of the normal dog heart. RCA, right coronary artery; LCA, left coronary artery; RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle.

Louden et al. AJP 157: 123-134; 2000

Summary of Endothelin

- Distribution and density of endothelin vasoconstrictive receptors co-localize with site specific toxicity in the dog heart
  - Site specific toxicity is associated with expression of the molecular target
- Density and distribution of ET receptor subtypes in the dog heart is associated with marked functional increases in coronary regional blood flow
- At certain anatomical sites in the dog heart profound ET receptor blockade causes disproportionate shift in blood flow that predisposes these sites to injury

Drug-Induced Vascular Injury: Potential Mode of Action

Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodelling of blood vessels

J. Clin Invest 116:1284-1291

Role of caveolin-1 in the regulation of the vascular shear stress response

J Clin Invest 116:1222-1225

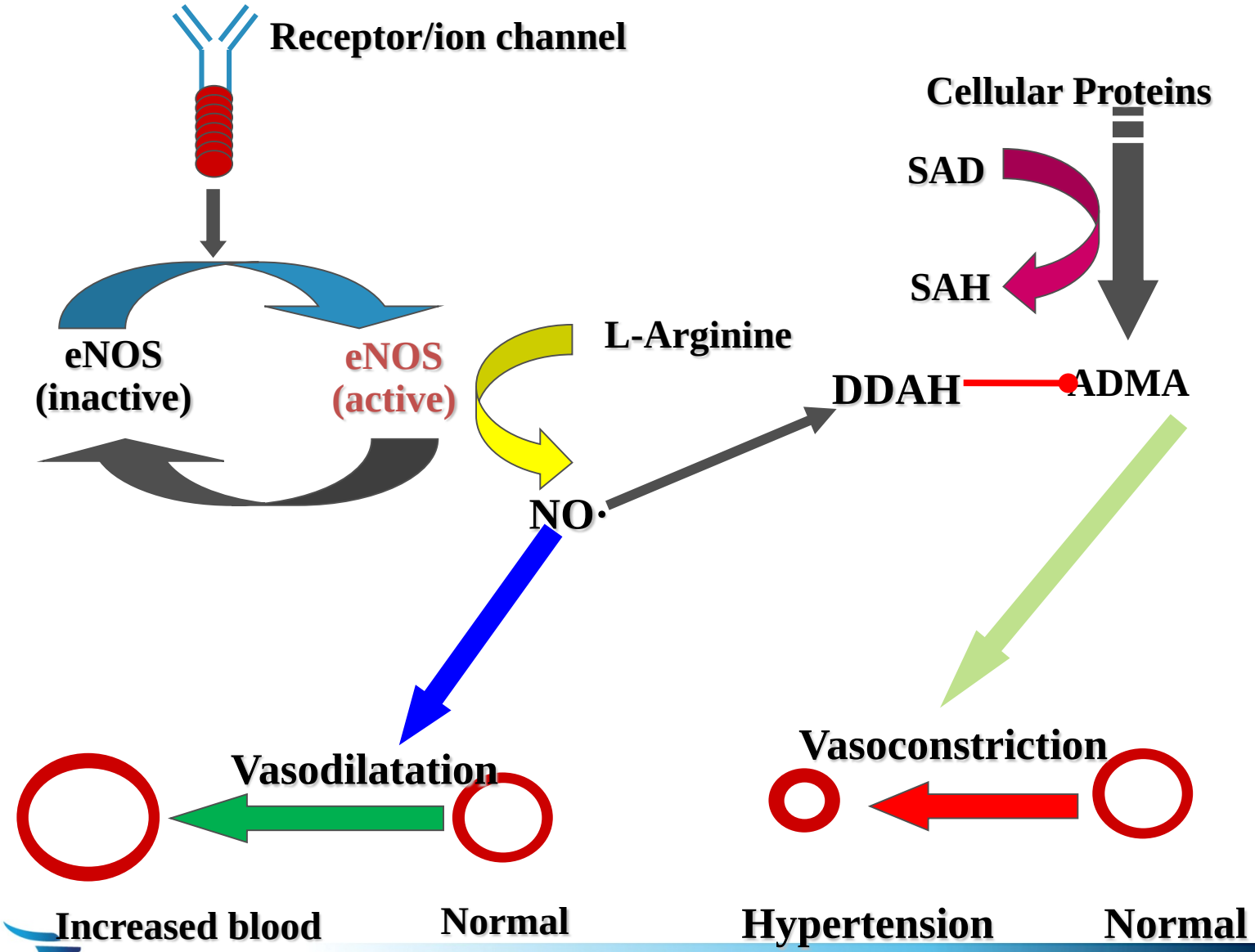
- ✓ **Role of Caveolin-1 and the NO Pathway in Vascular Injury induced by Fenoldopam**

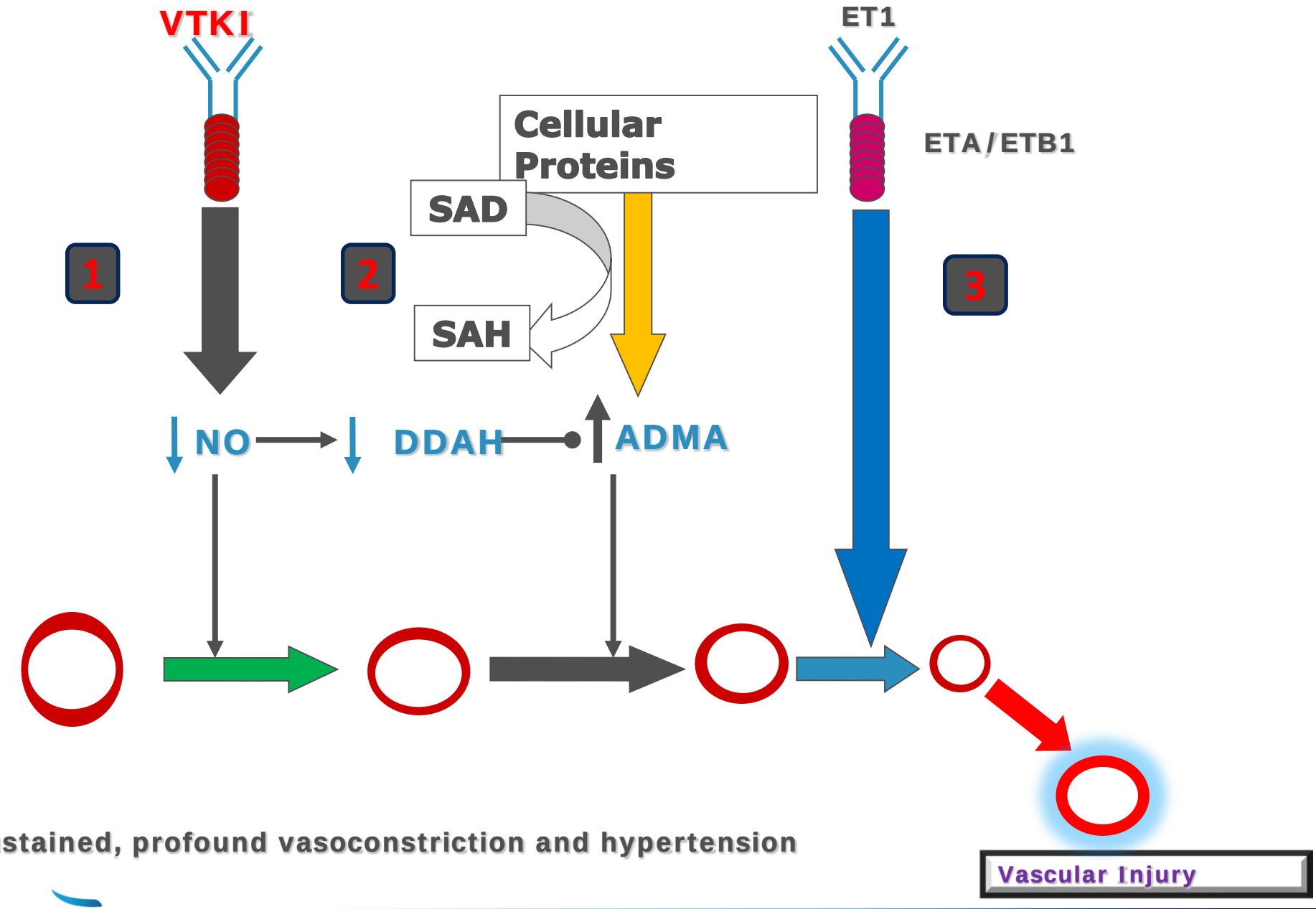
Drug-Induced Vascular Injury: Potential Mode of Action

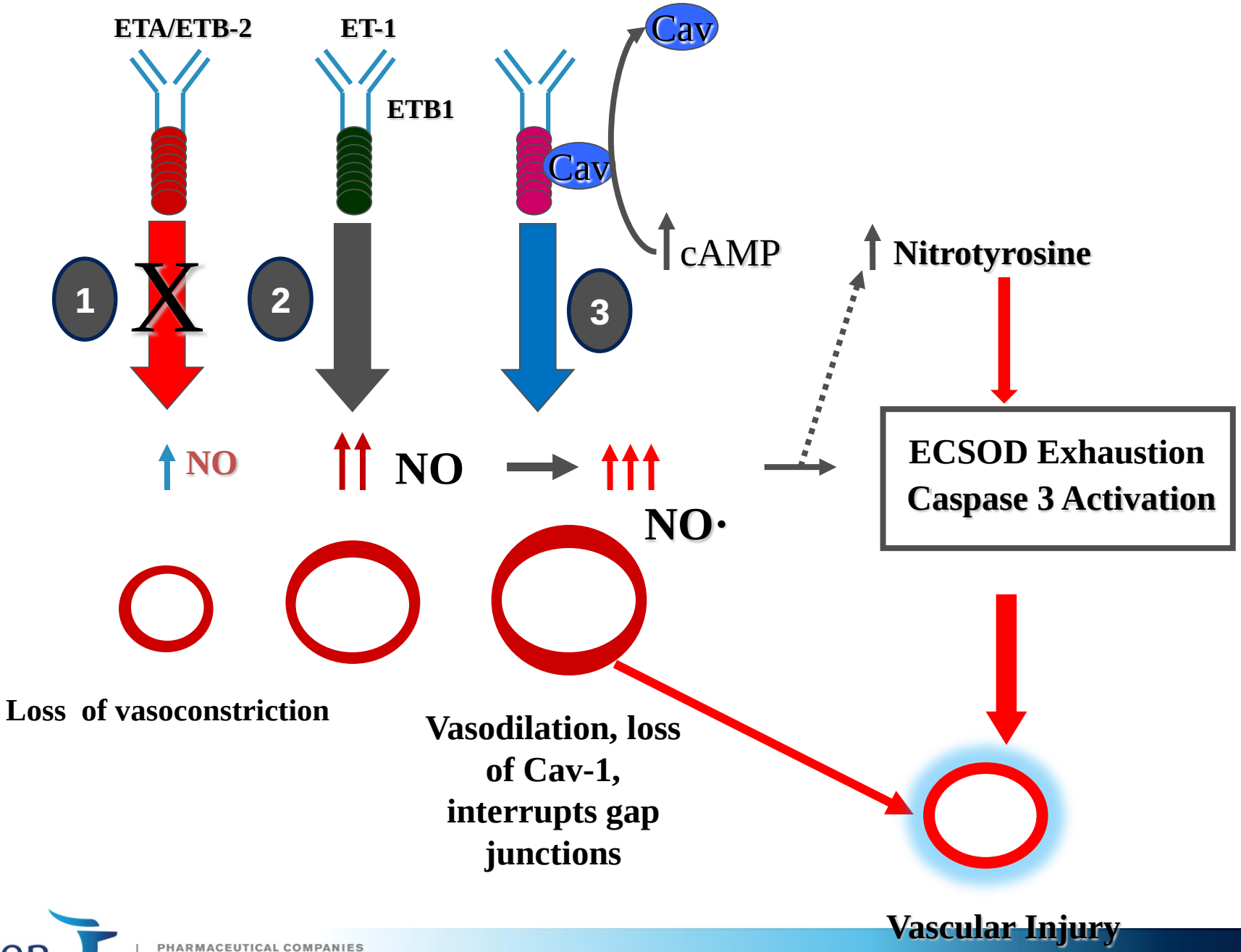
Caveolin-1 regulated nitric oxide pathway and increased c-AMP leads to functional hemodynamic, biochemical and structural alterations in endothelial cells, smooth muscle cells and the vascular wall.

- ✓ **Role of Caveolin-1 and the NO Pathway in Vascular Injury induced by Fenoldopam**

Regulation of Vascular Tone

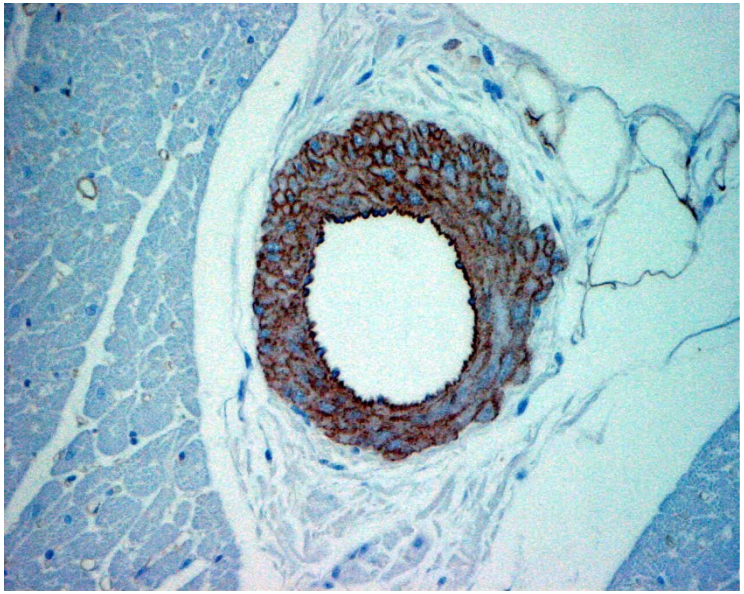



VTKI
ET1
Cellular Proteins
SAD
SAH
ETA/ETB1
1
2
3
NO
DDAH
ADMA
Sustained, profound vasoconstriction and hypertension
Vascular Injury

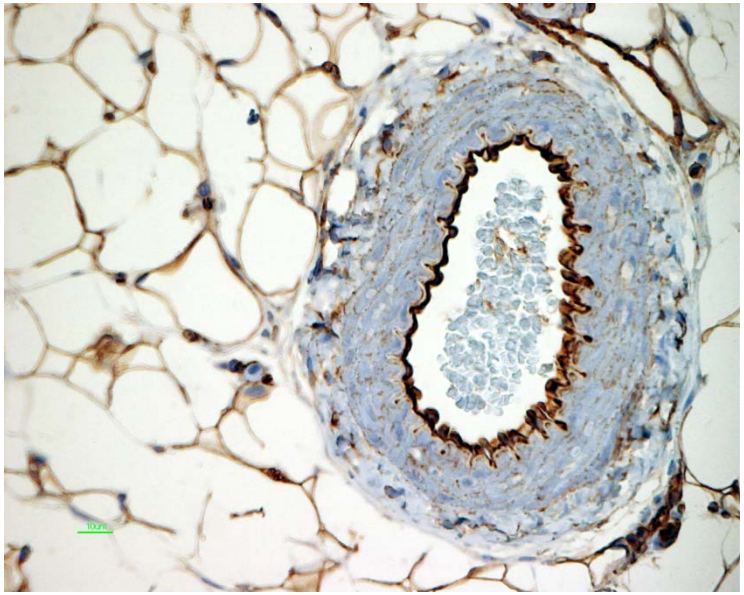

ETA/ETB-2
ET-1
ETB1
Cav
Cav
cAMP
Nitrotyrosine
1
X
2
3
NO
NO
NO·
ECSOD Exhaustion
Caspase 3 Activation
Loss of vasoconstriction
Vasodilation, loss of Cav-1, interrupts gap junctions
Vascular Injury

Novel Diagnostic Marker of Vascular Injury

Dog



Rat



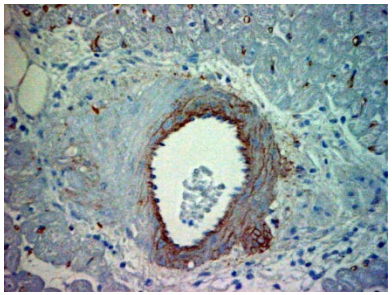
Caveolin-1

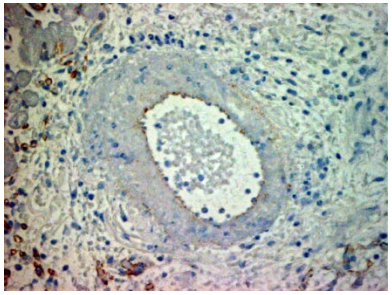
- expressed on endothelial and smooth muscle cells
- regulate vascular tone (NOS, NO and Ca+ dependent) endothelium dependent relaxation
- regulate tight junctions e.g., ZO-1 and claudin
- KO mice lacking Cav-1 display abnormal vascular contractions and inability to regulate vascular tone
- Co-localizes with receptors and enzymes associated with vascular injury

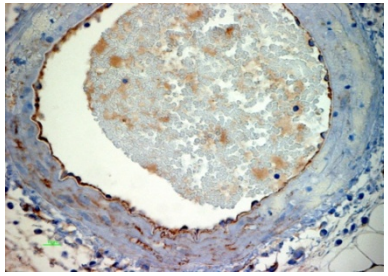
Caveolin-1 and NO Pathway in Drug-Induced Vascular Injury

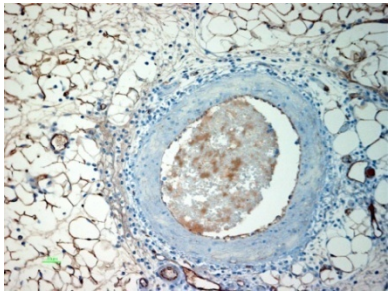
- **Evaluate Cav-1 expression in vascular injury**
  - paracellular permeability (tight junctions).
  - cell-to-cell communication/signaling (gap junction)
  - apoptosis (caspase 3 activation)
- **Determine if loss of Cav-1 precedes SMC death *in vitro***
  - apoptosis (caspase 3 activation and Annexin V expression)
  - do known classes of vascular toxicants decrease cav-1 *in vitro*
- **Assess effects of NO activity *in vivo***
  - ✓ peroxynitrite formation evaluation of nitrotyrosine
  - ✓ caspase-3 activation, status of tight junctions (ZO-1 and claudin)
  - ✓ will NO donors induce vascular injury comparable to fenoldopam
  - ✓ effects of blockade of NO *in vivo*

Caspase 3 Activation with Fenoldopam Treatment

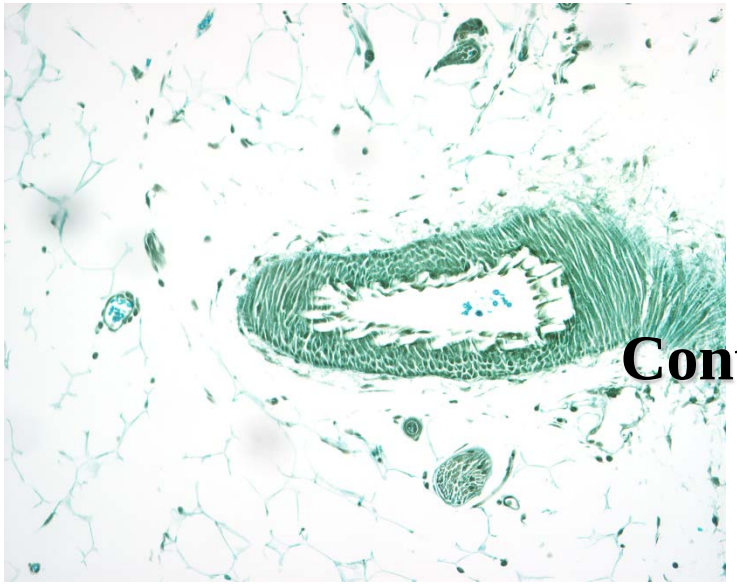




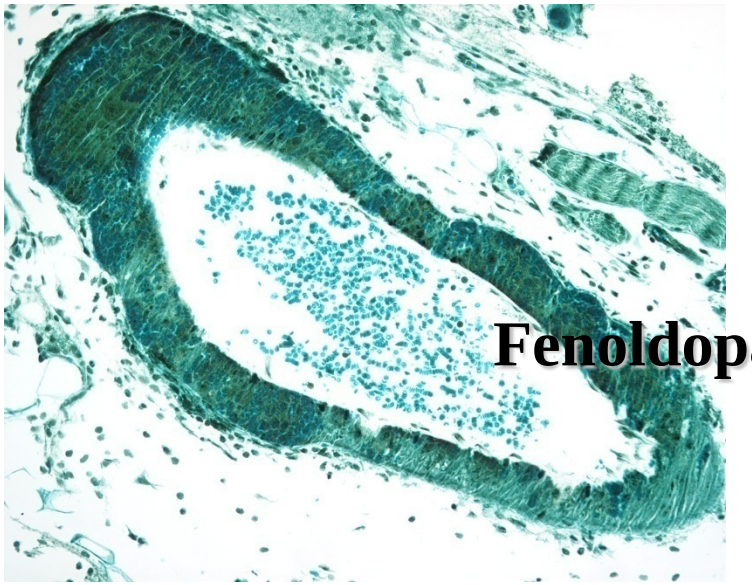




Cav-1 regulated Caspase-3 is activated in drug-induced vascular injury.

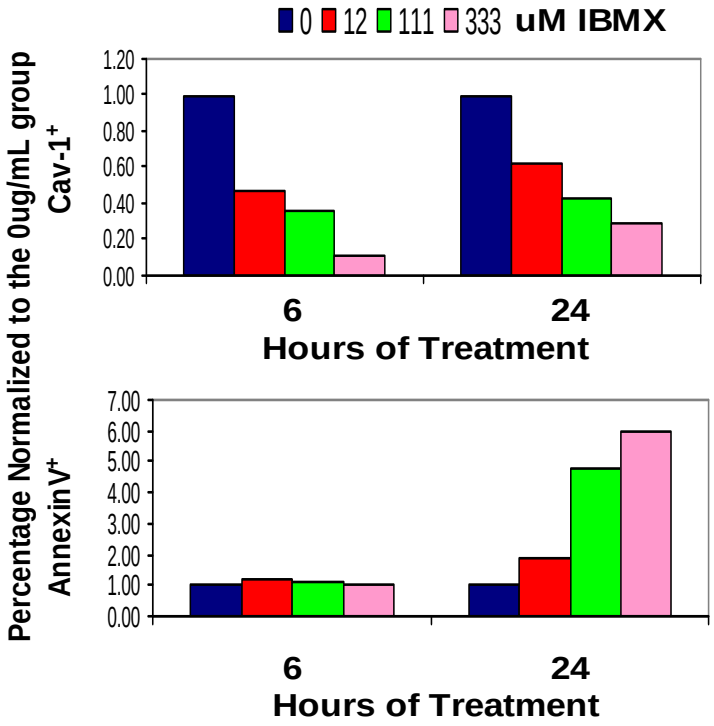


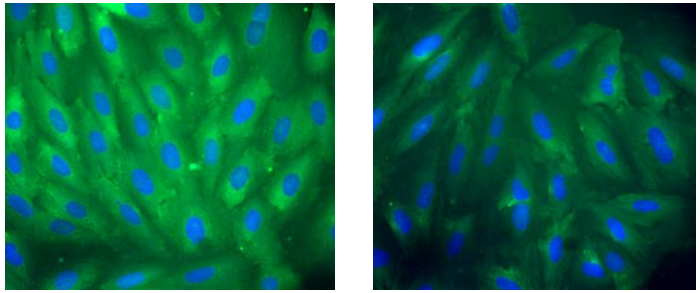
Control

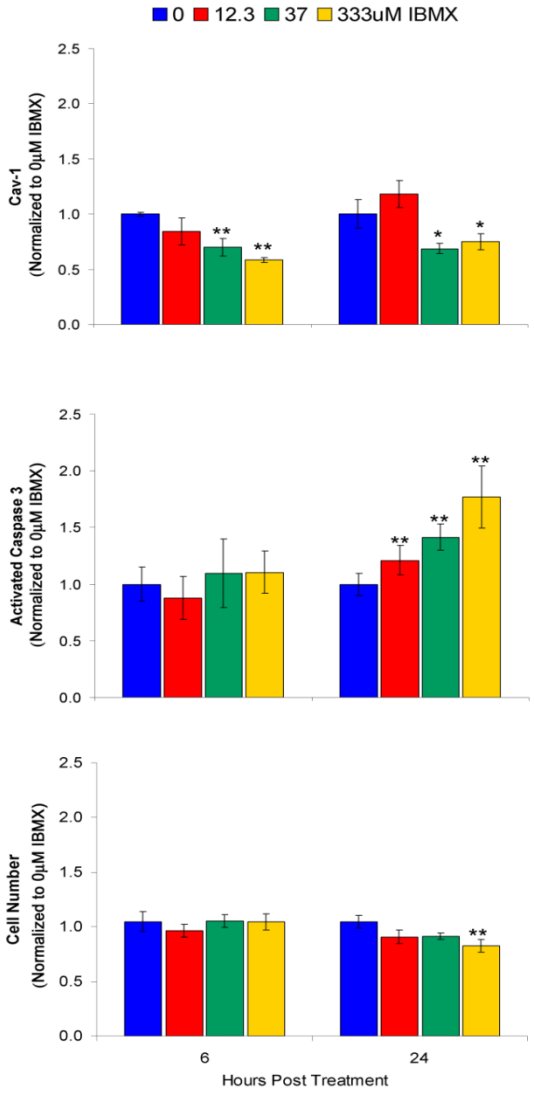


Fenoldopam

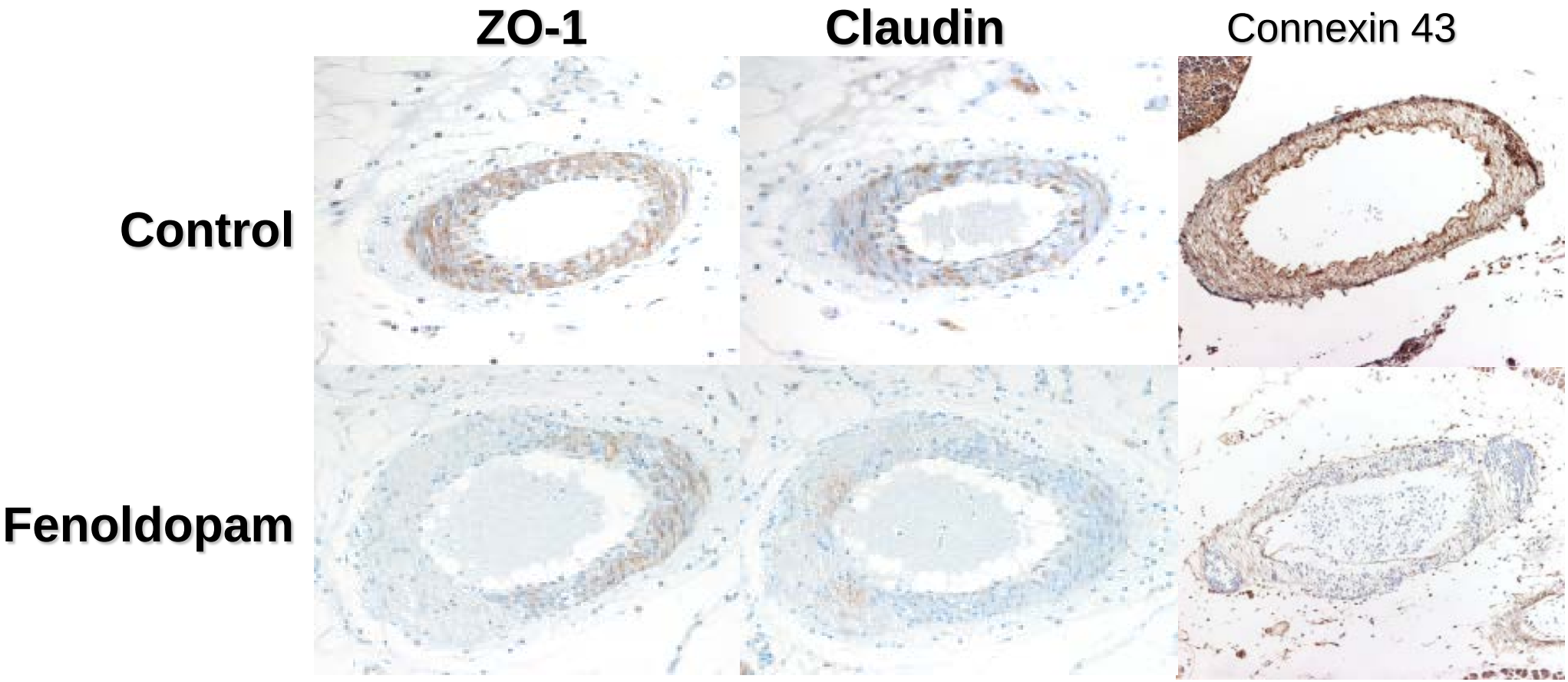
Evaluate Cav-1 expression in vascular injury







Tight & Gap Junctions in Vascular Injury



Cav-1 regulated tight & gap junctions are lost in drug-induced vascular injury

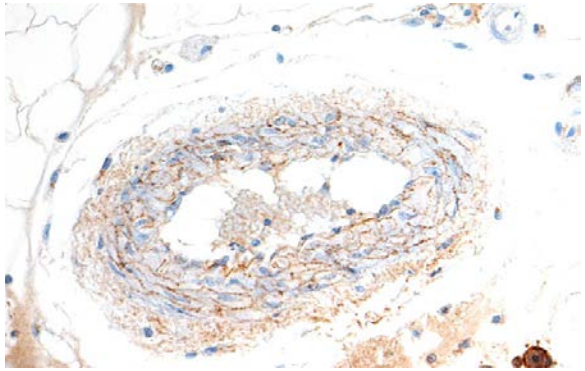
Fenoldopam Treatment and Nitrotyrosine Expression

Nitrotyrosine:

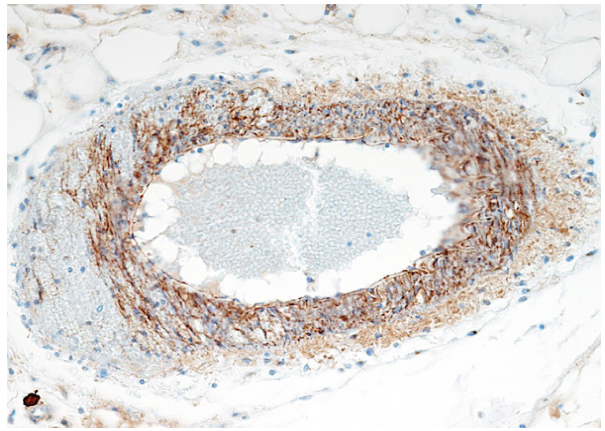
- footprint of peroxynitrite,
- NO-derived free radical produced when NO binds to superoxide
- Can be measured in biological fluids including plasma

Nitrotyrosine

Control



Treated



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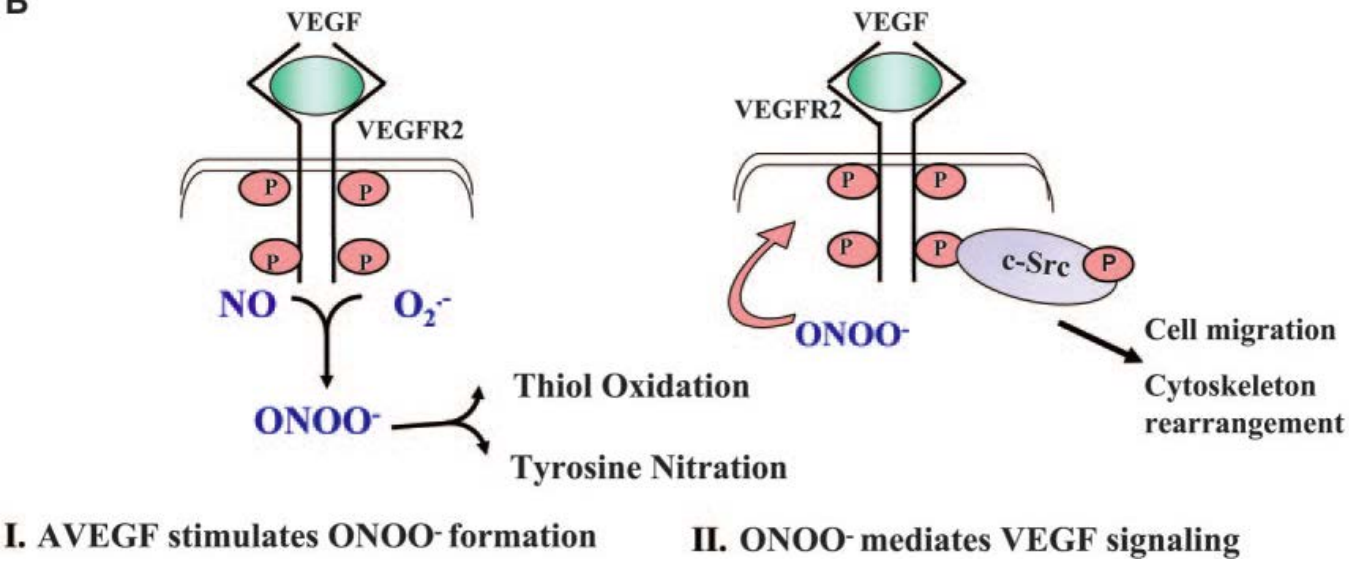


I. AVEGF stimulates $ONOO^-$ formation

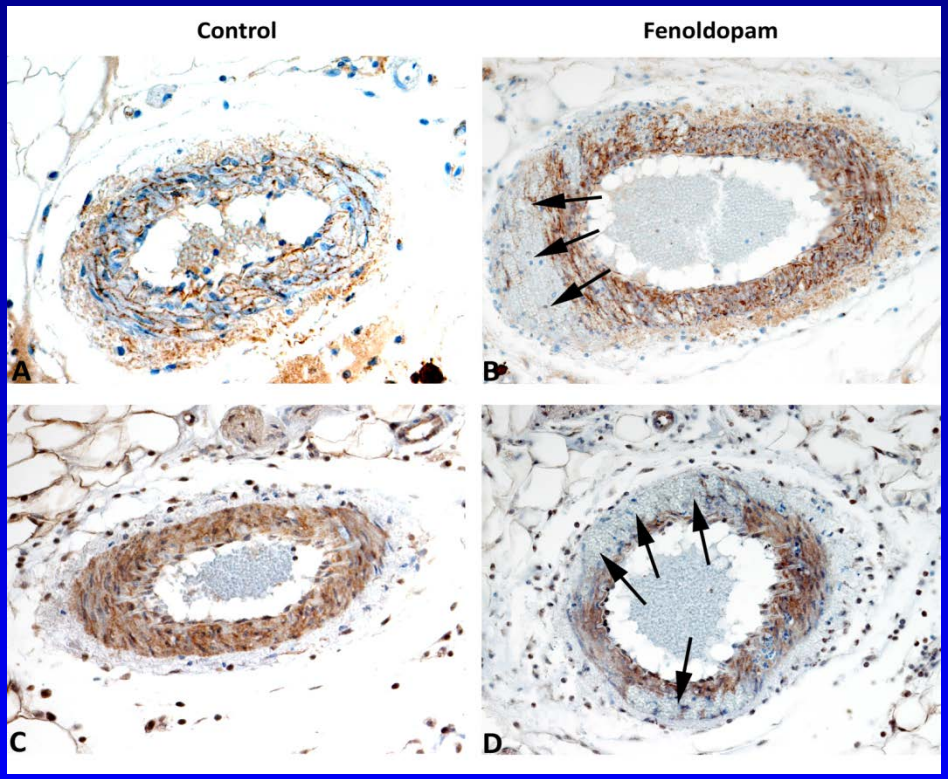
II. $ONOO^-$ mediates VEGF signaling

El-Remessy et al., FASEB 21:2528;2007

Extracellular Superoxide dismutase (ecSOD)

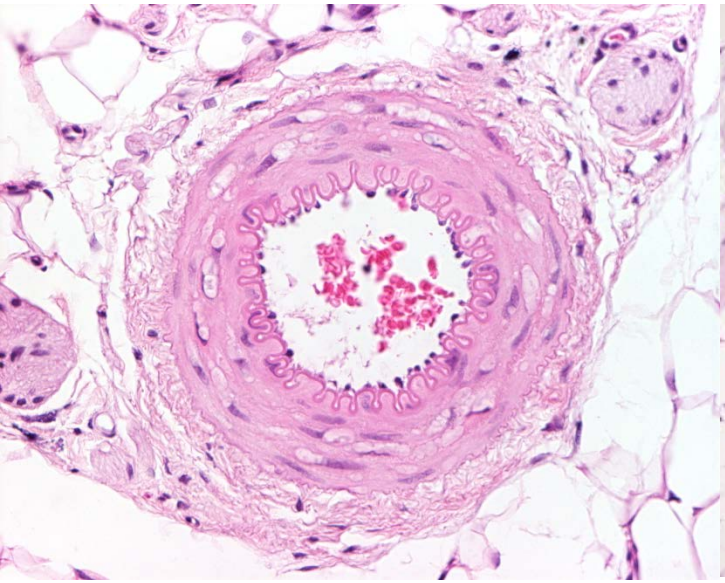
nitrotyrosine →

ecSOD →

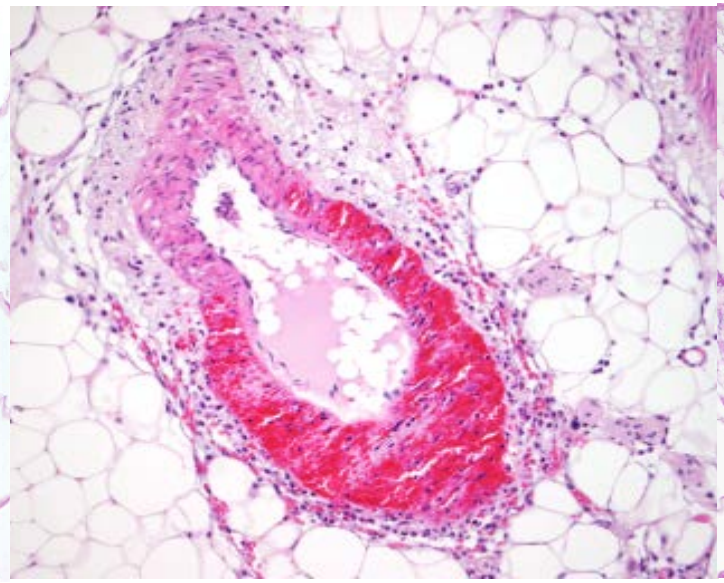


- ✓vessel wall major antioxidant
- ✓~highest in vessel wall (10-fold)
- ✓principal regulator of enothelium-derived NO bioactivity
- ✓SMC major site of synthesis
- ✓can be released into circulation
- ✓protects NO from superoxide
- ✓exogenous NO upregulates ecSOD

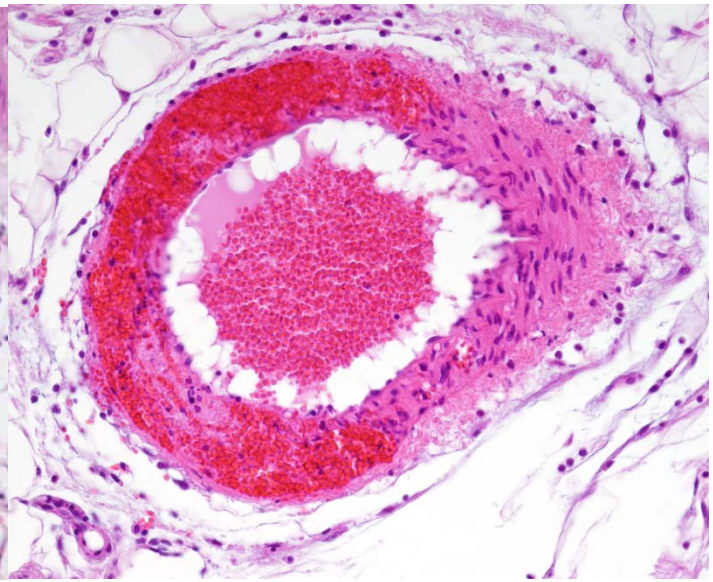
Sodium Nitroprusside (NO donor) vs. Fenoldopam



Control

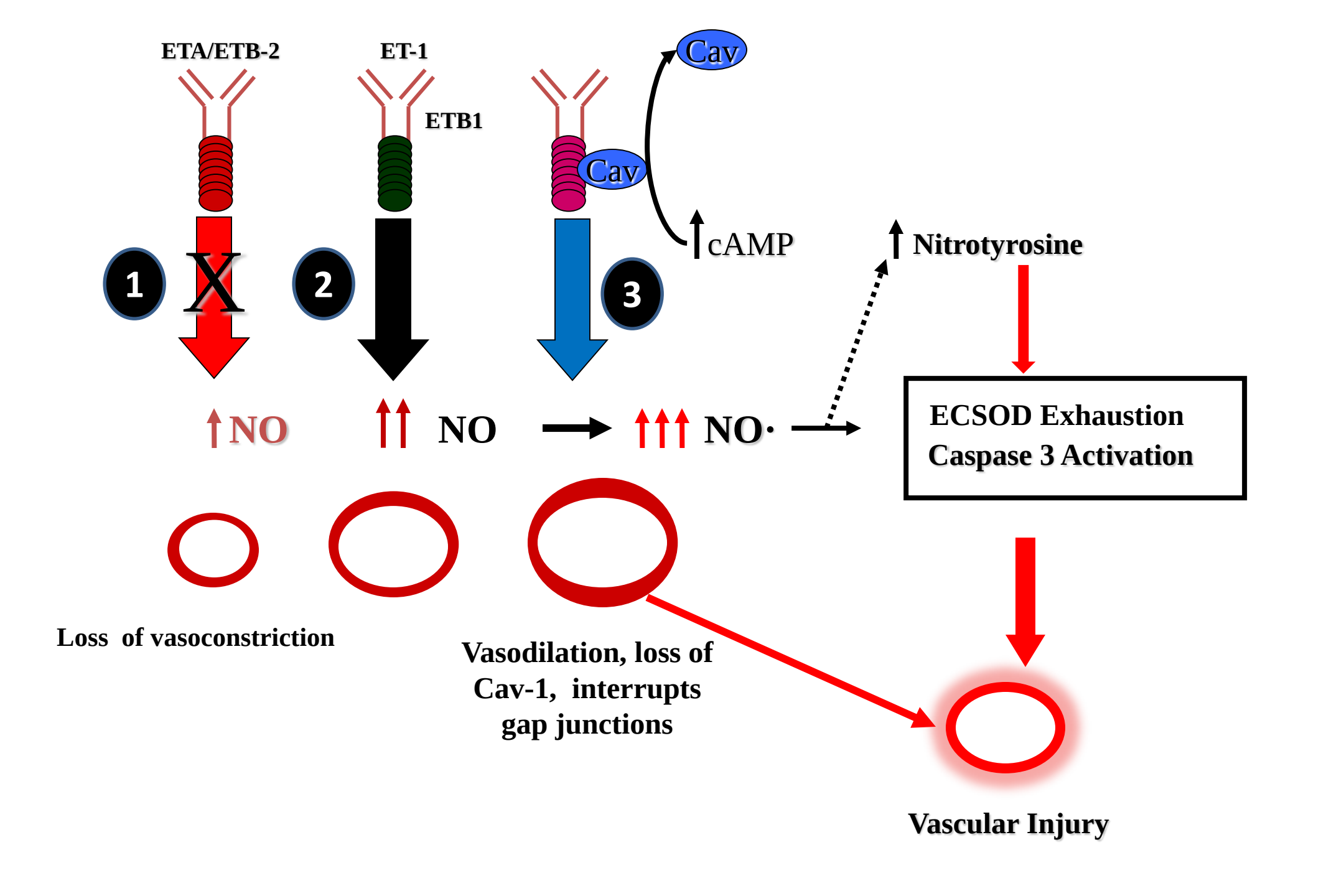


50ug/kg/minute sodium nitroprusside



100μg/kg/minute Fenoldopam

NO induced lesions morphologically indistinguishable from fenoldopam


ETA/ETB-2
ET-1
ETB1
Cav
Cav
cAMP
1
2
3
X
NO
NO
NO·
Nitrotyrosine
ECSOD Exhaustion
Caspase 3 Activation
Loss of vasoconstriction
Vasodilation, loss of Cav-1, interrupts gap junctions
Vascular Injury

Intervention Studies

- Co-administer fenoldopam with
  - L-NAME (a specific NOS inhibitor)
  - Tempol an ecSOD mimetic
  - Evaluate incidence and severity of vascular lesions
- **L-NAME and/or TEMPOL**
  - Intervention strategy not 100% successful
  - Reduced the incidence of fenoldopam –induced vascular injury (50-75%)
  - Significantly reduced the severity of vascular lesions

NOS, CAV-1 Summary

- Dysregulation of NOS pathway plays an important role in drug-induced vascular injury
- Caveolin-1, nitrotyrosine and ecSOD are potential candidate markers for drug-induced vascular injury

Human Relevance

- Species specific response
  - regulation of functional hemodynamics
    - complex regulation by autonomic nervous system (e.g., nitroglycerin; minoxidil) and baroflex mechanisms
  - influence of cardiac anatomy on RCBF
    - dog, pigs, rat
  - vascular wall ecSOD: human >>> dog and rat
    - 10-fold greater antioxidant activity in the vascular extracellular space compared to other tissues
- No clinical evidence of vascular lesions humans
  - several approved drugs cause vascular lesions pre-clinically
  - minoxidil-like vascular lesions not seen in humans
  - successful clinical investigation with ETRA and PDEI

Clinical Evaluation of Blood Flow

- Flow-mediated dilatation (FMD) of the brachial artery assessed by high-resolution ultrasound
  - a noninvasive approach to examine vasodilator function in vivo
  - widely believed to reflect endothelium-dependent and largely nitric oxide-mediated arterial vasodilatation
  - used as a surrogate marker of vascular health.

Vascular Lesions in Dogs

- Dog hypersensitivity to vasodilator-induced hemodynamic changes
  - Marked hemodynamic response compared to humans at comparable exposures
  - Several approved drugs from different classes reproducibly induce this effect in dogs
    - Cardiac glycosides, PDE Inhibitors, ETRAs, Potassium Channel openers, Mixed channel openers, Adenosine agonists, Isoprenaline (pure vasodilator)
  - Dog hemodynamic is regulated differently vs humans with regards to sympathetic vs parasympathetic influence
- Lesion is driven by severe coronary hypertension and tachycardia
  - Clinical objective is to restore MAP to within normal limits not induce hypotension and tachycardia

Key Summary Points

- Sensitivity of dogs to develop vascular lesions as a result of administering vasodilating agents, relates to the marked hypotensive hemodynamic response seen in this species rather than a direct toxic insult.
  - Vascular lesions in PAH patients is unlikely to occur because therapy is aimed at restoring arterial pressure to within normal limits not induce severe hypotension.

- Humans are not likely to develop drug-related arterial lesions similar to the dog during long-term use.
  - Severe cardiovascular pathology has been observed in beagle dogs but **not in man** given minoxidil (Sobota 1980 & 1989), despite considerably greater exposure in man to minoxidil through topical administration and for the treatment of hypertension

- The epidemiological data from man, treated with drugs that cause coronary arterial lesions in dogs, clearly indicate that therapeutic use of these drugs are not associated with an increased human risk of vascular injury
  - Based on these observations, it is safe to conclude that the findings of coronary arterial lesions in dogs can lead to an erroneous and irrelevant extrapolation to man.

Acknowledgements

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  - David Brott*
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