The Endothelium: Pharmacology of Vascular Targets And Reactive Oxygen Injuries

The Infrastructure of our Body: The Superhighways and Back Roads

Generation of Vascular Clotting

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Last Update: June 22, 2021
This Presentation is the First in a Series of Three Topics on:

• The Endothelium – Its Histology and Functions and General Biology

• The Endothelium Signaling Pathways that are at Risk from Ionizing Radiation, and,

• A walk-through the Basic Physics of Radiation Injury and how ionizing energies have underlying uncertainties of the absorbed dose due to the heterogeneity of targets and target vulnerabilities

The Endothelium is one of the Most Vulnerable Tissues to Radiation

The ENDOTHELIUM:
A one cell thick, point of initial drug biodistribution, weighing ~1.5 kg, containing 1.2 trillion targets, covering 400 sq meters (6 tennis courts) of surface area, blood and lymph vessels ....and a miracle of bioengineering.
The ANATOMY and HISTOLOGY:
A one cell thick, point of initial drug biodistribution, weighing ~1.5 kg, containing 1.2 trillion targets, covering 400 sq meters (6 tennis courts) of surface area, blood and lymph vessels ....and a miracle of bioengineering.
A One Cell Thick Network of Cells Serving as a Barrier, First Line Protector, Oxygen/Nutrition Delivery and a Communication Tool

Single Cell array all intricately connected via a variety of “hand holds” each with an important role for operation as a single entity “organ”

Programmed signal cascades from normal operation to immune functions to injury resolution

Tight Junctions: “Claudins/ Occludin

Gap Junctions: “communications” Connexin TMP

Adherens Junctions: “permeabilities” E-caderhin/ catenin

Focal tissue adhesions: Desmoglein

https://www.slideshare.net/deeberto/vascular-endothelium-in-health-and-disease-final
Endothelial Progenitor Cells (EPCs) are Bone Marrow Derived and Differentiate to Specific Roles Via Paracrine Exposures

- Endothelium uniquely contains Weibel-Palade bodies (vWF stores)
- Serves as a blood barrier as well as a multifunctional and permeable paracrine and endocrine delivery functions.
- Involved in immune functions, growth regulation, coagulation and extracellular matrix elements, and modulates blood flow as well as blood vessel tone.
- Pathologies occur following decreased NO or increased endothelin (ET-1)

Pathologies (dysfunctions) fall into 6 main camps:
- Apoptosis, adhesions, lipid accumulations, vasoconstriction, growth, thrombosis

https://www.slideshare.net/SweetyKalantri/endothelial-cell-in-health-disease-seminar
**Reactive Oxygen Species (ROS) Effects:**

- ROS inhibits endothelium-dependent vasodilation pathways, i.e., NO (nitric oxide) and Endothelium-Derived Hyperpolarizing Factor (EDHF) pathways.

- ROS shifts the balance in eicosanoid action from dilatory and antithrombotic to constrictive and thrombotic phenotypes.

- Superoxide anions reduce NO bioavailability, reduces guanylyl cyclase (GC; lowering GTP reduces GDP and thence cGMP) and inactivates Ca-activated K channels ➔ vascular smooth muscle relaxation.

- Peroxynitrites also inhibit GTP, SOD and decreases EDHF activity.

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**Imbalance between generation of reactive ROS and antioxidant defense systems represents the primary cause of endothelial dysfunction.** Endothelial dysfunction can lead to vascular damage. Endothelial activation (aka “injury”) is characterized by becoming a pro-inflammatory and pro-thrombotic phenotype of the lining the lumen of blood vessels.

*Maria Angela-Incalz, et al.* Vascular Pharmacology, 100: Jan2018, Pages 1-19

[https://www.slideshare.net/SweetyKalantri/endothelial-cell-in-health-disease-seminar](https://www.slideshare.net/SweetyKalantri/endothelial-cell-in-health-disease-seminar)

Nitric Oxide (NO)-Sensitive Guanylyl Cyclase (NO-sens-GC)

Nitric oxide (NO)-sensitive guanylyl cyclase (NO-sens GC) is generally accepted as the most important receptor for the signaling molecule NO.

- **GC is also known as “soluble” or “cytosolic GC”** though a membrane-bound counterpart does also exist.

- **NO is a physiological activator** that binds to the prosthetic heme group of the GC enzyme (protoporphyrin ring with a central iron atom).

- Two novel classes of GC activators have been identified, YC-1 and YC-1-like substances, that act as NO sensitizers.

- BAY 58-2667 stimulates NO-sens GC independently from NO and also preferentially activates the heme-free form of the enzyme.

- Membrane-bound, peptide-activated GCs are structured like the cytosolic enzymes but are not stimulated by NO.

- NO-sens GC isolated from rat and bovine lung and brain show α₁ and β₁ subunits. However, human variants of the α₁ and β₁ (α₃ and β₃) of NO-sensitive GC represent similar subunits rather than new isoforms.

- YC-1 and BAY 58-2667 represent new classes of activators of NO-sensitive GC.

NO-sens GC is a hemoprotein and presence of the prosthetic heme group is mandatory for the activation of the enzyme by NO. Removal of the heme group abolishes NO-induced activation, which can be restored after a heme structure is reconstituted.

**physiological activations:**
- Ion channels
- Protein kinases
- Phosphodiesterases

- *Is this a route of ROS repair?*

[https://www.ahajournals.org/doi/full/10.1161/01.RES.0000082524.34487.31](https://www.ahajournals.org/doi/full/10.1161/01.RES.0000082524.34487.31)
Regulation of Gene Expression: NO and ANP Paths to Cyclic GMP

**Cyclic GMP**, produced in response to nitric oxide and natriuretic peptides, is a key regulator of vascular smooth muscle cell contractility, growth, and differentiation, and is implicated in opposing the pathophysiology of hypertension, cardiac hypertrophy, atherosclerosis, and vascular injury/restenosis.

**Cytoplasmic soluble guanylate cyclases** (sGCs) are activated by nitric oxide (NO) to generate cGMP. **Receptor guanylate cyclases** (rGCs) are activated by natriuretic peptides [atrial natriuretic peptide (ANP) or B- and C-type natriuretic peptides (BNP and CNP)].

- cGMP effector proteins include cGMP-dependent protein kinase (PKG) I and II, cyclic nucleotide (cNT) regulated ion channels, and phosphodiesterases (PDEs; hydrolyze cGMP and/or cAMP).
- PKG is the major intracellular cGMP target. PKG I is highly expressed in platelets, smooth muscle, glomerular mesangial cells, cardiomyocytes, and many endothelial and neuronal cells.
- PKG II is encoded by a different gene limited expression.
- **Apoptosis, a radiation injury event, is PKG mediated.**


[https://www.ahajournals.org/doi/pdf/10.1161/01.RES.0000103311.52853.48](https://www.ahajournals.org/doi/pdf/10.1161/01.RES.0000103311.52853.48)
Molecular Pathway Processes Involving cGMP Regulation of Gene Expression

**MAP Kinase Phosphatase-1 (MKP-1):**
MKP-1 mRNA expression is increased by NO-releasing agents, ANP, and cGMP analogues in endothelial and smooth muscle cells. MKP-1 dephosphorylates and inactivates the MAP kinases (Erk-1/2, p38, and c-Jun/JNK).

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**Vascular Endothelial Growth Factor (VEGF):**
VEGF mediates angiogenesis and vascular permeability. In normoxic VSMCs NO donors increase basal VEGF mRNA levels in a cGMP-dependent fashion. VEGF expression rises in balloon-injured arterial walls by hypoxia. Treating hypoxic VSMCs or endothelial cells with NO or cGMP decreases VEGF mRNA and NO synthesis inhibition increases VEGF mRNA.

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**VSMC Differentiation/Phenotypic Modulation:**
In response to vascular injury, VSMCs change their state of differentiation from a highly differentiated, “contractile” phenotype to a dedifferentiated “synthetic” phenotype. In the synthetic phenotype they proliferate, migrate, and produce extracellular matrix proteins (i.e. osteopontin and thrombospondin), thus contributing to neointima formation after vascular injury.

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CgMP decreases expression of cell cycle-promoting genes, such as cyclin A, D1, and E.

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Pro- and antiapoptotic effects of cGMP have been described in different cell types. In VSMCs, cardiomyocytes, and endothelial cells, NO, natriuretic peptides, and cGMP analogues increase apoptosis, and the effect appears to be PKG mediated.

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ET-1 is a potent vasoconstrictor and mitogenic peptide produced by proteolytic cleavage from an inactive precursor and secreted by endothelial cells.

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**Regulation of Cell Proliferation:**
Depending on the cell type, cGMP can have pro- or antiproliferative effects. In VSMCs, mesangial cells, and various fibroblasts, cGMP inhibits proliferation, and the effect is mostly mediated by PKG, although it may involve PKA cross-activation under some conditions.

https://www.ahajournals.org/doi/10.1161/01.res.0000103311.52853.48
Endothelial dysfunction

**Endothelial dysfunction** is a systemic pathological state of the endothelium (the inner lining of blood vessels) and can be defined as an imbalance between vasodilating and vasoconstricting substances produced by the endothelium. Endothelial dysfunction can result from and/or contribute to several disease processes, as occurs in

- hypertension
- atherosclerosis
- diabetes
- septic shock

Endothelial dysfunction is a major pathophysiological mechanism that leads towards coronary artery disease and other atherosclerotic diseases.

### Modulators of Endothelin -1

<table>
<thead>
<tr>
<th>ETα receptor</th>
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<tbody>
<tr>
<td>Antagonist-</td>
</tr>
<tr>
<td>BQ-123</td>
</tr>
<tr>
<td>FK 139317</td>
</tr>
<tr>
<td>TTA 386</td>
</tr>
<tr>
<td>Ambrisentan</td>
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<tr>
<td>Sitaxentan</td>
</tr>
<tr>
<td>Atrasentan</td>
</tr>
<tr>
<td>Zibotentan</td>
</tr>
<tr>
<td>Non peptide selective antagonist-</td>
</tr>
<tr>
<td>PD 151242 , L 754142 ,PD 156707 ,</td>
</tr>
<tr>
<td>BMS 182874</td>
</tr>
<tr>
<td>SB 234551</td>
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<th>ETβ receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist-</td>
</tr>
<tr>
<td>Sarafotoxin (aα1-ET1)</td>
</tr>
<tr>
<td>BQ 3020</td>
</tr>
<tr>
<td>IRL 1620</td>
</tr>
<tr>
<td>Antagonist-</td>
</tr>
<tr>
<td>BQ 788, RO468443</td>
</tr>
<tr>
<td>IRL2500, A192621</td>
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<tr>
<td>RES 7011</td>
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</tbody>
</table>

**Antagonist- block both ETα & ETβ**

- TAK 044
- Bosentan
- SB 209670
TNF and DAMP/PAMP Processes Involving NF-KB Regulation of Gene Expression In Endothelial Cell (EC) Activation

NF-KB = [ p50-p65-IkBα ] complex

We will cover the Notch, NF-KB and other pathways more in Part 2.
Healthy Endothelium Reduces ROS, Increases PGI2, and Decreases Nitrotyrosine

Oxidative stress is involved in the pathogenesis of microangiopathic and macroangiopathic complications. In diabetics, elevated nitrotyrosine is seen.

NOTE: Nitrotyrosine is a biomarker for 'nitrating species' rather than being specific for Peroxynitrite (ONOO⁻). There is interesting evidence that AT-1 receptor blockers (i.e., Irbsartan) can also work as intracellular antioxidants by reducing nitrotyrosine formation.

https://link.springer.com/article/10.1007/s00125-004-1487-3
https://www.slideshare.net/deeberto/vascular-endothelium-in-health-and-disease-final
ROS Species and the Cascade of Reactions that Control Exposure

NO is a stored but limited reservoir and a corrective path from free radical oxygen species.

NO synthesis is part of an armament of several control paths and reactions that keep ROS under control.
The standard path is ROS $\rightarrow$ SOD $\rightarrow$ peroxide $\rightarrow$ (catalase or glutathione peroxidase (GP) $\rightarrow$ Water
Other reactions that support ROS reduction:

**Carboxy-PTIO:** Carboxy-PTIO (a K salt; a water soluble and stable nitric oxide radical scavenger) can have a rapid reaction with nitric oxide (NO) to produce nitrogen dioxide (NO$_2$). (oxyhemoglobin scavenges similarly)

**Fenton Reaction:** Deferoxamine is a drug to ameliorate free hydroxyl ions by complexing them with Fe$^{+3}$

**Haber-Weiss Reaction:** Controls peroxide and free radical oxygen species to be made into hydroxyl ions (requires Cu or Fe) $\rightarrow$ then renal elimination supported by mannitol (mannitol, an osmotic diuretic, is used in the perioperative setting in the belief that it exerts reno-protective properties)
### Vasoactive Factors – Dilatory and Constrictive

#### Vasodilation Factors:
- NO (nitric oxide)
- Prostacyclin (PGI2; along with PGE2)
- Endothelium derived hyperpolarizing factor (EDHF)

#### Vasoconstrictive Factors:
- Thromboxane A2 (TXA2)
- Endothelin-1 (ET-1)
- Prostaglandin H2 (PGH2)
- ROS (when NO release is nulled or inhibited)

### Vasodilators

<table>
<thead>
<tr>
<th>Nitric Oxide</th>
</tr>
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<tbody>
<tr>
<td>maintenance of basal vasodilator tone of the blood vessels</td>
</tr>
<tr>
<td>reduce platelet and monocyte stickiness</td>
</tr>
<tr>
<td>Reduce oxidation of LDL</td>
</tr>
<tr>
<td>Reduce release of superoxide free radicals.</td>
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<table>
<thead>
<tr>
<th>Prostacyclin</th>
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<tbody>
<tr>
<td>It is synthesized from arachidonic acid</td>
</tr>
<tr>
<td>It mainly involve PGI2 and PGE2</td>
</tr>
<tr>
<td>It relaxes the vascular smooth muscle</td>
</tr>
<tr>
<td>It helps in the release of NO from endothelium</td>
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<table>
<thead>
<tr>
<th>Endothelium derived hyperpolarization factor</th>
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<tbody>
<tr>
<td>Relax smooth muscle</td>
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### Synthesis Requires: Ca+2 calmodulin; No receptor for NO

### Vasoconstrictors

<table>
<thead>
<tr>
<th>Endothelin-1</th>
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<tbody>
<tr>
<td>ET-1 exerts vasoconstrictor actions through stimulation of ETA receptors in vascular smooth muscle and vasodilator Actions through stimulation of ETB receptors in endothelial cells.</td>
</tr>
<tr>
<td>Stimulates cell proliferation.</td>
</tr>
<tr>
<td>increase the expression of several genes,</td>
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<table>
<thead>
<tr>
<th>Prostaglandins</th>
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<tbody>
<tr>
<td>It involves PGH2</td>
</tr>
<tr>
<td>It is a vasoconstrictor.</td>
</tr>
<tr>
<td>It is precursor for prostanoids.</td>
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<tbody>
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<td>ROS</td>
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<tr>
<td>free radical inhibit the release of NO and leads to vasoconstriction.</td>
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Vasoactive Factors – Dilatory and Constrictive: The Role of NO

Types of Nitric Oxide Synthase (NOS):
As a biogas, NO rapidly crosses cell membranes to activate the intracellular target;
• NOS 1 (a NOS or n NOS; 155 kD)
  • Located at central and peripheral neuronal cells
  • Brain, spinal cord, platelets
  • Ca+2 dependence for neuronal communications
  • Constitutive

• NOS II (inducible; I NOS; 125 kD)
  • Most nucleated cells (esp, macrophages)
  • Independent of intracellular Ca+2
  • Regulation depends on novo synthesis
  • Inducible in the presence of inflammatory cytokines and bacterial liposaccharides

• NOS III (c NOS or e NOS; 135 kD)
  • Present on vascular endothelial cells and neuronal cells
  • Ca+2 dependent
  • Vascular regulation (dialation)

• Bacterial NOS (bNOS)
  • Gm + bacteria; defense against antibiotics

Biologic Role of NO:
• Relaxation of vascular smooth muscle cells
• Inhibition of platelet aggregation and adhesion
• Active role in brain long term memory
• Vasodilation
• Reduces leukocyte adhesion/ rolling
• Potent stimulant of cell division, maturation and differentiation/
• Wound healing and cell repair
**Endothelium Derived Hyperpolarizing Factor (EDHF):**
- Not one factor alone
- The endothelium mediates relaxation as well as contraction
- Endothelium-dependent contractions are from physical and chemical stimuli (i.e., hypoxia pressure, stretch, etc), autocoids, local and circulating hormones
  - Endothelin-1
  - Thromboxane A2 (TXA2)
  - Prostaglandin H2 (PGH2)
  - Angiotensin II
  - and, Reactive Oxygen Species (ROS); ROS is also a [potent driver or EDGFs (E-D growth factors)]
  - **Endothelium-derived hyperpolarizing factors** (EDHFs), like H$_2$O$_2$ can activate PKG1α in a cyclic guanosine monophosphate-independent manner

**The Biology of EDHF:**
The endothelium controls vascular tone by:
- Releases NO and prostacyclin for hyperpolarization of the underlying smooth muscle
- Key players: Arachidonic acid metabolites derived from COX, LOX and CytP450 pathways, H2O2, CO, H2S and peptides released by endothelial cells
- These activate **different families** of K channels leading to hyperpolarization of SMCs and lead to relaxation/dilation


ENDOTHELIN-1 (ET-1):

- ET-1 was first identified in 1988 as an endothelial peptide with both vasoconstrictive and vasodilatory properties.
- Inflammatory mediators, growth factors, thrombin, cytokines, Angiotensin II, and ROS can promote ET-1 synthesis.
  - ACE2 receptors are prominent on endothelium and are the major receptor for COVID-19.
  - ACE2: Ang II (ROS generator) ➔ Ang 1,7 (ROS brake)
- NO inhibits ET-1 synthesis (also NO donors).
- ET-1 is mostly secreted by vascular endothelium and is the prominent isoform and most potent vasoconstrictor known.
  - isoforms include: ET-1, ET-2 and ET-3.
- Endothelin receptors are four types: ETA, ETB1, ETB3 and ETc.
- Binding to ETA mediates vasoconstriction via Ca release.
- ETB1 mediates vasodilation ➔ release of NO (feedback loop).
- ETB3 mediates vasoconstriction.
- ETc is not yet defined as to its exact function.

**Endothelial cell production of ET-1:**

<table>
<thead>
<tr>
<th>PrePro-ET-1</th>
<th>Pro(Big) ET-1</th>
<th>ET-1</th>
<th>released</th>
<th>(endothelial cell) ET-B1</th>
<th>NOS activation</th>
<th>NO</th>
<th>[cAMP]</th>
<th>Dilate</th>
</tr>
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<tbody>
<tr>
<td>203 aa</td>
<td>39 aa</td>
<td>21 aa</td>
<td>&quot;</td>
<td>(smooth muscle cell) ETA and ET-B3</td>
<td>cell [Ca]</td>
<td>Constrict</td>
<td></td>
<td></td>
</tr>
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https://cvphysiology.com/Blood%20Pressure/BP026
https://cvphysiology.com/Blood%20Flow/BF012
ET\(_A\) and ET\(_B\) receptors are coupled to a Gq-protein and receptor activation leads to the formation of IP\(_3\), which causes the release of calcium by the sarcoplasmic reticulum (SR) and increased smooth muscle contraction/vasoconstriction.

When ET-1 binds to the endothelial ET\(_B\) receptors, the formation of nitric oxide (NO) is stimulated. In the absence of smooth muscle endothelin receptor stimulation, this NO causes vasodilation.

https://cvphysiology.com/Blood%20Flow/BF012

https://www.jbc.org/action/showPdf?pii=S0021-9258%2819%2984125-X
Tight Junction Proteins and Signaling Pathways in Cancer and Inflammation: A Functional Crosstalk

Tight Junction proteins signal to the cell interior either directly or through recruiting other signaling molecules to regulate cell proliferation, migration, survival and differentiation. Inflammatory cells generate reactive oxygen species and proinflammatory mediators which may enhance the mutation rate of cells, induce DNA damage and increase genomic instability.

**The GI System is also a specialized endothelium:**
In IBD, Tight Junction proteins change in expression and localization which causes segment-specific alterations in paracellular barrier and channel functions. These changes generally result in increased paracellular transport of solutes and water, typically mediated by up-regulated claudin-2 and down-regulated barrier forming claudins.

Disruption of the epithelial tight junctions can increase intestinal permeability, as well as possibly damage the intestinal barrier by forming tissue lesions and punctures that could lead to a leaky intestinal epithelium. Localized presence of reactive oxygen species can disrupt these functional junctures by denaturing processes.

Bhat> et al., 2019; Tight Junction Proteins and Signaling Pathways;
The Endothelium and Signaling Pathways: SUMMARY

The Endothelium is a major controller to our physiology and homeostasis. Like any highway network, it requires trafficking of components, opportunity to correct local environments and repair itself when damaged.

The histology reveals a thin squamous “tile-like” layout that at “rural roads” has multiple fenestration to allow for fluids to reach end tissues. The entire endothelium has “fit for purpose” histologic patterns related to

The chemical signaling that predominates is Nitric Oxide (NO) and it serves FOUR purposes:
• vasotone, ROS control, reduce LDL oxidations, and reduce platelet and monocyte stickiness.
ET-1 is mostly secreted by vascular endothelium
• Inflammatory mediators, hypoxia, vascular sheer stress and ROS can promote ET-1 synthesis
• NO inhibits ET-1 synthesis
• Overall balance if “tone” and dilation/constriction
• PrePro-ET-1 ➔ Pro(Big) ET-1 ➔ ET-1 is an important path
The Endothelium and Signaling Pathways: SUMMARY

The Endothelium is a major controller to our physiology and homeostasis. Tight Junction proteins that signal to the cell interior either directly or through recruiting other signaling molecules, are important variable in assessing endothelial damage. Their disruption causes errors in regulating cell proliferation, cell migration, error repair for survival and even failure of differentiation where replacement strategies fail.

NO synthesis is part of an armament of several control paths and reactions that keep ROS under control. The standard path is ROS $\rightarrow$ SOD $\rightarrow$ peroxide $\rightarrow$ (catalase or glutathione peroxidase (GP) == Water

$\Rightarrow$ Other reactions that support ROS reduction: Fenton Rx, Haber-Weiss Rx and the

In the GI Tract: Disruption of the epithelial tight junctions can increase intestinal permeability, as well as possibly damage the intestinal barrier by forming tissue lesions and potential exists for septic events.
What Happens When Endothelium is Exposed to Damaging Doses of Radiation?
The Endothelium: Part 2.

The Complex Mix of GAGs, NF-κβ, Anti-Phospholipid Antibodies, Notch Signaling, and much more

Injury to the Endothelium is a Complex Array of Processes to Minimize Injuries and Support Repair

Oxidative injury and Generation of Anti-Phospholipid Ab

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