

# Redox Physiology

**Interplay of oxidative stress, inflammation, lipid, and cell death signaling pathways during tissue injury and regeneration in vivo: implications for physiology/pathology**

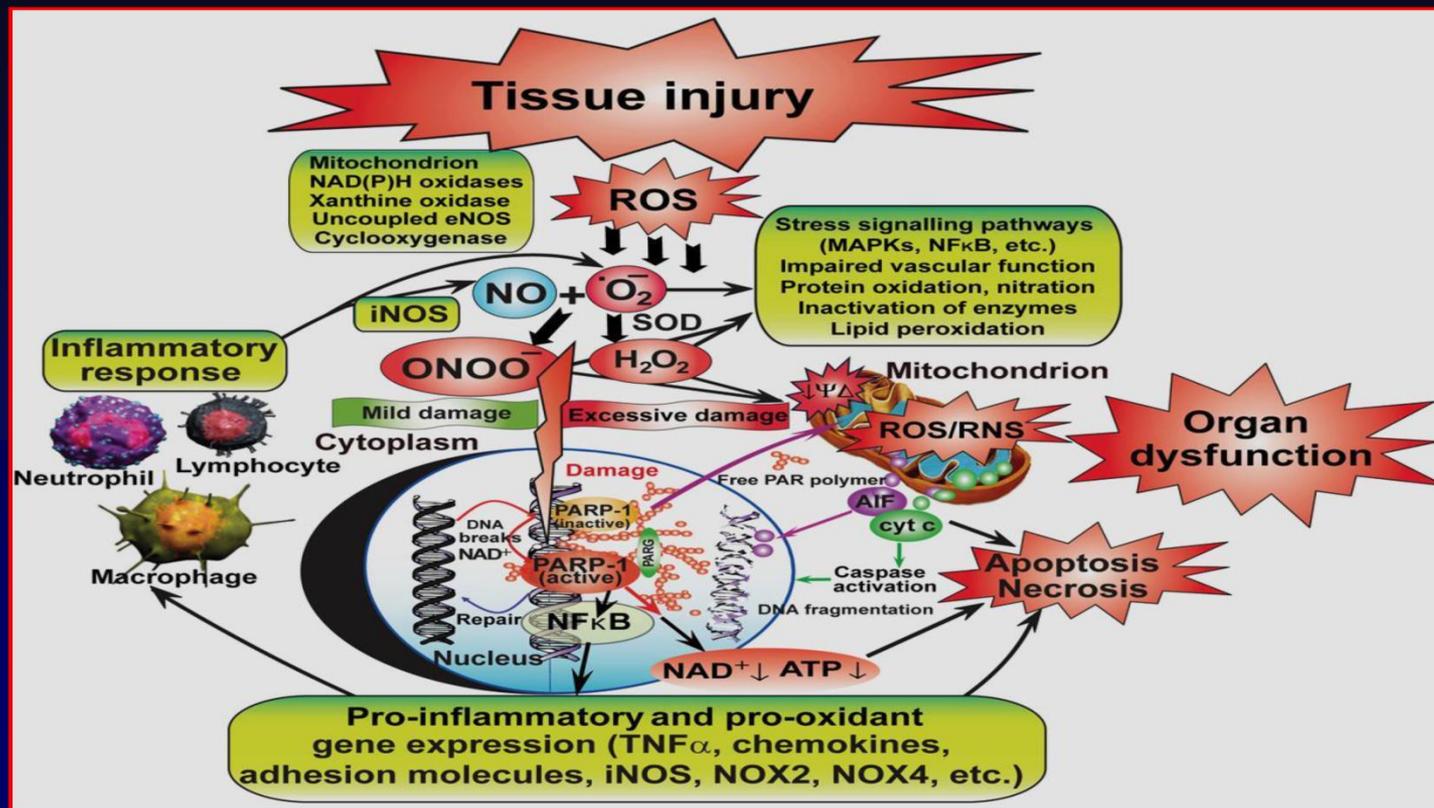
**Pal Pacher M.D., Ph.D., F.A.H.A., F.A.C.C.**

*Section on Oxidative Stress Tissue Injury, Laboratory of Physiological Studies, National Institutes of Health, NIAAA, Bethesda, MD, U.S.A.*



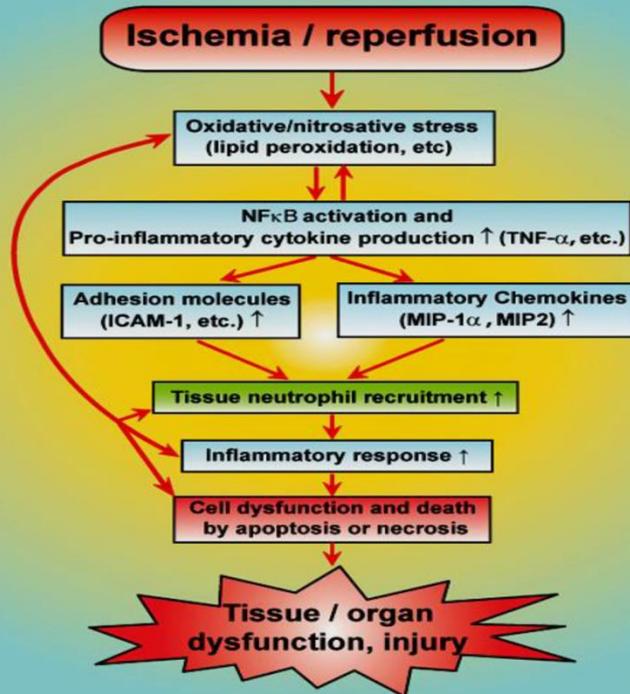
# Oxidative/Nitrosative Stress

Interplay of oxidative/nitrosative/nitrative stress, inflammation and cell death pathways in tissue injury



# Ischemia/Reperfusion Injury

**Interplay of oxidative/nitrative stress, inflammation with cell death in tissue injury**

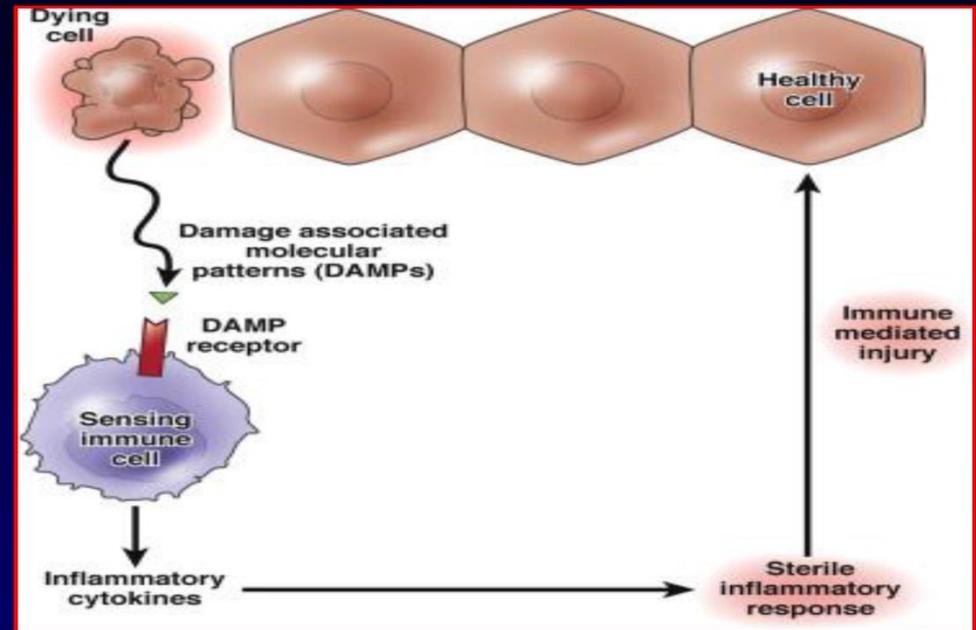
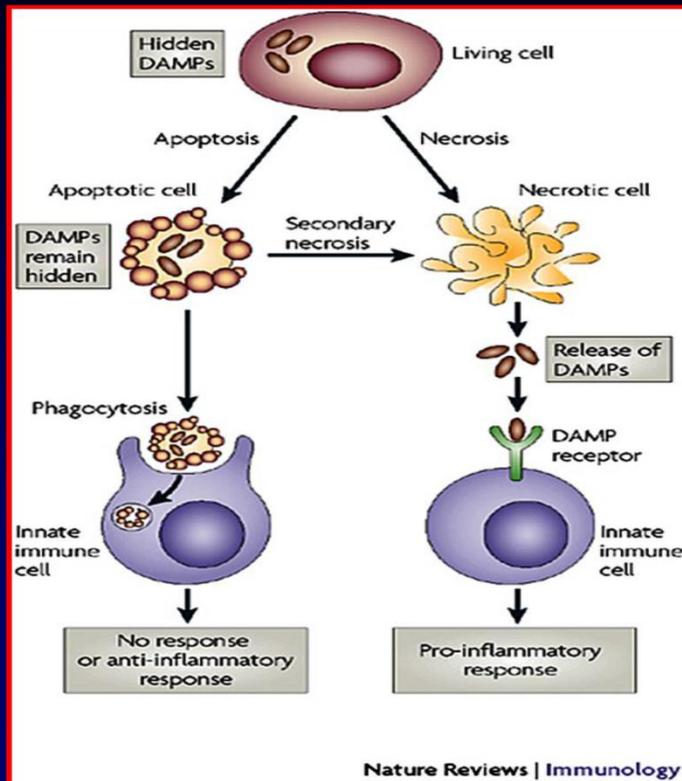


**Ischemia/reperfusion (I/R) injury:**

- myocardial infarction,
- stroke,
- organ transplantation,
- vascular surgery,
- hemorrhagic shock,
- etc.

# Cell Death and Sterile Inflammation

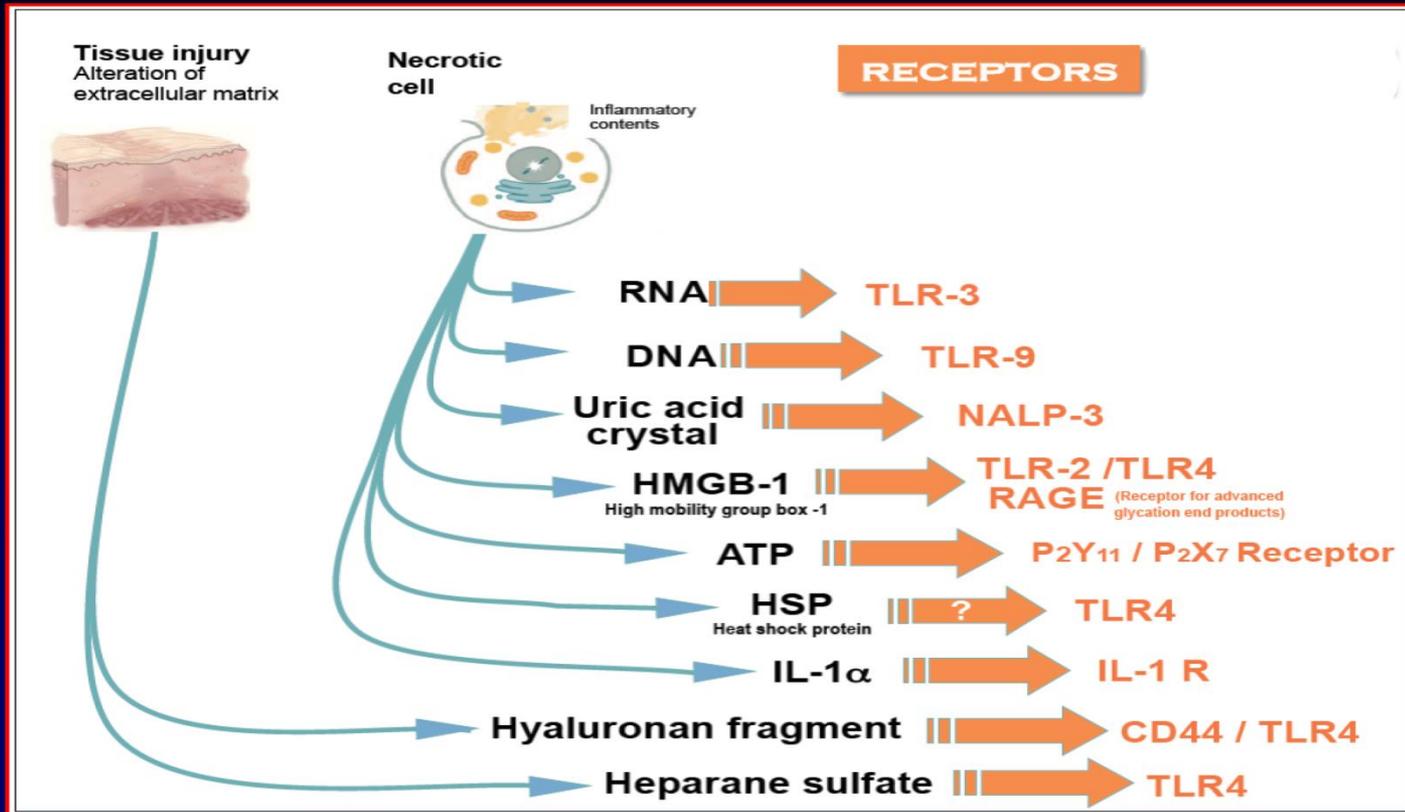
**Damage/danger associated molecular pattern molecules (DAMPs/alarmins), link cell death and sterile inflammation**



*Kubes and Mehal Gastroenterology 2012*

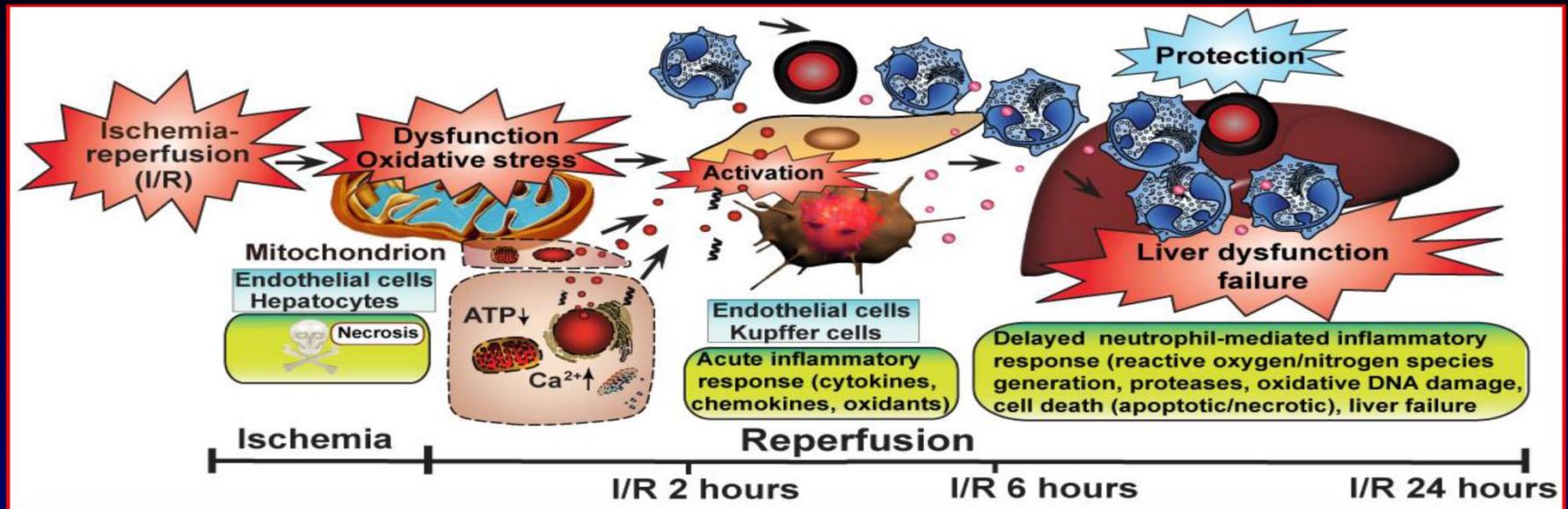
# Pattern Recognition Receptors

Various DAMPs released by injured tissues and necrotic cells activate innate immune response through different pattern recognition receptors (PRRs)



# Hepatic Ischemia/Reperfusion Injury

**Hepatic ischemia/reperfusion (I/R) injury: simplified**



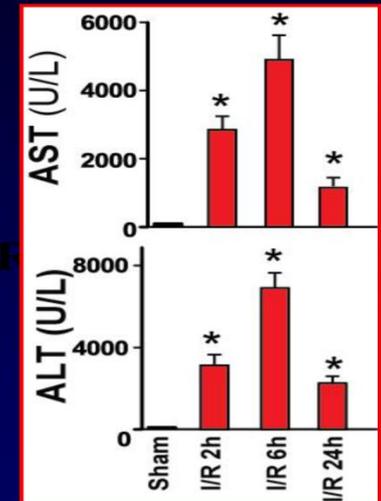
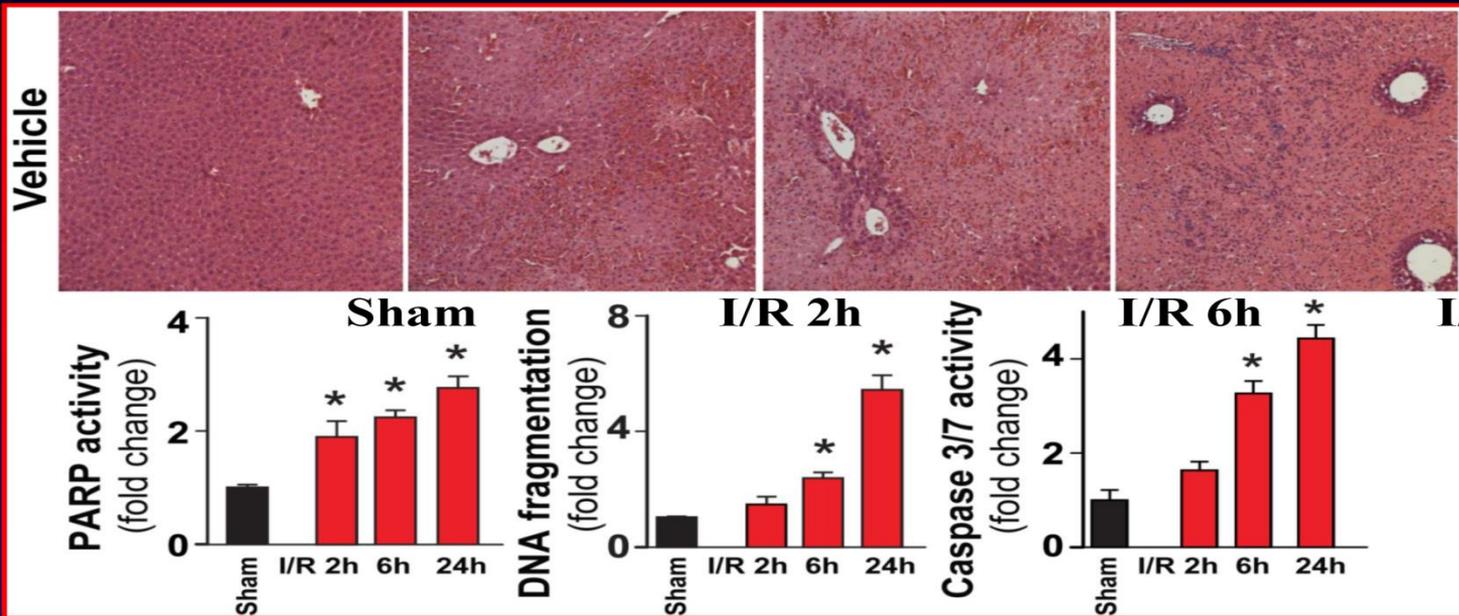
*Mukhopadhyay et al. FRBM 2012*

Mouse model of partial (70%) hepatic ischemia (1h)/reperfusion (2, 6 and 24 hours) injury

# Increased Cell Death

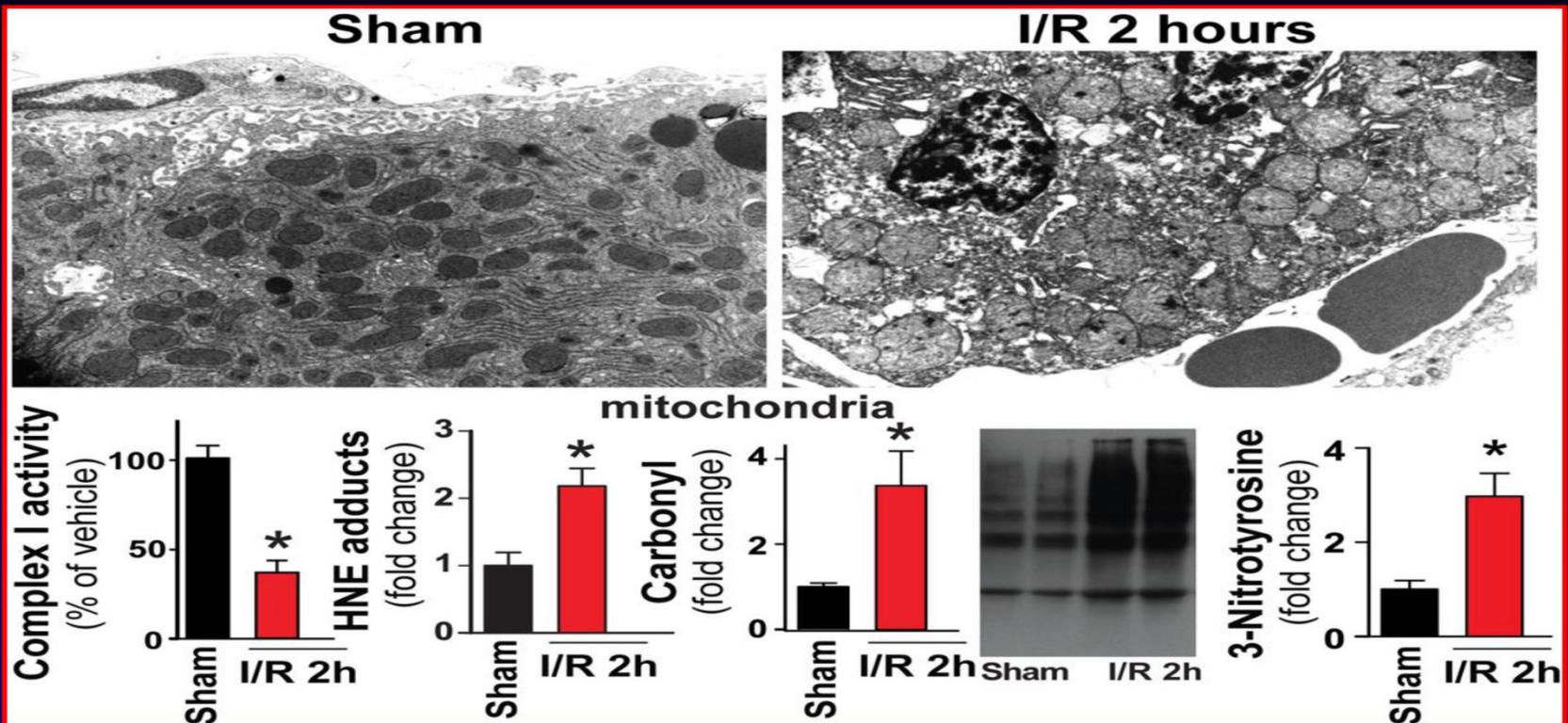
## Hepatic I/R injury: **increased cell death**

Mouse model of partial (70%) hepatic ischemia (1h)/reperfusion (2, 6 and 24 hours) injury



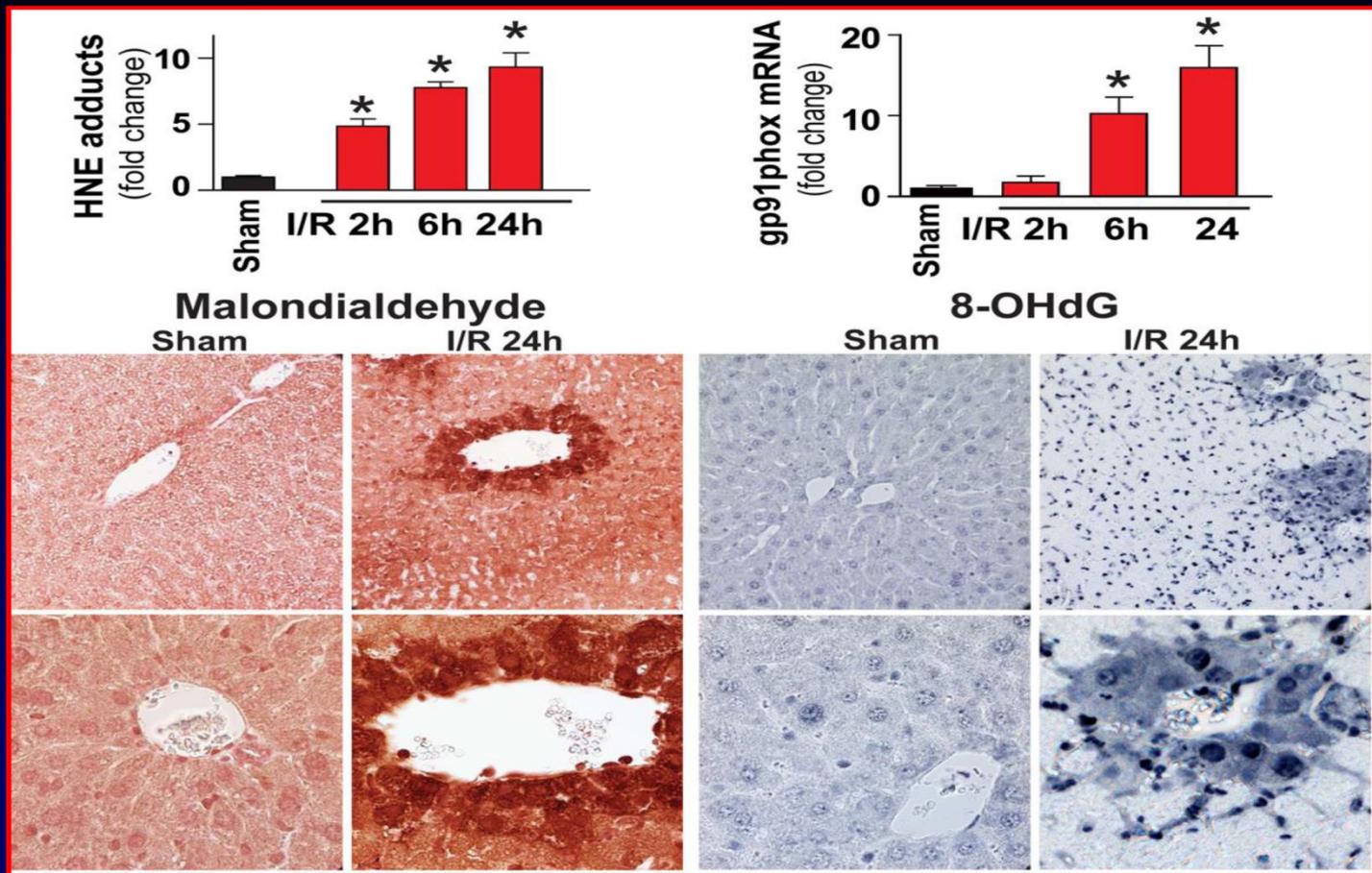
# Early mitochondrial dysfunction and oxidative/nitrative stress

Hepatic I/R injury: **early mitochondrial dysfunction and oxidative/nitrative stress**



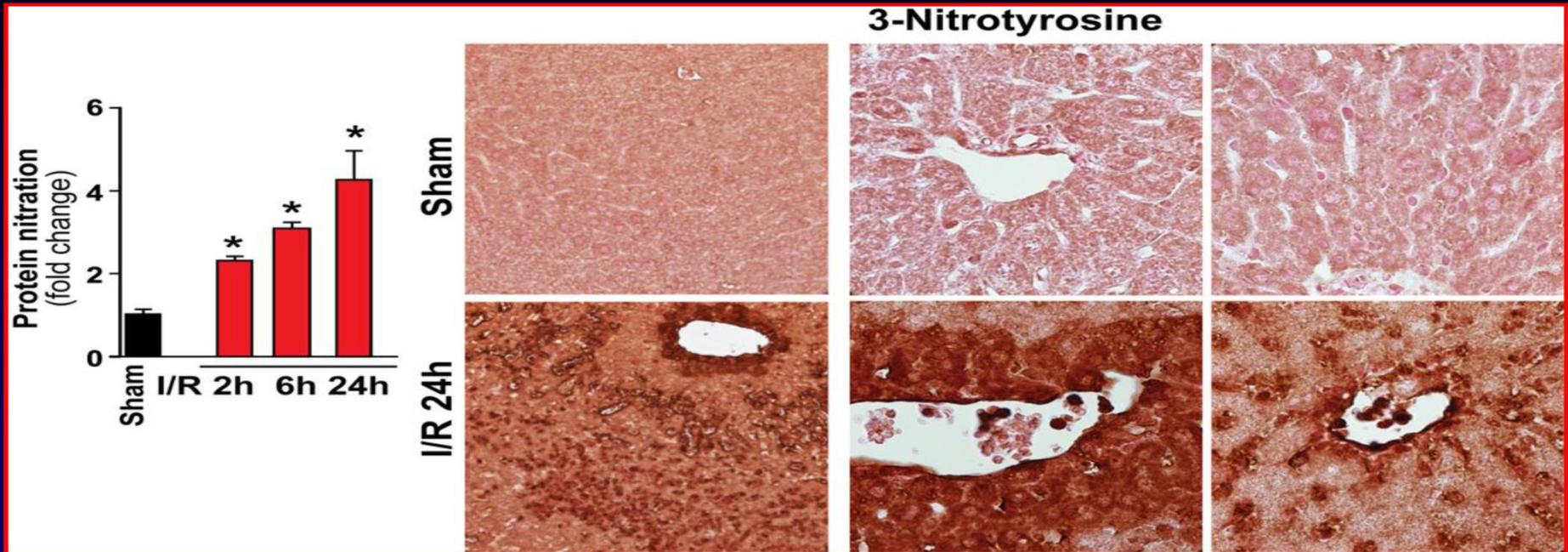
# Increased Oxidative Stress

Hepatic I/R injury: time-dependent **increase in oxidative stress**



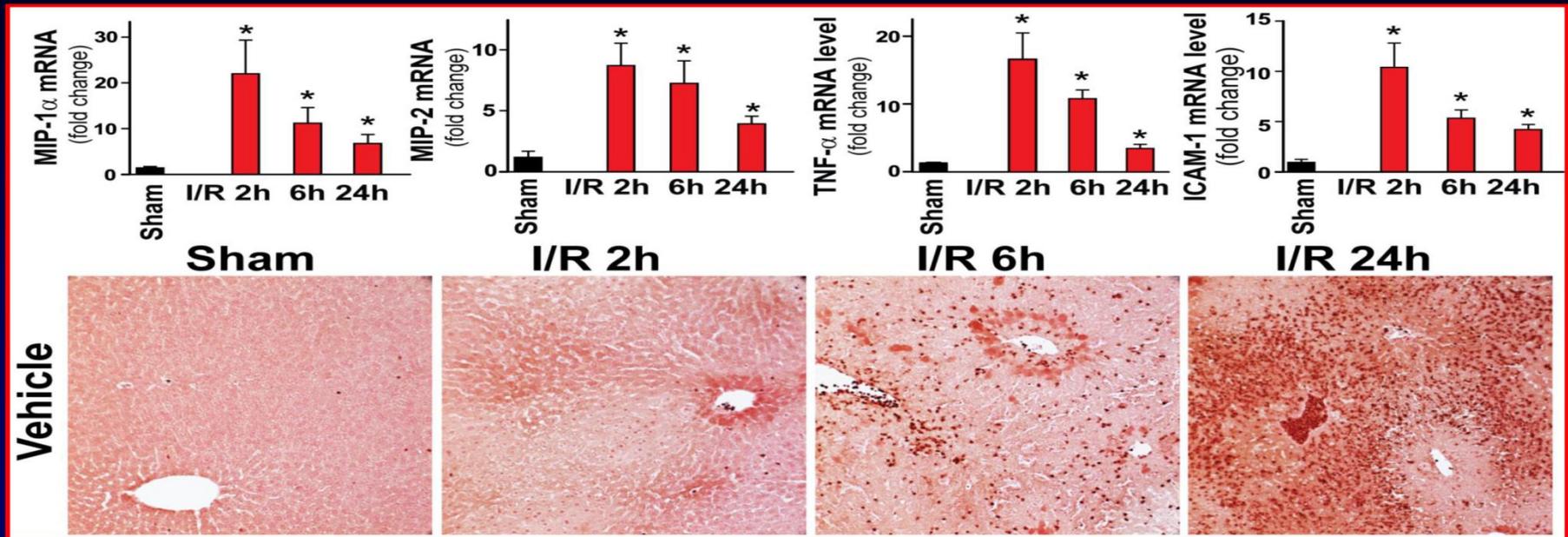
# Increased Nitritive Stress

Hepatic I/R injury: **increased nitritive stress**



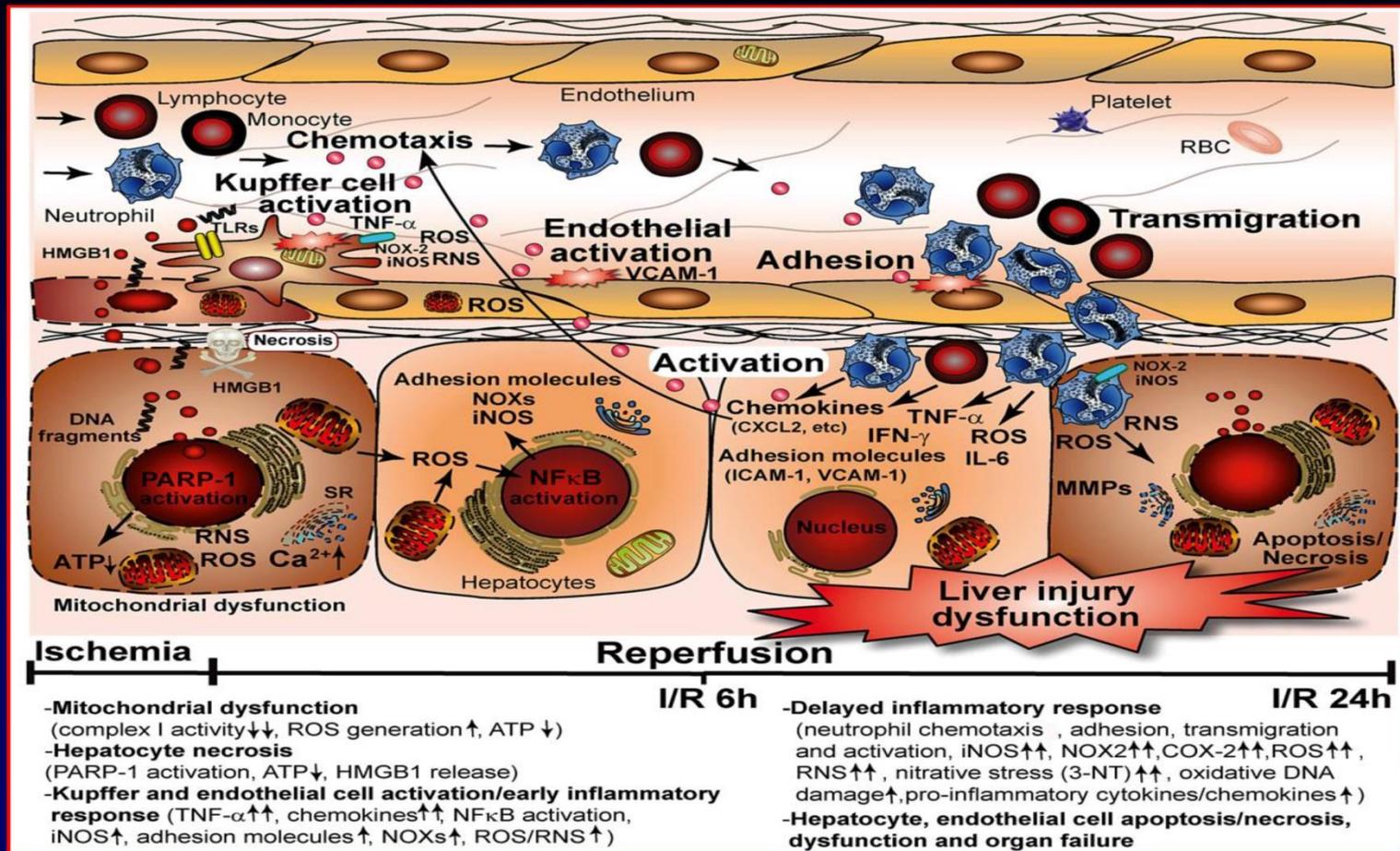
# Increased acute inflammatory response and delayed neutrophil infiltration

**Hepatic I/R injury: increased acute inflammatory response and delayed neutrophil infiltration**



# Mechanisms, Complexity

## Hepatic I/R injury: mechanisms, complexity



# Cannabis sativa (marijuana)

**Cannabis sativa (marijuana): most ancient medicinal plant used in humans, reach source of antioxidant and anti-inflammatory agents**

Review

Cell  
PRESS

## Non-psychoactive plant cannabinoids: new therapeutic opportunities from an ancient herb

Angelo A. Izzo<sup>1,4</sup>, Francesca Borrelli<sup>1,4</sup>, Raffaele Capasso<sup>1,4</sup>, Vincenzo Di Marzo<sup>2,4</sup> and Raphael Mechoulam<sup>3</sup>

<sup>1</sup>Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy

<sup>2</sup>Institute of Biomolecular Chemistry, National Research Council, Pozzuoli (NA), Italy

<sup>3</sup>Department of Medicinal Chemistry and Natural Products, Hebrew University Medical Faculty, Jerusalem, Israel

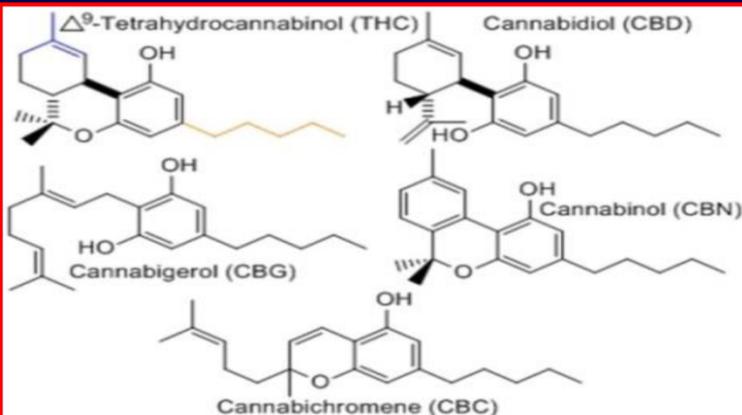
<sup>4</sup>Endocannabinoid Research Group, Italy

Proc. Natl. Acad. Sci. USA  
Vol. 95, pp. 8268–8273, July 1998  
Medical Sciences

## Cannabidiol and (–) $\Delta^9$ -tetrahydrocannabinol are neuroprotective antioxidants

A. J. HAMPSON<sup>\*†</sup>, M. GRIMALDI<sup>‡</sup>, J. AXELROD<sup>\*</sup>, AND D. WINK<sup>§</sup>

<sup>\*</sup>Laboratory of Cellular and Molecular Regulation, National Institutes of Mental Health, Bethesda, MD 20892; <sup>†</sup>Laboratory of Adaptive Systems, National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892; and <sup>§</sup>Radiology and Biology Branch, National Cancer Institute, Bethesda, MD 20892



**Cannabis sativa:**  
**421 chemical compounds,**  
**80 terpeno-phenols not detected**  
**in any other plants**



UN FARMACO A BASE DE CANNABIS

**SATIVEX**

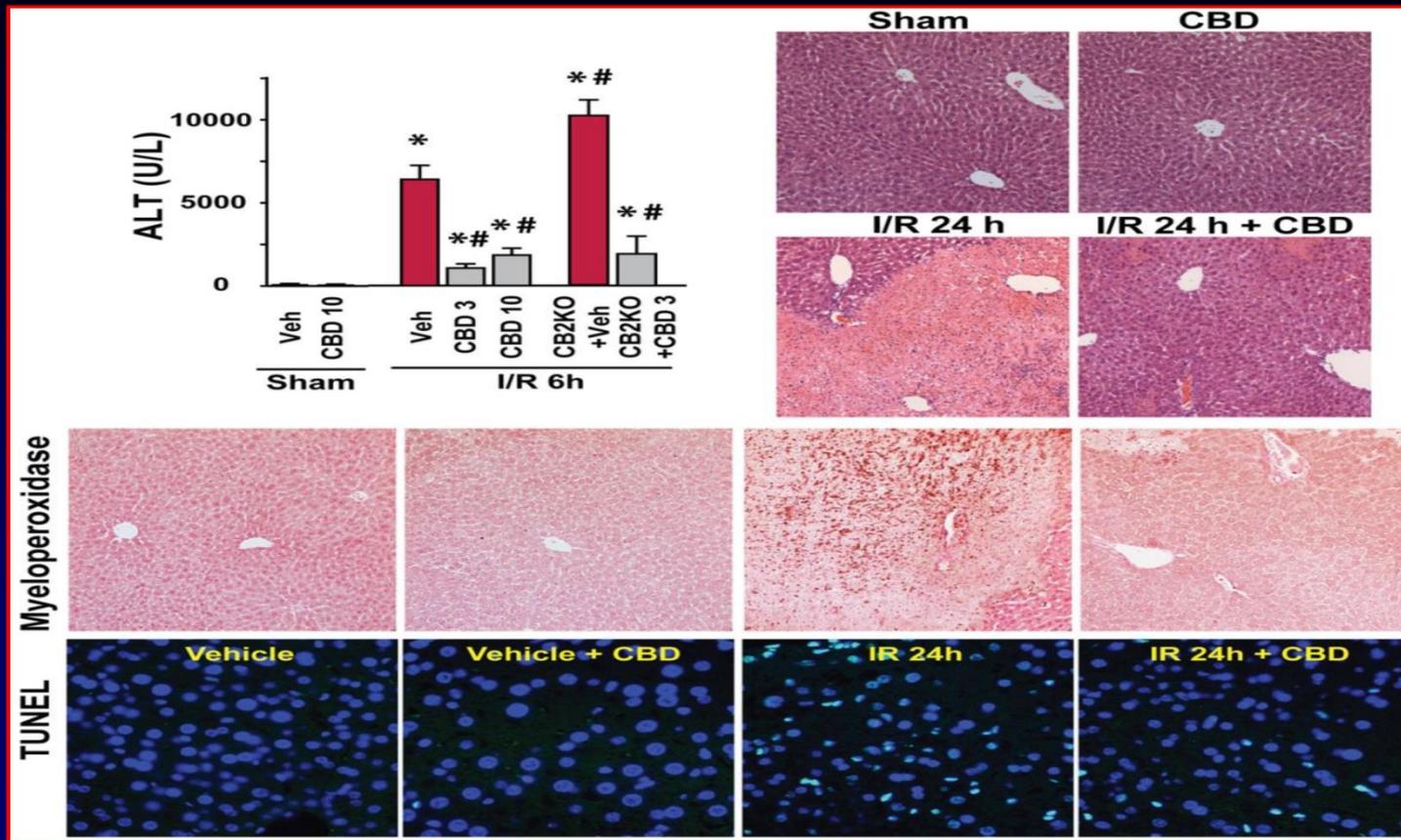
- Medicamento que se comercializa en Canadá
- Extracto puro de cannabis
- Pulverización sublingual

COMPONENTES ACTIVOS POR BOLSAS

- 2,7 mg tetrahidrocannabinol (THC)
- 2,5 mg cannabidiol (CBD)
- 5% Otros cannabinoides

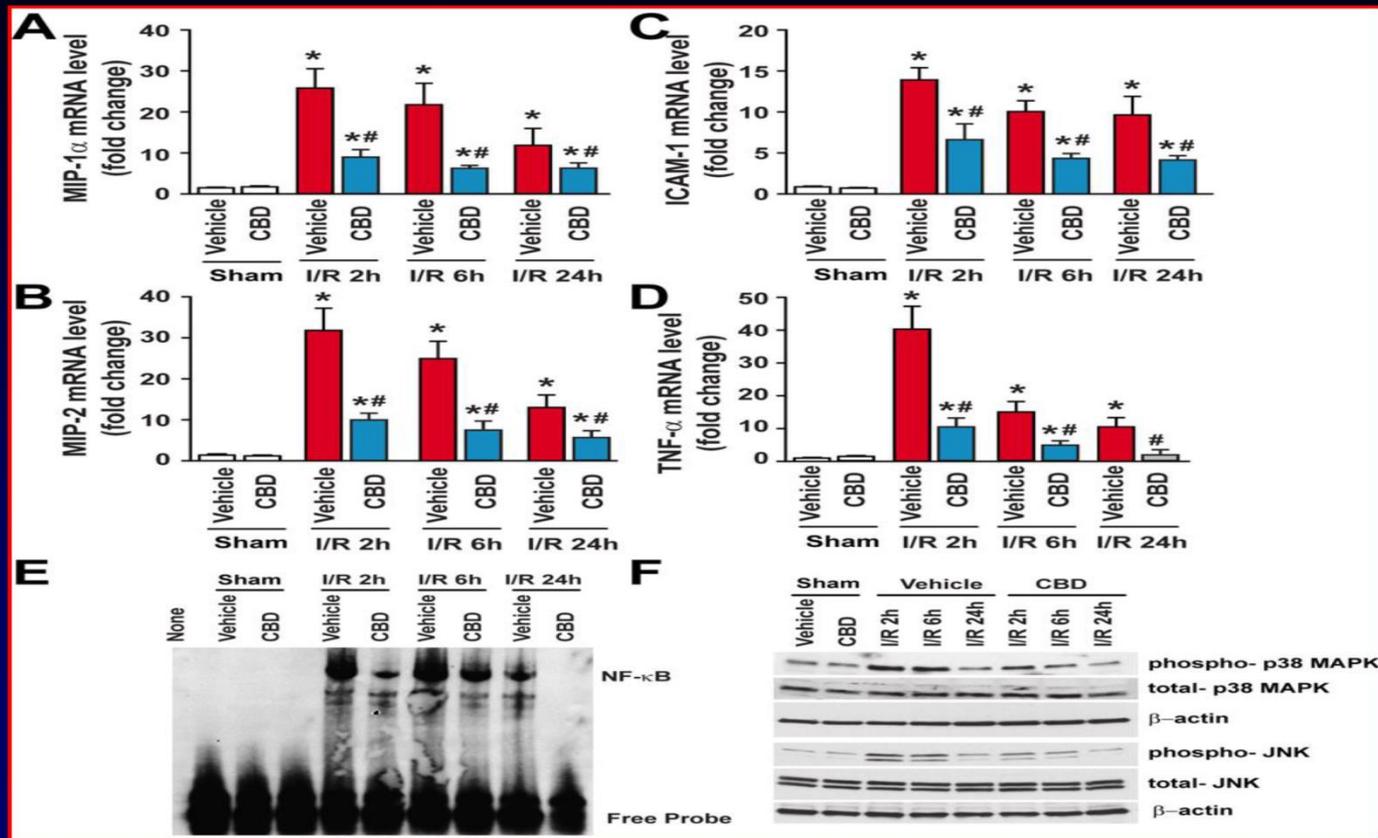
# CBD

## CBD attenuates I/R-induced cell death and neutrophil infiltration in the liver



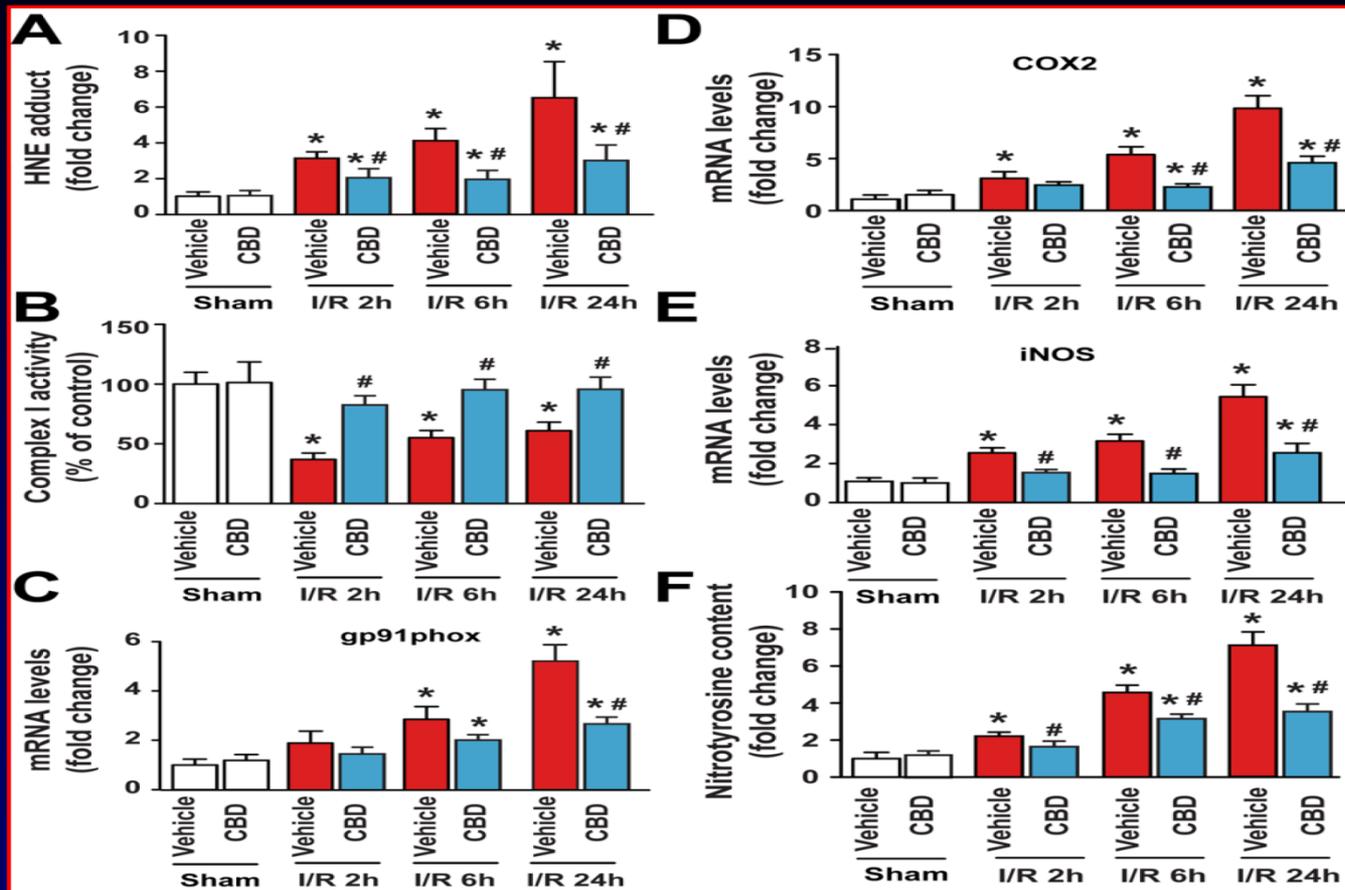
# CBD

## CBD attenuates I/R-induced inflammatory and cell death signaling in the liver



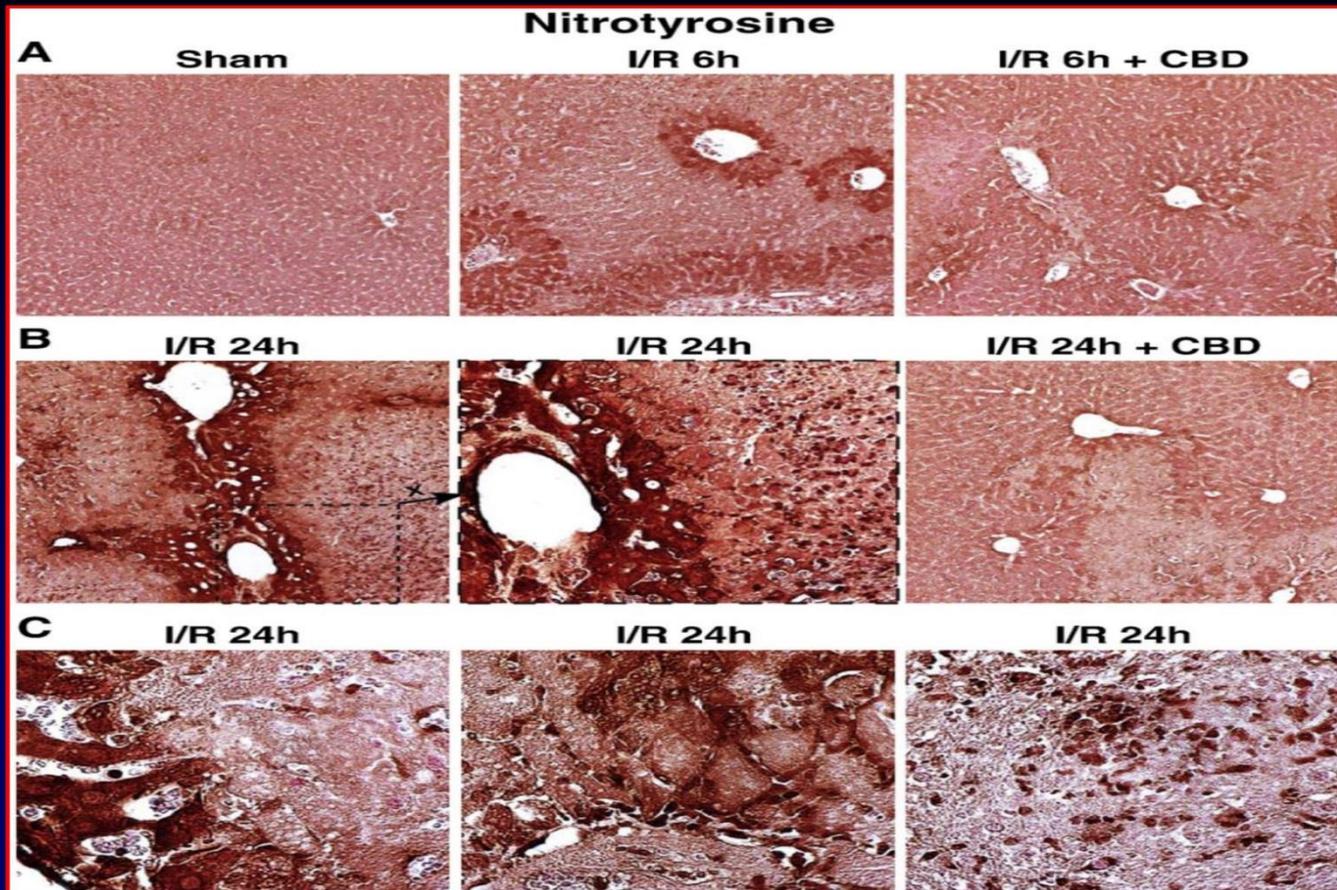
# CBD

## CBD attenuates hepatic I/R-induced oxidative stress, mitochondrial dysfunction, iNOS, COX2 and NOX2 expression



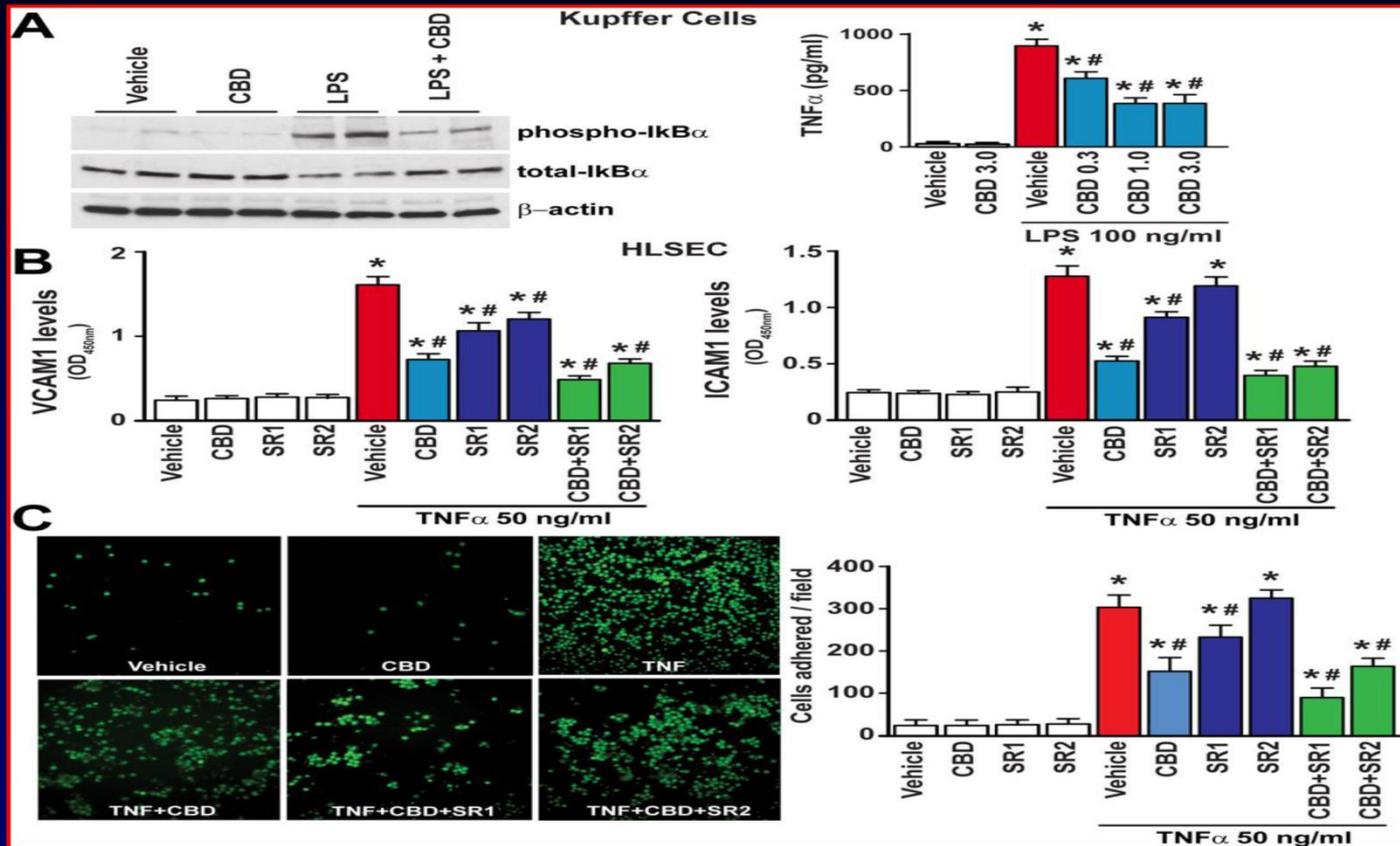
# Hepatic I/R-Induced Nitritative Stress

**CBD attenuates hepatic I/R-induced nitritative stress**



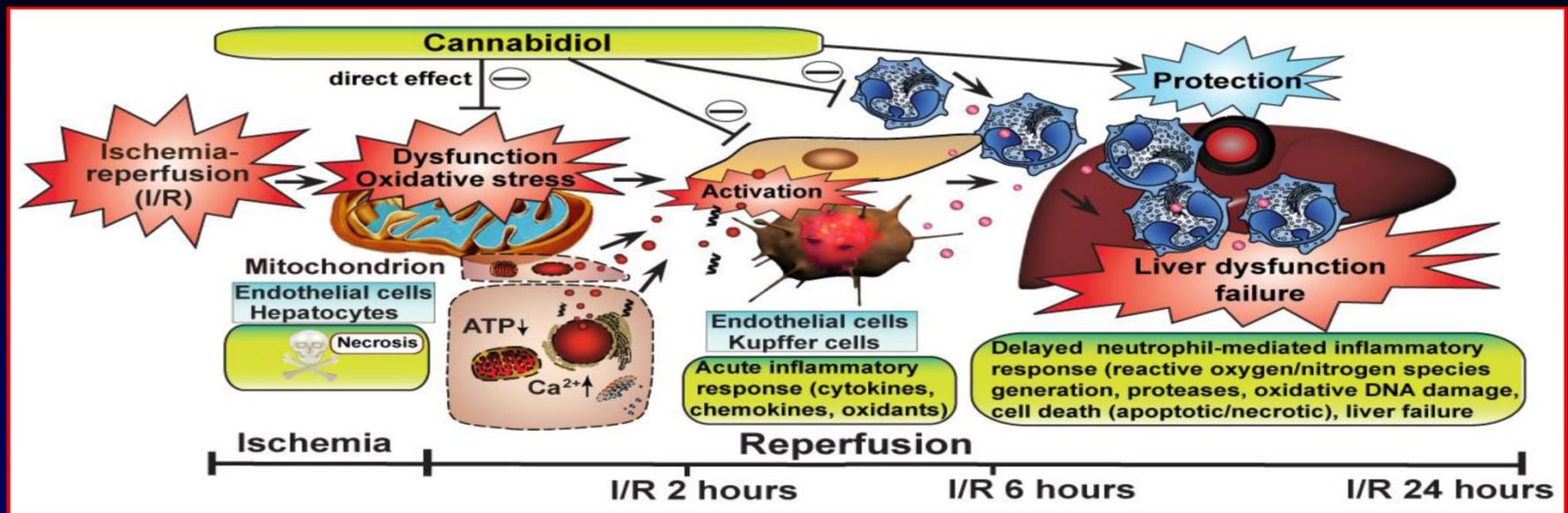
# CBD

**CBD attenuates inflammatory response in Kupffer cells, activation of primary liver sinusoidal endothelial cells (HLSEC), and adhesion of neutrophils to the activated endothelium, independent from CB<sub>1/2</sub>**



# Protective Effects of CBD

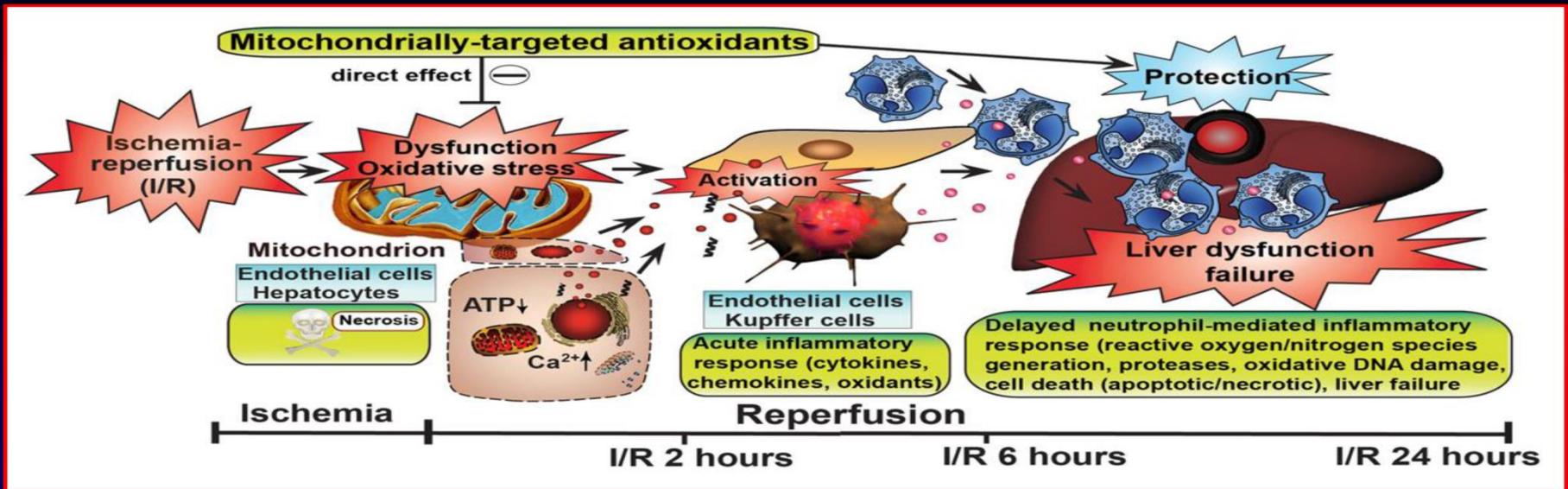
## Protective effects of CBD in hepatic I/R: mechanisms



**Conclusion:** cannabidiol attenuates mitochondrial dysfunction, oxidative/nitrative stress, key inflammatory and cell death signaling pathways in liver injury; targets multiple mechanisms and cell types.

# Protective Effects of Mitochondrially Targeted Antioxidants

## Protective effects of mitochondrially targeted antioxidants in hepatic I/R: mechanisms



**Conclusion:** mitochondrial dysfunction and oxidative/nitrative stress triggers key inflammatory and cell death signaling pathways in liver injury, but targeting mitochondrial dysfunction is only effective at very early stages of I/R injury.

# **ECS and Cannabinoid Receptors**

**Interplay of the lipid endocannabinoid system (ECS) with oxidative stress and inflammation: implications for tissue injury.**

**Role of cannabinoid 1 and 2 receptors, endocannabinoids and endocannabinoid degradation pathways.**

# Cannabinoid Research

## CANNABINOID RESEARCH

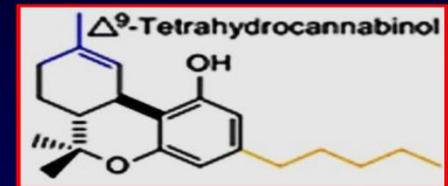
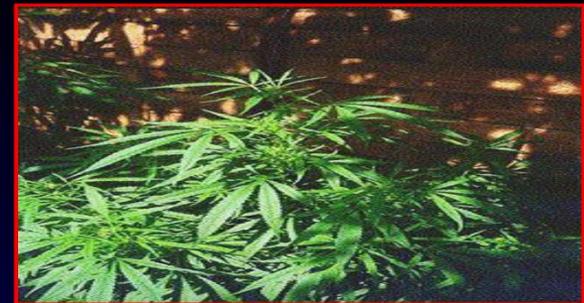
- 1964: bioactive ingredient in plant ( $\Delta^9$ -THC) identified
- Specific CB receptors identified
  - radioligand binding, mapping receptors in brain
  - 1990: CB1 receptor cloned in brain
  - 1993: CB2 receptor cloned in spleen
  - 1994: CB1-selective antagonist introduced (SR141716A)
  - 1997: CB2-selective antagonist introduced (SR144528)
  - 1998: CB1 and CB2 knockout mice generated
- Signaling via  $G_i/o$ ,  $G\beta\gamma$ , MAPK, etc.
- Endogenous ligands identified
  - 1992: anandamide (arachidonyl ethanolamide)
  - 1995: 2-arachidonyl glycerol (2-AG)



ANANDAMIDE



2-ARACHIDONOYL GLYCEROL (2-AG)



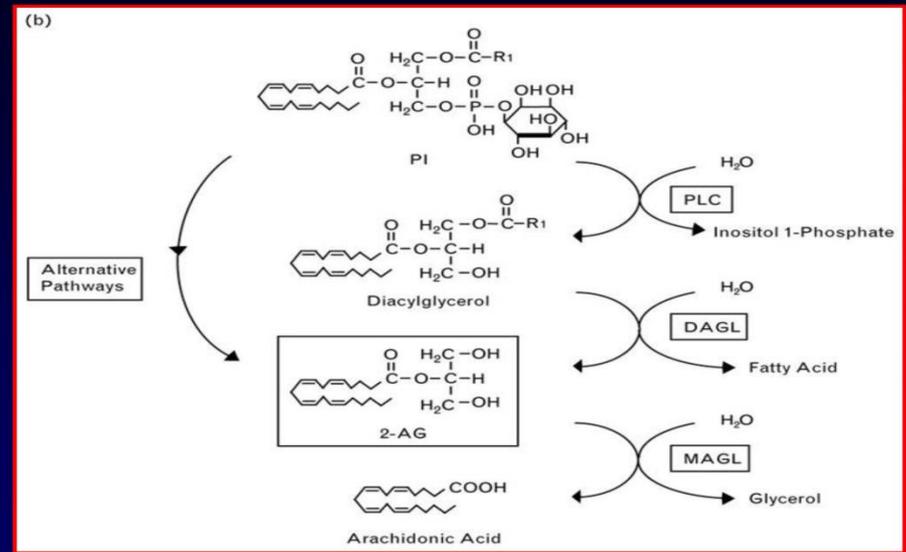
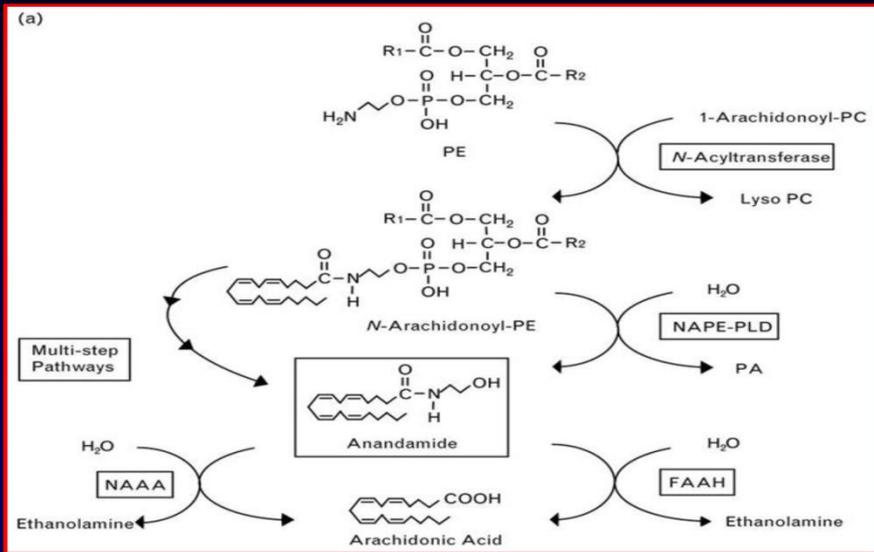
Cannabis sativa:  
421 chemical compounds,  
80 terpeno-phenols not detected  
in any other plants

-Sativex approved in Canada,  
UK, Spain

-Human trials with  
SR141716A/Rimonabant  
(Acomplia), was marketed in  
40 countries

# Endocannabinoids

## Endocannabinoids: biosynthetic pathways



(a) Anandamide. (b) 2-Arachidonoylglycerol (2-AG). DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D; NAAA, *N*-acylethanolamine-hydrolyzing acid amidase; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PLC, phospholipase C.

# Cannabinoid 1 Receptor

## Cannabinoid 1 receptor (CB<sub>1</sub>)-mediated effects with relevance to cardiovascular risk/cardiometabolic diseases

### *Metabolism*

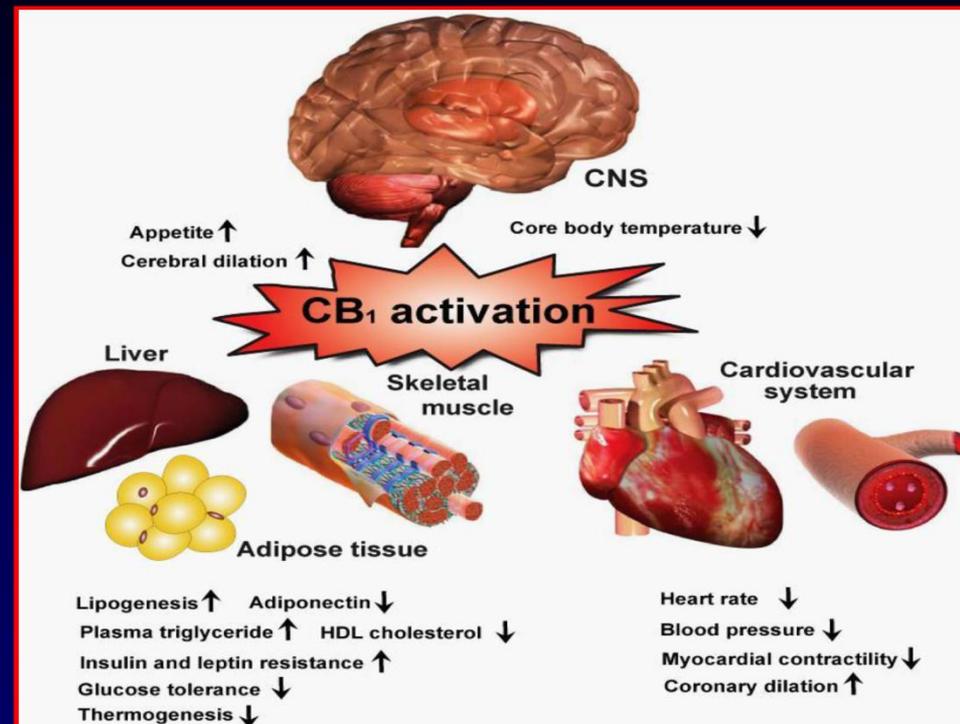
Quarta, Mazza , Obici , Pasquali , Pagotto U.  
*Trends Mol Med. 2011 Sep*

Silvestri and Di Marzo V. *The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. Cell Metab. 2013 Apr*

### *Various diseases*

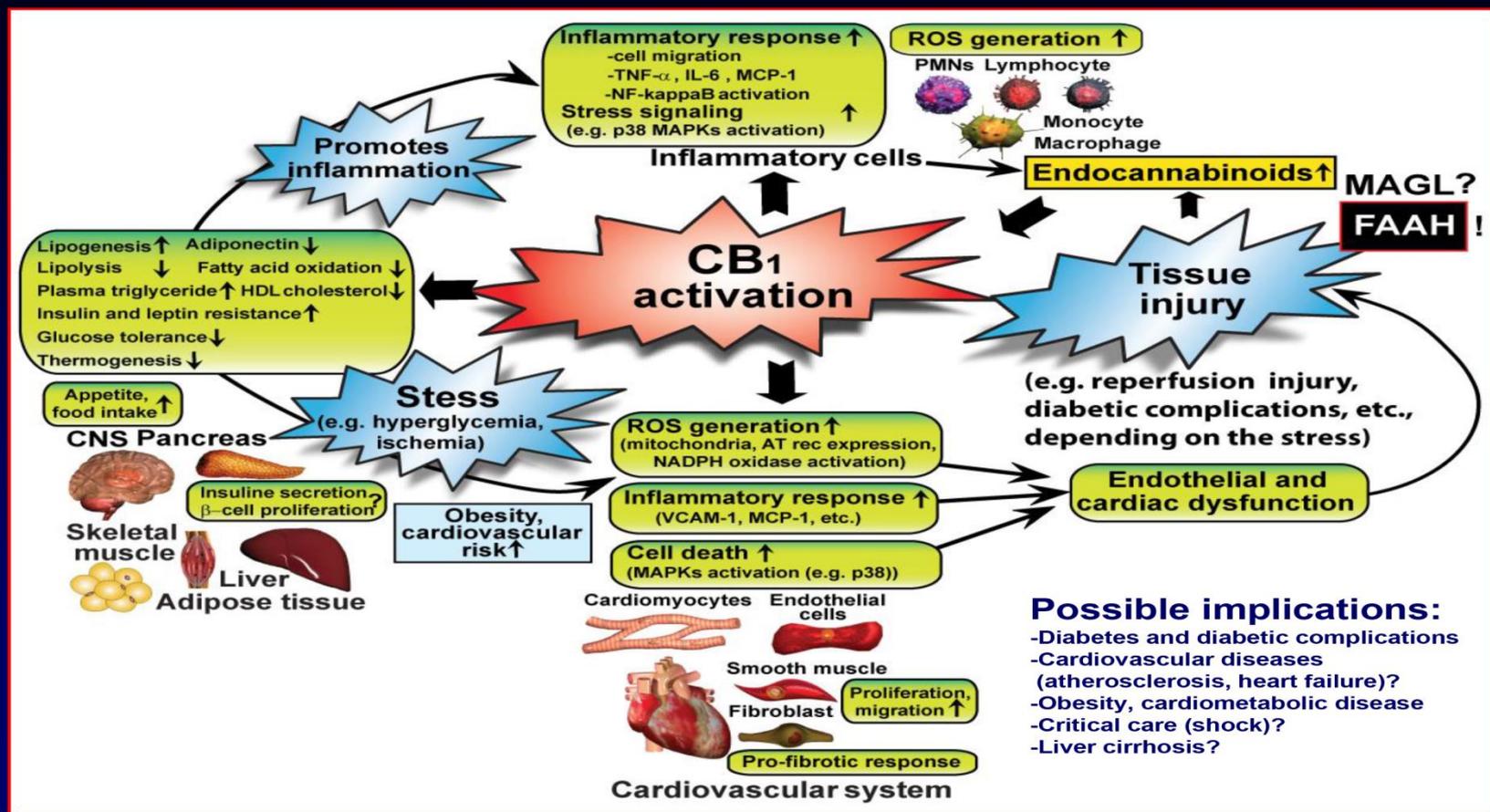
Pacher, Batkai, Kunos. *The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacological Reviews 2006*

Pacher, Kunos. *Modulating the endocannabinoid system in human health and disease-successes and failures. FEBS J. 2013 May;*



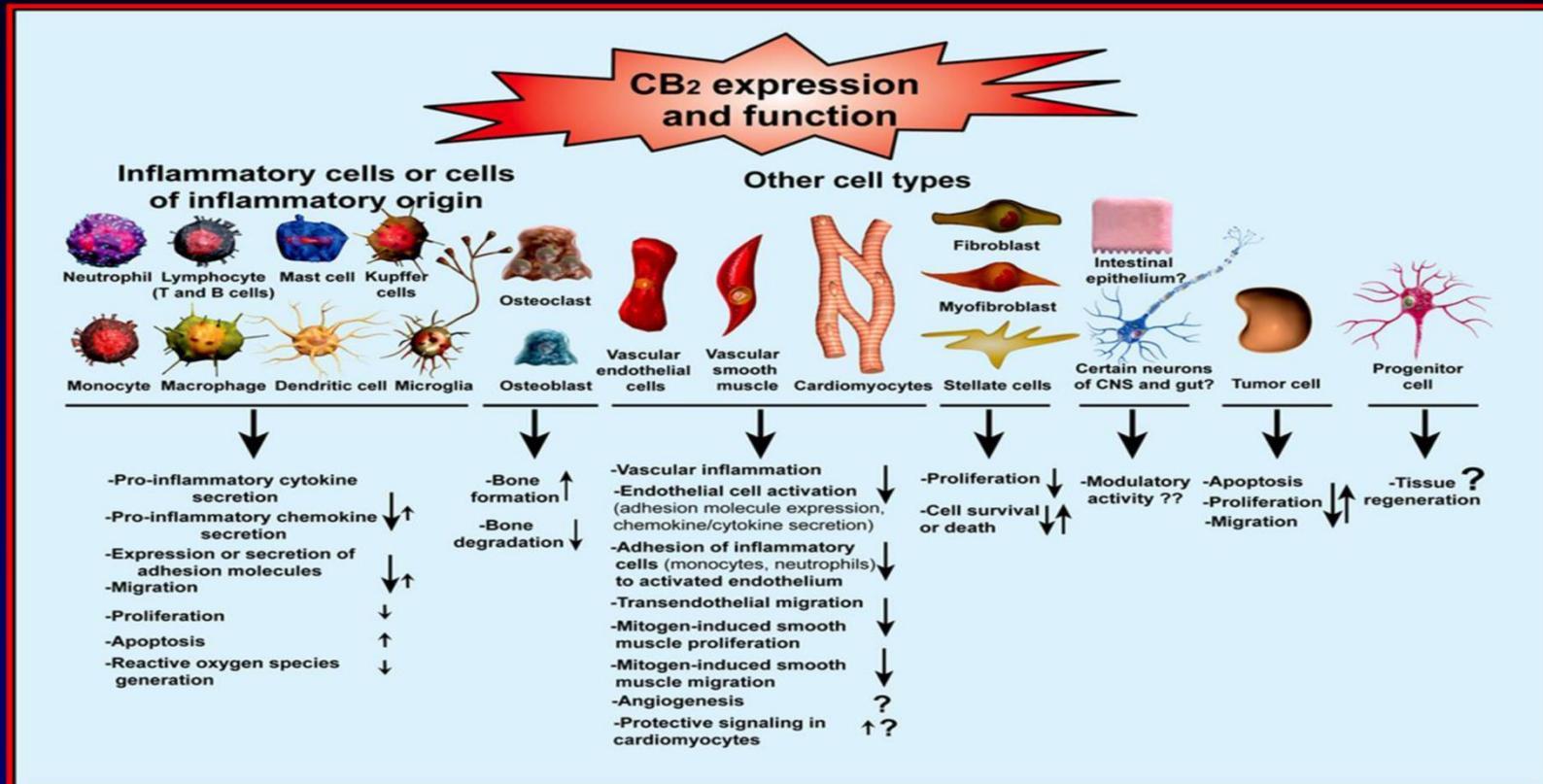
# Implications for Tissue Injury

## Interplay of CB1 receptors with oxidative stress and inflammation: implications for tissue injury



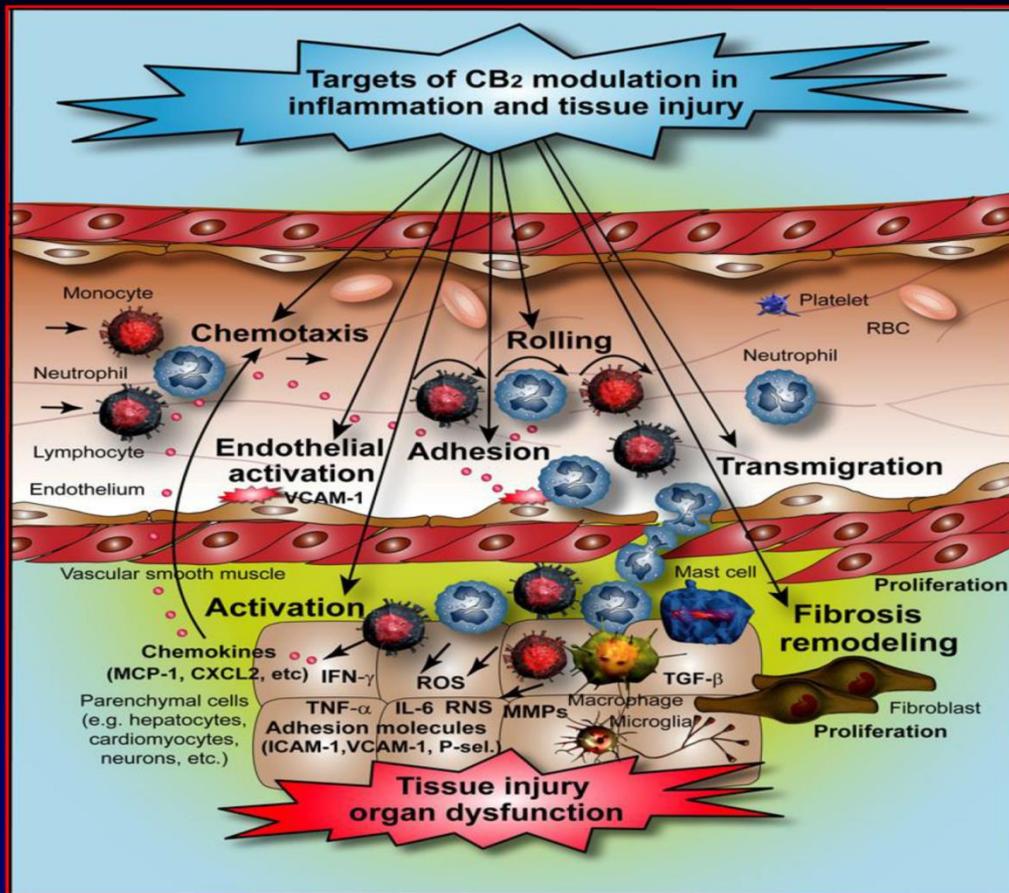
# Cannabinoid 2 Receptor

## Cannabinoid 2 receptor (CB<sub>2</sub>)-mediated effects with relevance to inflammatory diseases



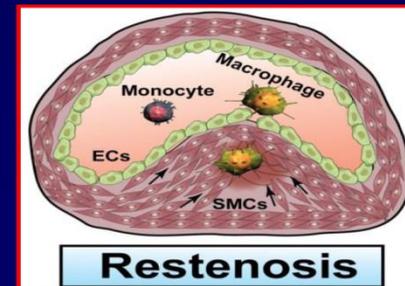
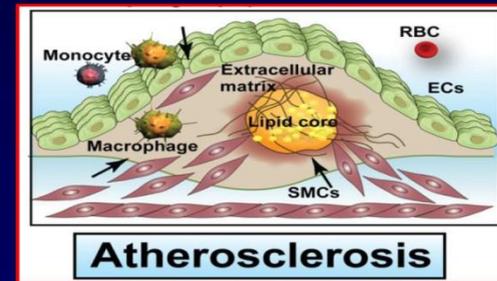
# Implications for Tissue Injury

## Interplay of CB<sub>2</sub> receptors with oxidative stress and inflammation: implications for tissue injury



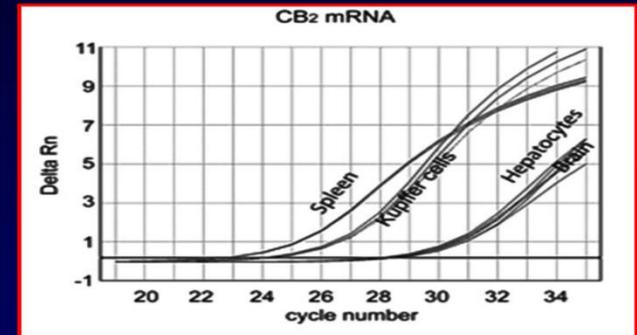
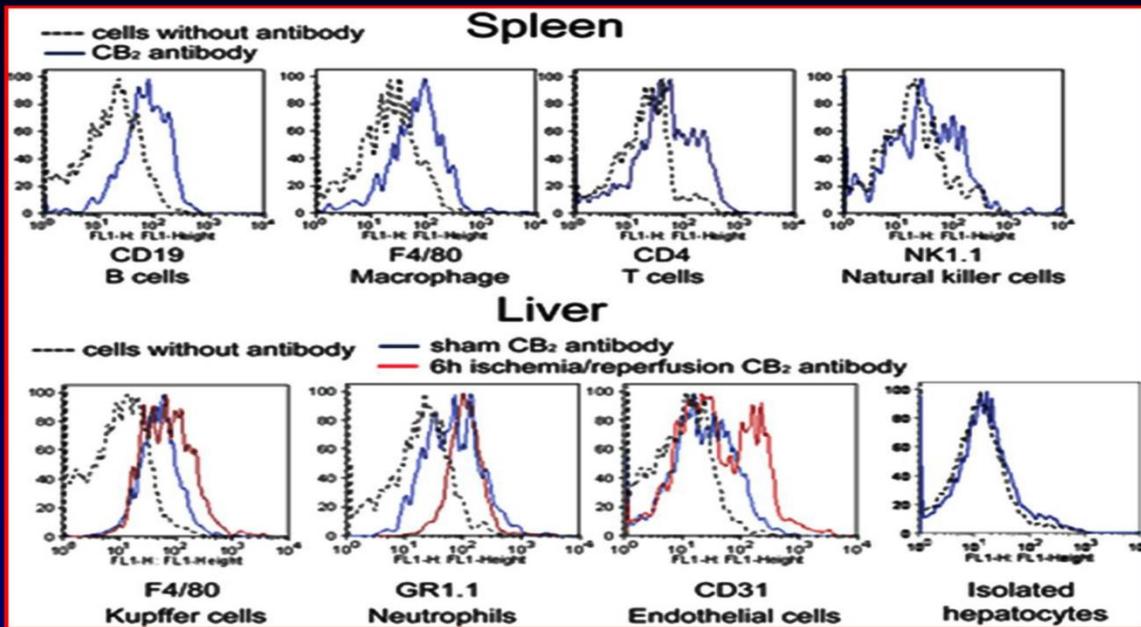
### Potential benefits of CB<sub>2</sub> R modulation in “sterile” cardiovascular and inflammatory diseases

- Myocardial infarction, stroke, organ transplantation, vascular surgery
- Restenosis, atherosclerosis
- Autoimmune, neuroinflammatory, gastrointestinal, kidney diseases, etc



# Hepatic Ischemic/Reperfusion Injury

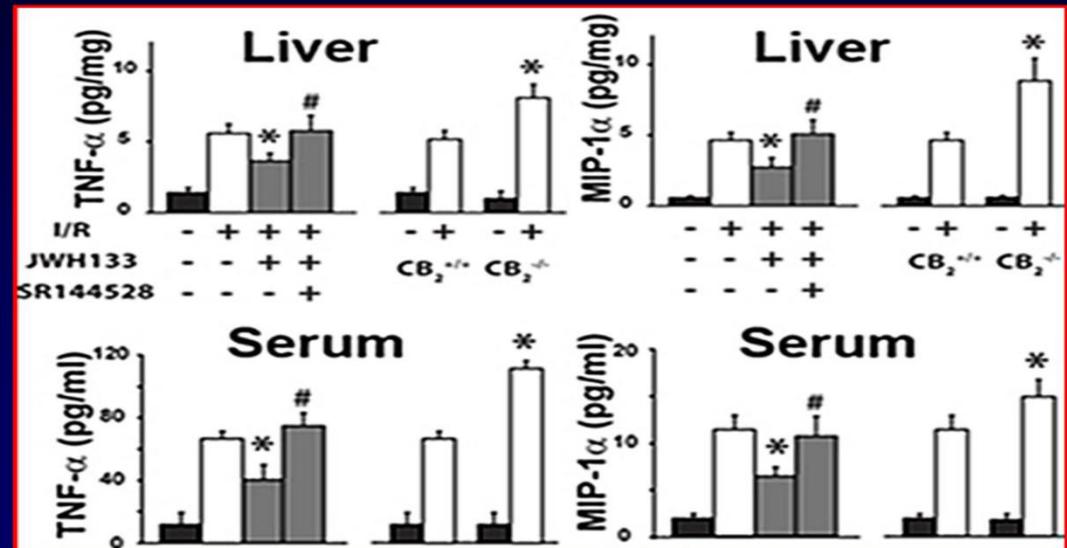
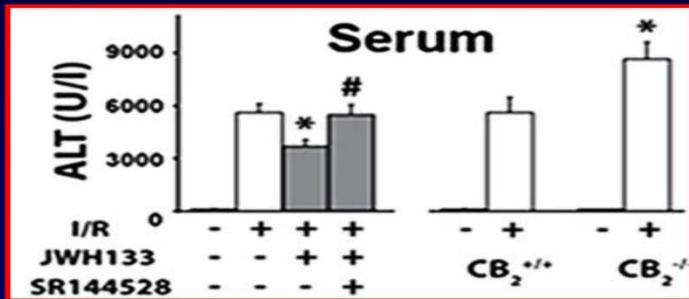
## Hepatic ischemic/reperfusion injury: role of CB<sub>2</sub>



# Hepatic Ischemic/Reperfusion Injury

## Hepatic ischemic/reperfusion injury: role of CB<sub>2</sub>

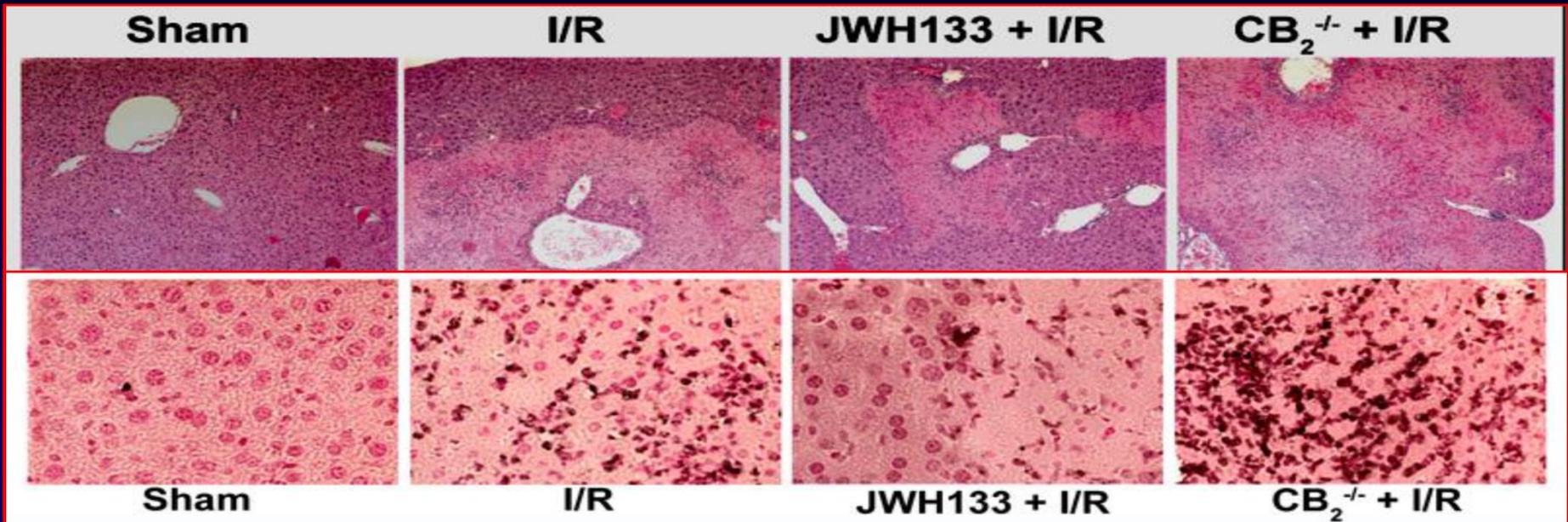
**CB<sub>2</sub> agonist JWH133 attenuates I/R-induced hepatic necrosis (serum alanine aminotransferase (ALT) ) and acute inflammation (serum and/or liver TNF $\alpha$ , MIP-1 $\alpha$ /CCL3, liver myeloperoxidase activity (MPO)). Enhanced I/R-induced inflammation in CB<sub>2</sub><sup>-/-</sup> mice.**



# Hepatic Ischemic/Reperfusion Injury

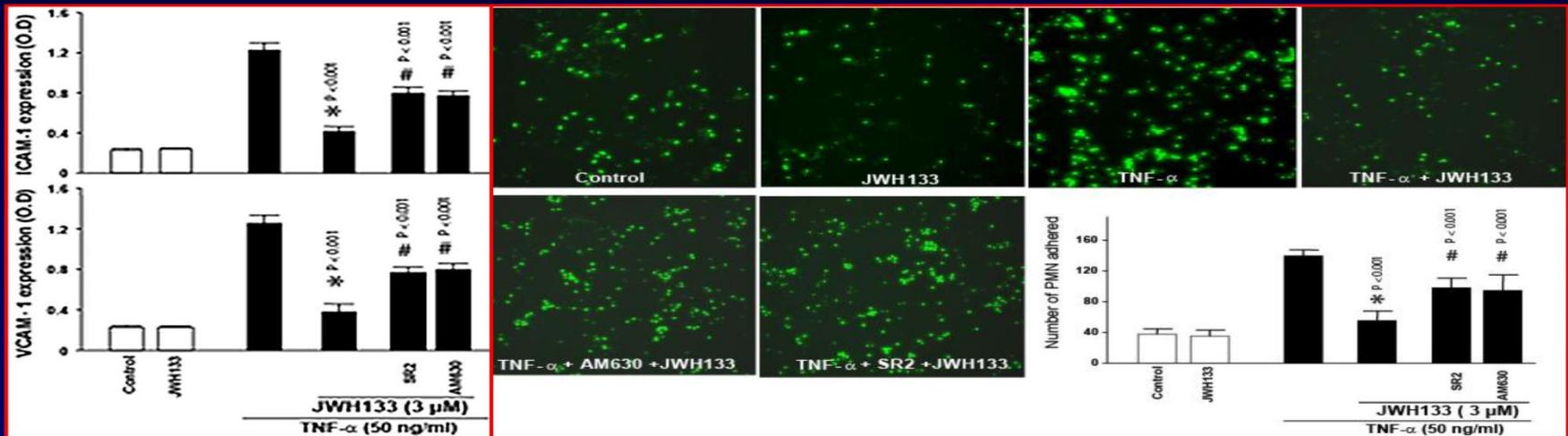
**Hepatic ischemic/reperfusion injury: role of CB<sub>2</sub>**

**CB<sub>2</sub> activation attenuates delayed histological damage and inflammatory (neutrophil) cell infiltration 24 hrs following reperfusion, increased injury in CB<sub>2</sub><sup>-/-</sup> mice.**



# Hepatic Ischemic/Reperfusion Injury

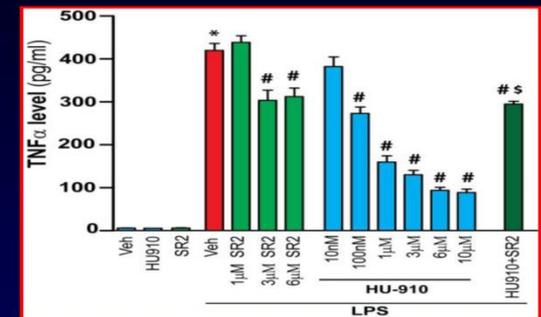
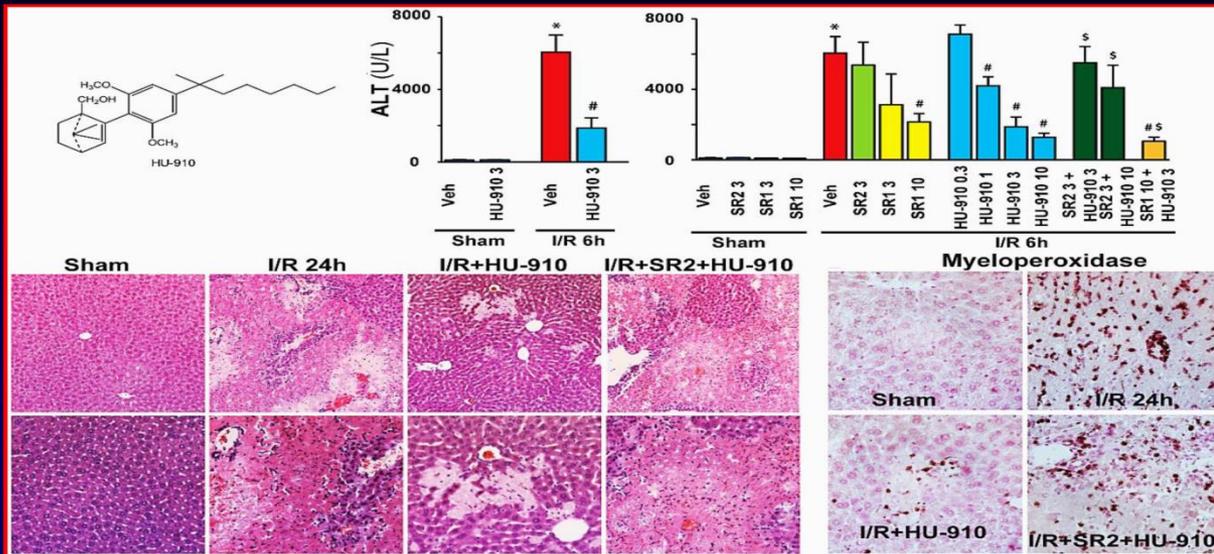
**Hepatic ischemic/reperfusion injury: role of CB<sub>2</sub>**  
**CB<sub>2</sub> agonists decrease TNF $\alpha$ -induced overexpression of adhesion molecules ICAM-1 and VCAM-1 in human liver endothelial cells, and adhesion of neutrophils to human sinusoidal endothelial cells.**



Batkai et al. *FASEB J* 2007; Rajesh et al. *J Leukoc Biol* 2007.

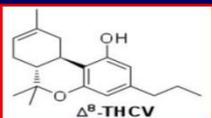
# Hepatic Ischemic/Reperfusion Injury

**Hepatic ischemic/reperfusion injury: role of CB<sub>2</sub> HU-910 attenuates I/R-induced liver injury and neutrophil infiltration, as well as Kupffer cell activation.**



Horvath et al. *Br J Pharmacol* 2012 (new class of CB<sub>2</sub> agonists HU910), attenuation of Kupffer cell activation, opposing role of CB<sub>1</sub> in I/R)

Horvath et al. *Br J Pharmacol* 2012 (new class of CB<sub>2</sub> agonists HU910)



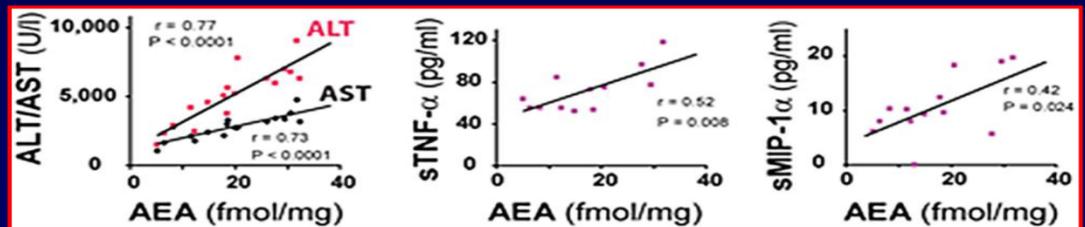
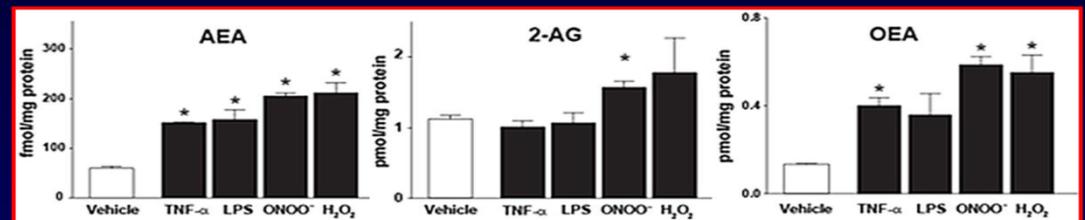
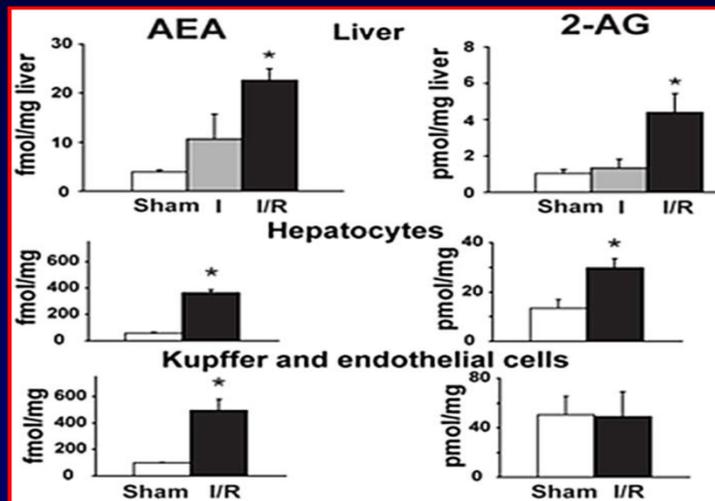
Synthetic analog of Δ<sup>9</sup> THCV, Δ<sup>8</sup> THCV is a potent CB<sub>2</sub> agonist *in vitro* and *in vivo*

Batkai et al. *Br J Pharmacol* 2012 (Δ<sup>8</sup> THCV is a potent CB<sub>2</sub> agonist), also opposing role of CB<sub>1</sub>

# Role of Endocannabinoids

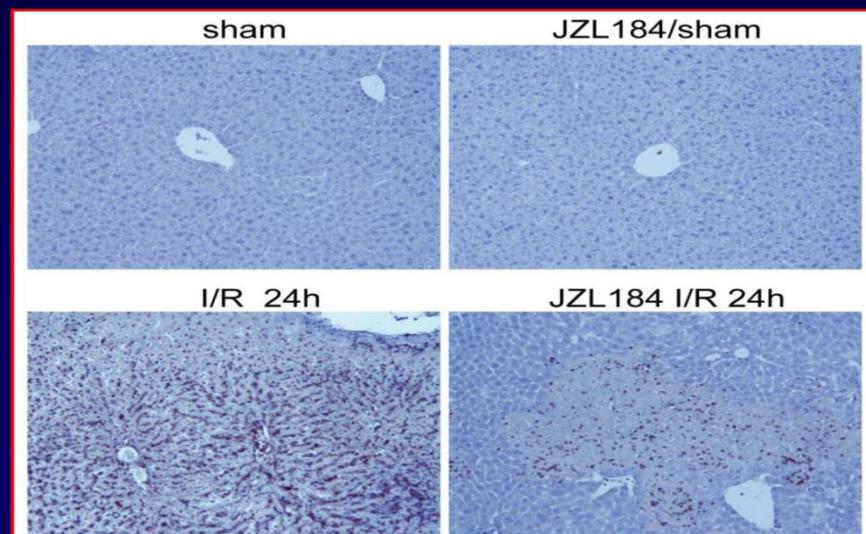
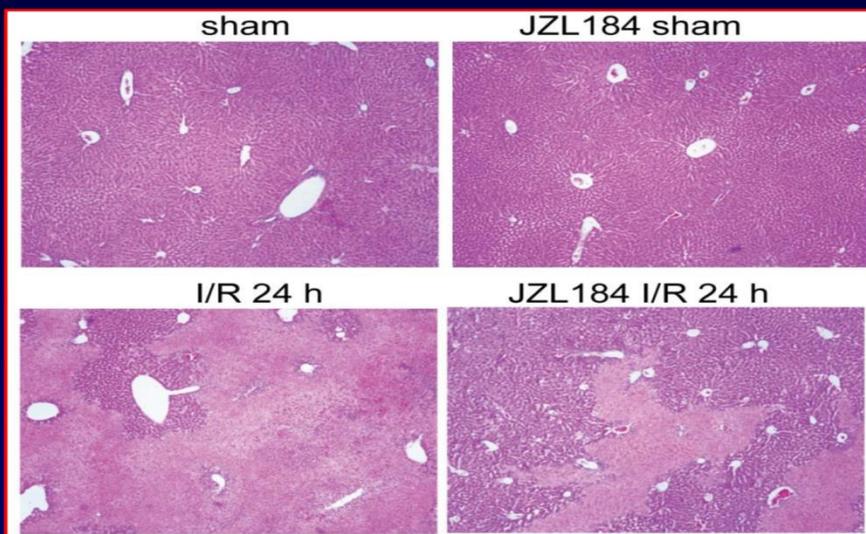
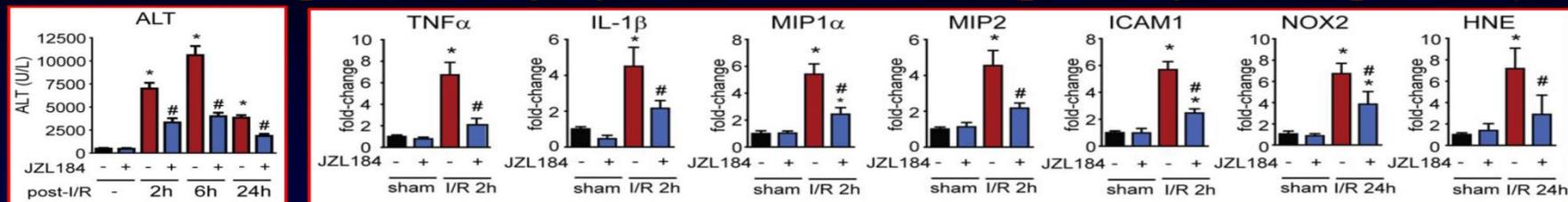
## Hepatic I/R injury: role of endocannabinoids?

Hepatic I/R (but not I) increases anandamide (AEA) and 2-arachidonoylglycerol (2-AG) levels in the liver, which originate from hepatocytes, Kupffer and endothelial cells. Inflammatory stimuli/oxidants increase endocannabinoid levels in isolated hepatocytes. Hepatic endocannabinoid levels during reperfusion injury positively correlate with markers of tissue damage and inflammation.



# Role of Endocannabinoids

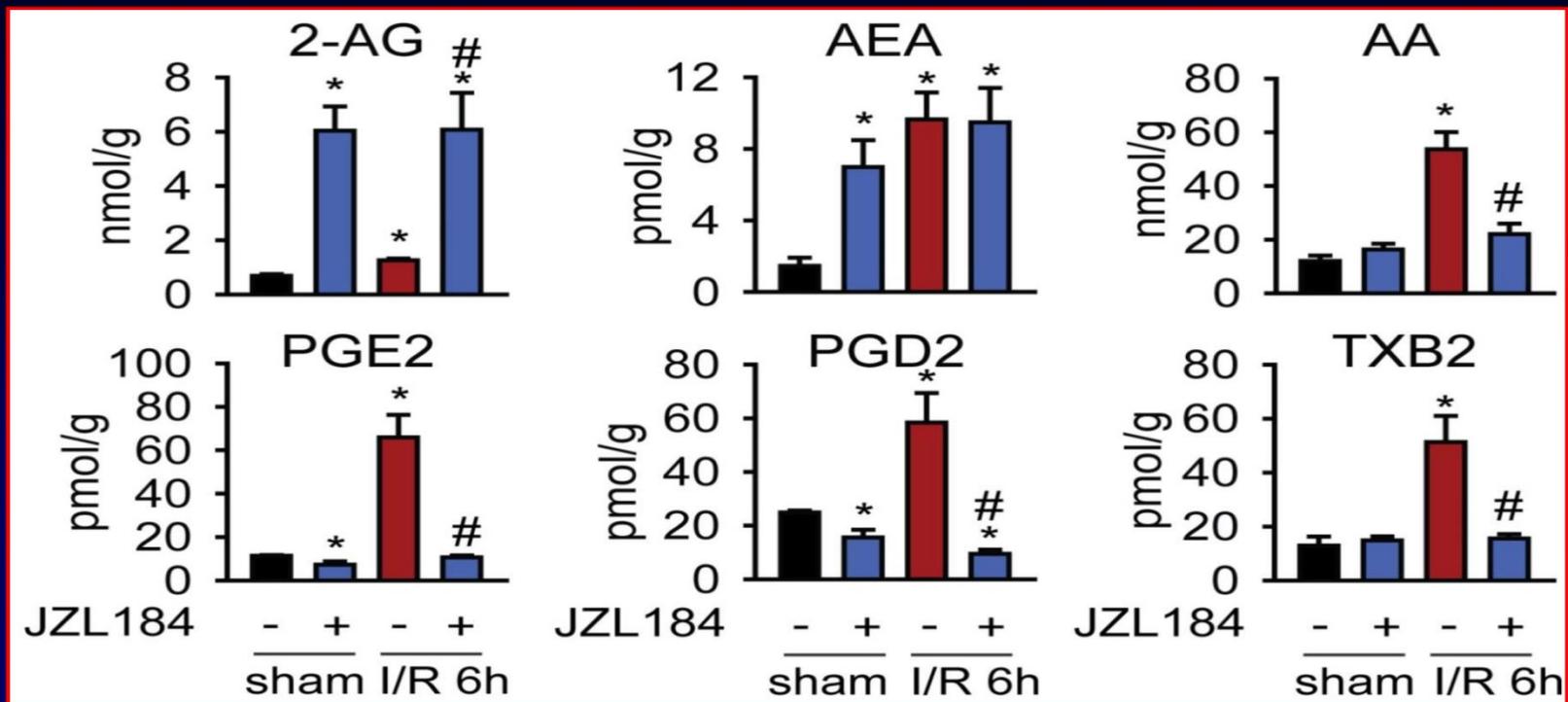
**Hepatic I/R injury: role of endocannabinoids.**  
**Monoacylglycerol lipase (MAGL) inhibition with JZL184 /genetic deletion attenuates hepatic I/R injury and inflammation (partially CB<sub>2</sub> dependent)**



# Role of Endocannabinoids

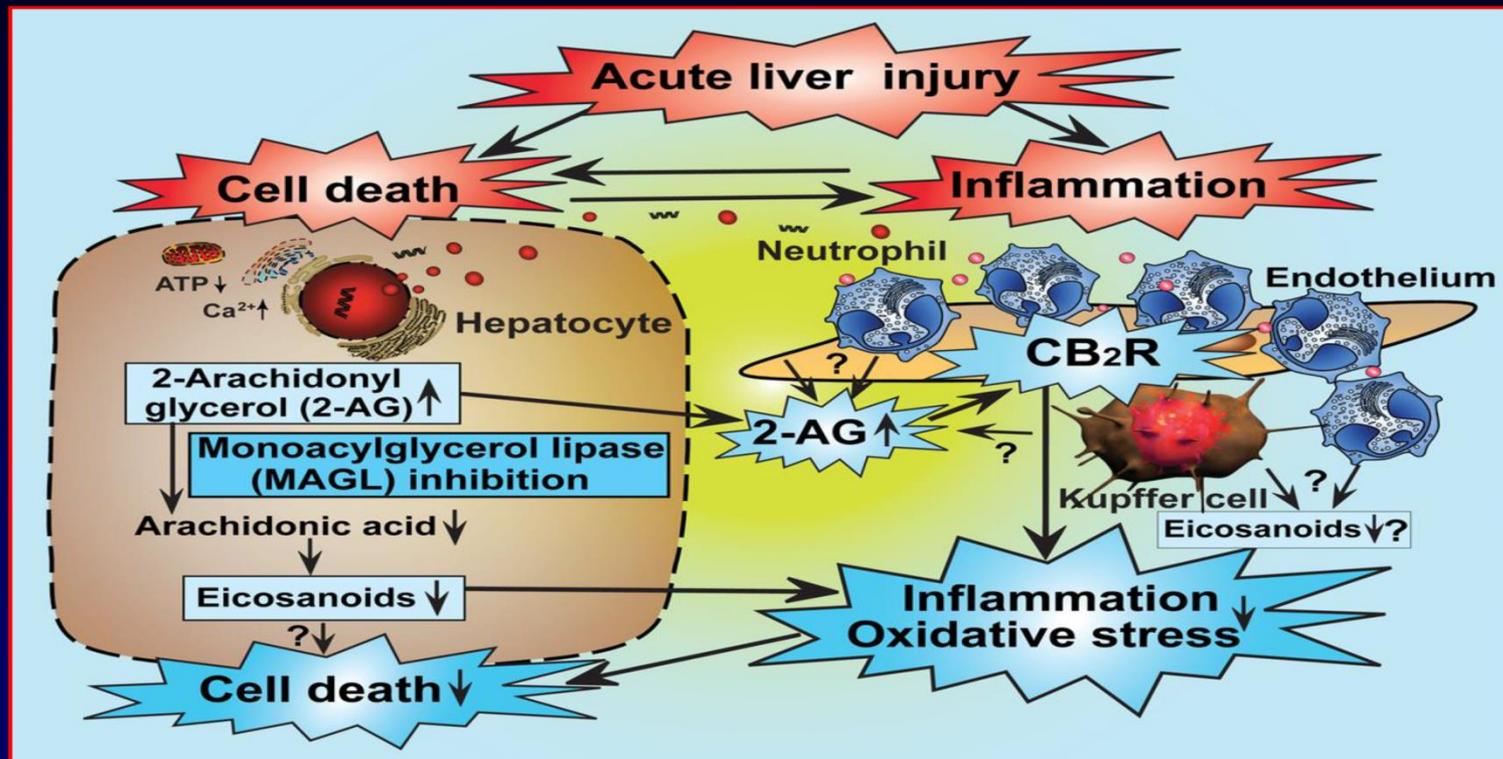
## Hepatic I/R injury: role of endocannabinoids.

*Monoacylglycerol lipase (MAGL) exerts dual controls over endocannabinoid and eicosanoid signaling and hepatic injury in mice*



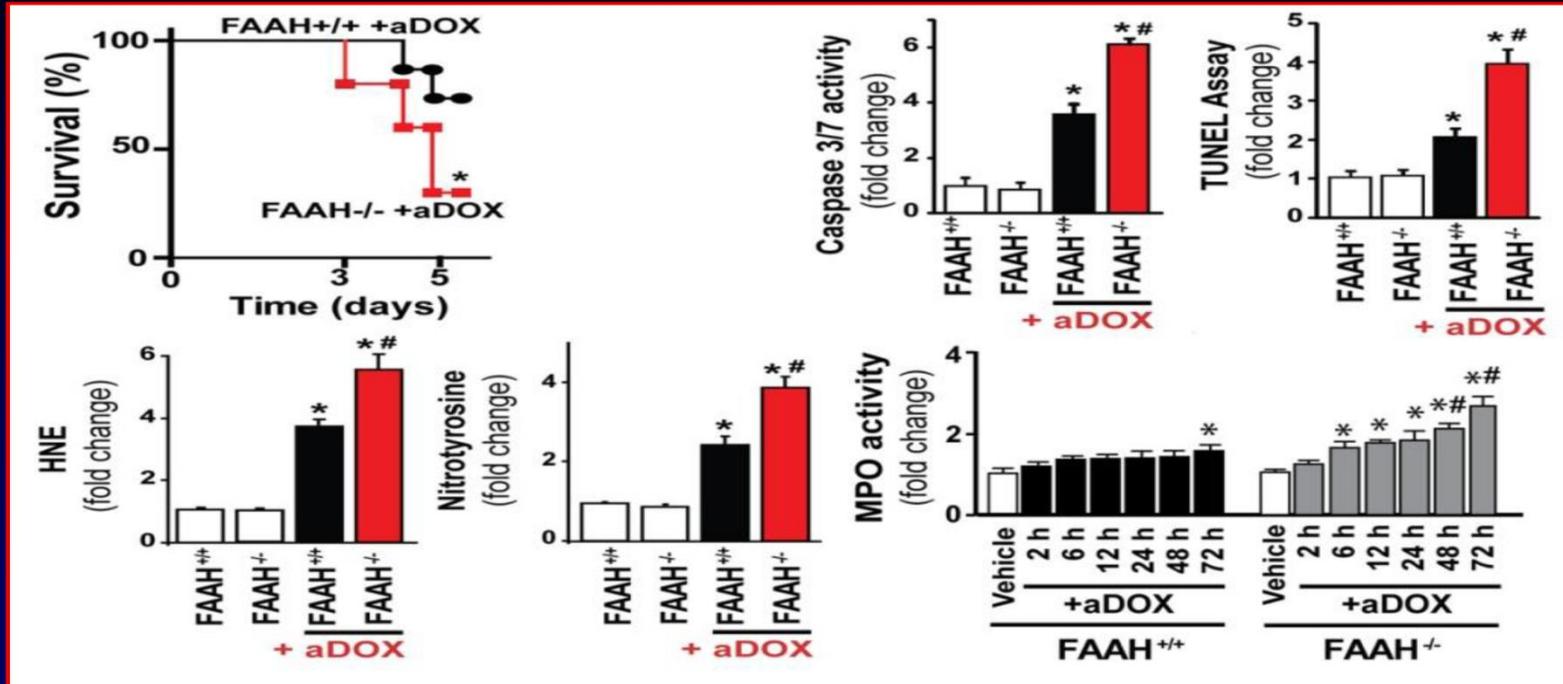
# Role of Endocannabinoids

**Hepatic I/R injury: role of endocannabinoids.**  
**Monoacylglycerol lipase (MAGL) exerts dual controls over endocannabinoid and eicosanoid signaling and hepatic injury in mice**



# Genetic Deletion of FAAH

Genetic deletion of the endocannabinoid degrading enzyme FAAH is associated with enhanced myocardial oxidative/nitrative stress, cell death and cardiac dysfunction in a model of drug-induced cardiomyopathy, which is partially CB1-dependent



FAAH deletion may promote oxidative stress and inflammation under pathological conditions

## Human relevance:

-Eur Heart J. 2011 Jun, *Quericioli et al.* Elevated endocannabinoid plasma levels are associated with coronary circulatory dysfunction in obesity.

# Summary

## Summary

- ECS is activated during reperfusion or in other types of tissue injury by oxidative/nitrosative stress and inflammatory stimuli;
- All cell types exposed to “stress” may release/produce endocannabinoids;
- Role of endocannabinoids in tissue injury ? : context dependent (cell/tissue- and time-dependent)
  - protective ? : CB<sub>2</sub>      -detrimental ? : CB<sub>1</sub>
  - AA metabolites
- selective CB<sub>2</sub> agonists may decrease endothelial cell activation and inflammatory cell (neutrophil, etc.) mediated damage during tissue injury
- peripherally restricted CB<sub>1</sub> antagonists may attenuate tissue inflammation and injury

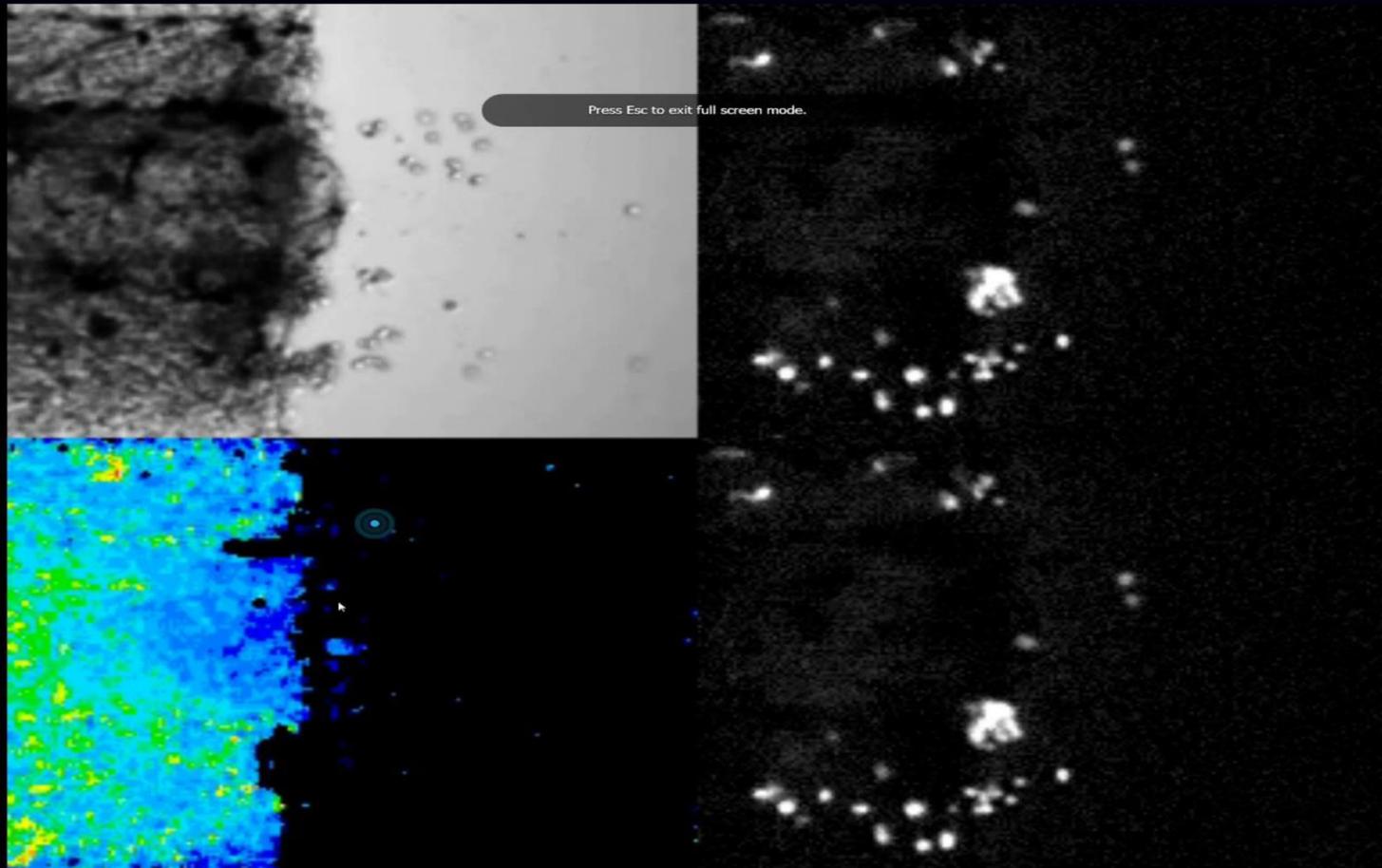
# Role of Reactive Oxygen Species

**Role of the reactive oxygen species in tissue repair and regeneration: implications for physiological and pathological processes**

**Amputation-induced reactive oxygen species (ROS) are required for *Xenopus tropicalis* tadpole tail regeneration**

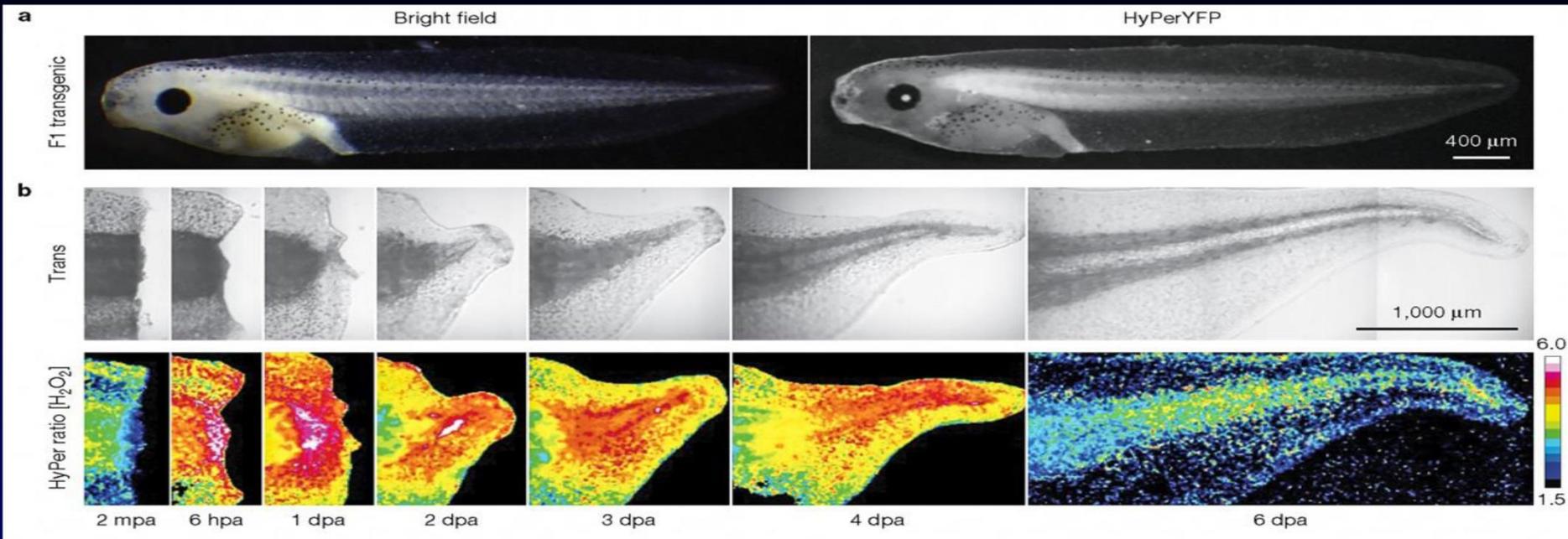
Love NR et al. *Nature Cell Biology* 2013. Febr

# Nature Cell Biology



# Xenopus Tadpole Tail Regeneration

## ROS are required for *Xenopus* tadpole tail regeneration

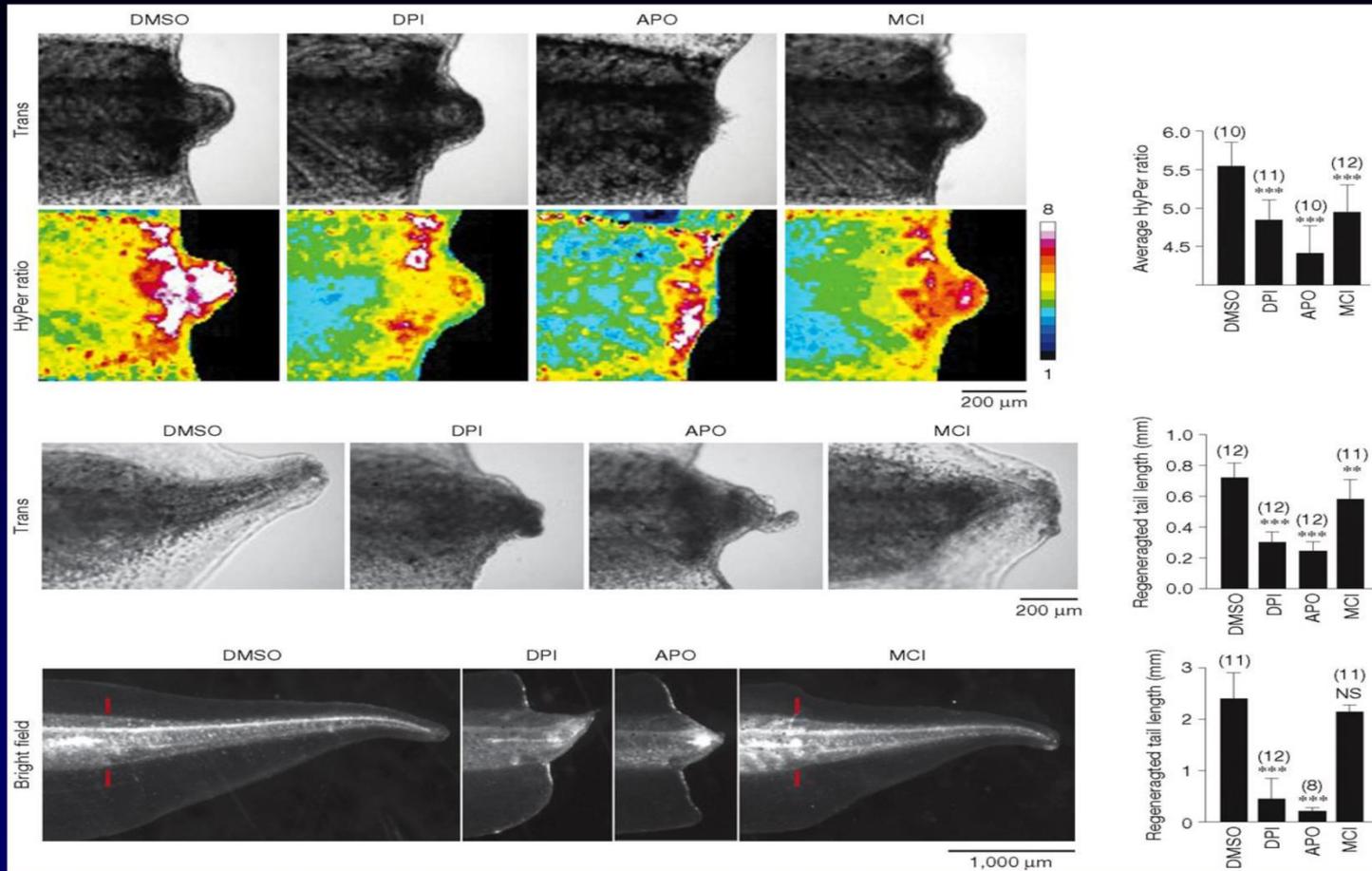


**Figure 1** Production of ROS during *Xenopus* tadpole tail regeneration. **(a)** Bright-field and fluorescence micrographs of a tadpole derived from the F1 generation of a transgenic *X. laevis* line that expresses the H<sub>2</sub>O<sub>2</sub> sensor HyPerYFP ubiquitously<sup>10</sup>. **(b)** HyPerYFP transillumination (Trans) imaging of a representative regenerating tadpole tail. [H<sub>2</sub>O<sub>2</sub>] is

derived from the excitation ratio of HyPerYFP490nm/HyPerYFP402 nm. mpa, minutes post-amputation; hpa, hours post-amputation; dpa, days post-amputation. Owing to the size of the regenerated tail, the 6 dpa time point panels are derived from the merging of three images.

# Xenopus Tadpole Tail Regeneration

ROS are required for *Xenopus* tadpole tail regeneration



# Conclusion

**Conclusion:** Better understanding the complexity of the interplay of oxidative stress, inflammation, lipid and cell death signaling pathways during tissue injury and regeneration is essential to successfully target these processes for therapeutic benefit.

# Acknowledgements:

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