

The Low-Dose Ozone Concept

Ozone as a Bioregulator

Renate Viebahn-Hänsler

indirect reaction **O₃** **direct reaction**

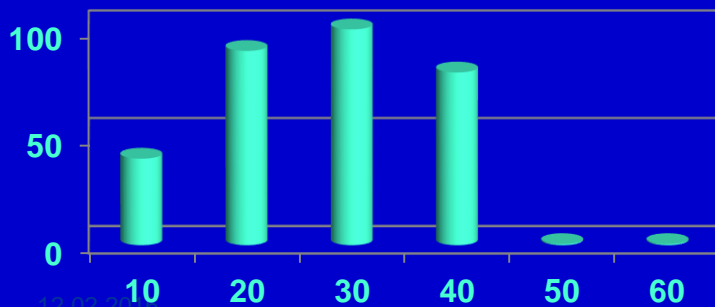


Ox radicals / H₂O₂ / peroxides
 bactericidal, fungicidal,
 virus inactivating

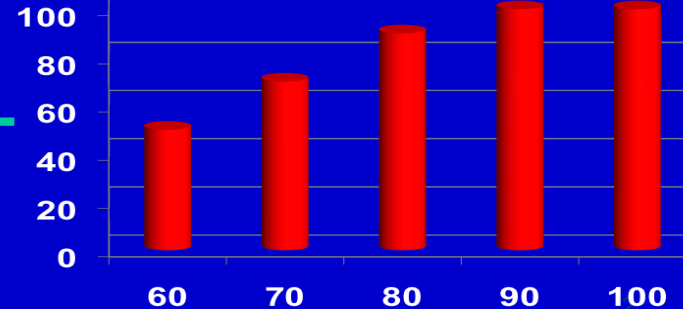
Systemic treatment
 chronic inflammations
 woundhealing

Topical treatment
 Wound cleansing
 Diabetic foot, ulcerate colitis

effectivity

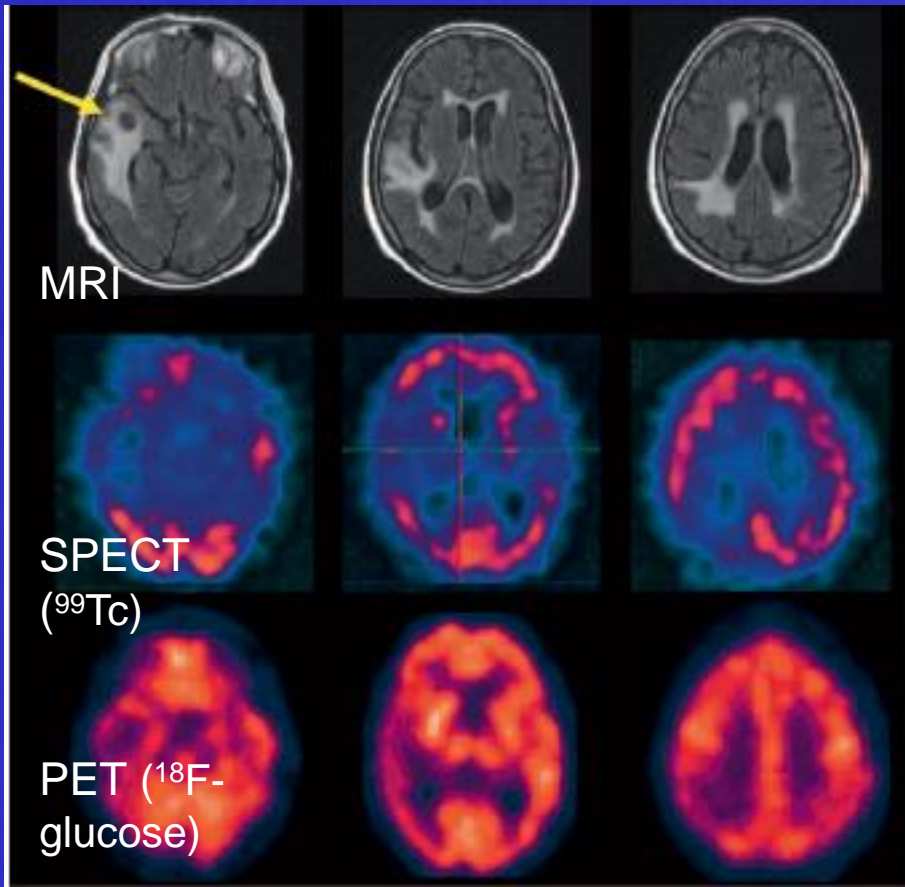


wound healing



12.02.2014

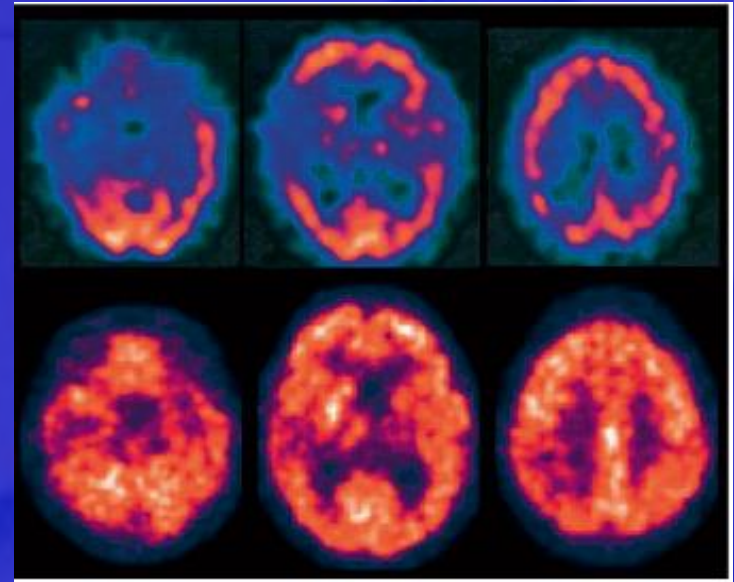
iv OZONOSAN
Ozone concentration in mg/L



Pat female 75 diab, high blood pressure, epilepsy.
 MRI: Meningioma with vasogenic edema –
 stereotactic radio surgery–Parkinson syndrome after
 8 months, L-DOPA, progression of bradypsychia –
 14 months: SIADH (antidiuretic hormone secretion
 syndrom) no changes in MRI (see above), areas of
 leukoencephalopathy (secondary to radiotherapy
 correlates to hypoperfusion and hypometabolism

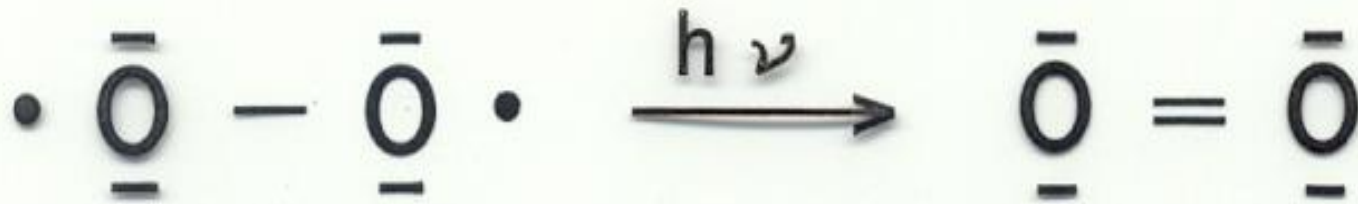
Leukoencephalopathy as radiation-
 induced brain injury (RBI) mediated
 by ischemia and hypometabolism

Clavo et al 2011
 Case Report Forsch Kompl Med



Functional imaging studies show an
 increase in blood perfusion and an overall
 increase in metabolism after 3 (5) months
 of ozone treatment (MAH 3/week; 30 mg/l).

Oxygen, Ozone Molecular Structures



Triplet Electron Structure

Singlet Electron Structure



Toxicity versus therapeutical benefit

Toxicity of ozone by inhalation
on the respiratory system and
extrapulmonary organs

Long term exposure (8h) etc
with concentrations up to 1 ppm in
animals →

oxidative dysstress

→ Chronic Inflammation

→ → O_2^- , OH-radicals...

→ dysfunction of antioxidants →

→ DOWNREGULATION OF
ANTIOXIDANTS

Therapeutic benefit by special
applications

Single dose, low concentration,
low dose →

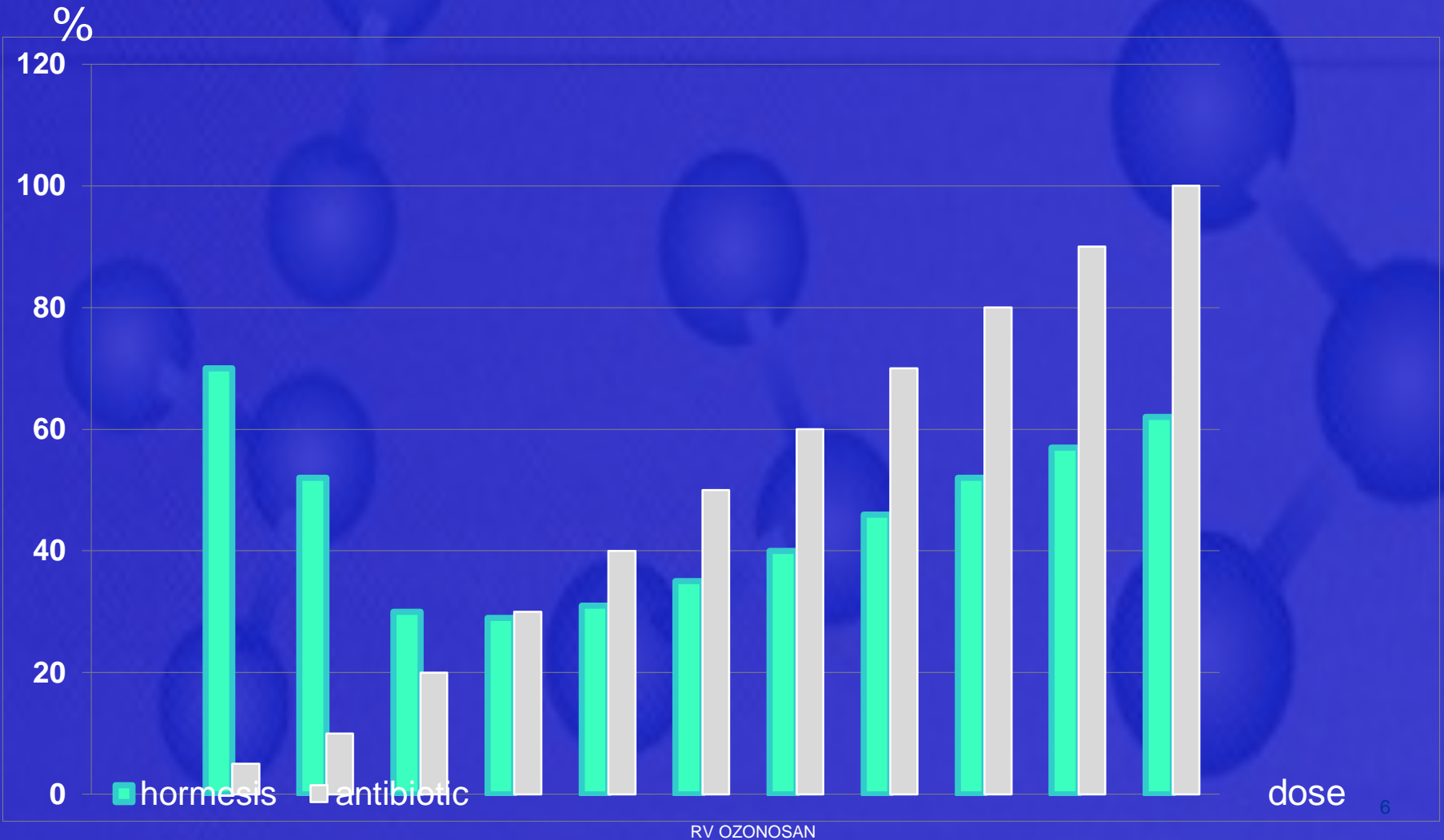
positive oxidative stress

→ →

BIOREGULATION OF
ANTIOXIDANTS

Hormesis

Efficacy-Dose



Medical Ozone

Medical Ozone is a mixture of pure Oxygen and pure Ozone

0,05 % O₃	-	5 % O₃ (Vol.)
99,95 % O₂	-	95 % O₂

1 µg /ml	-	100 µg/ml	O₃
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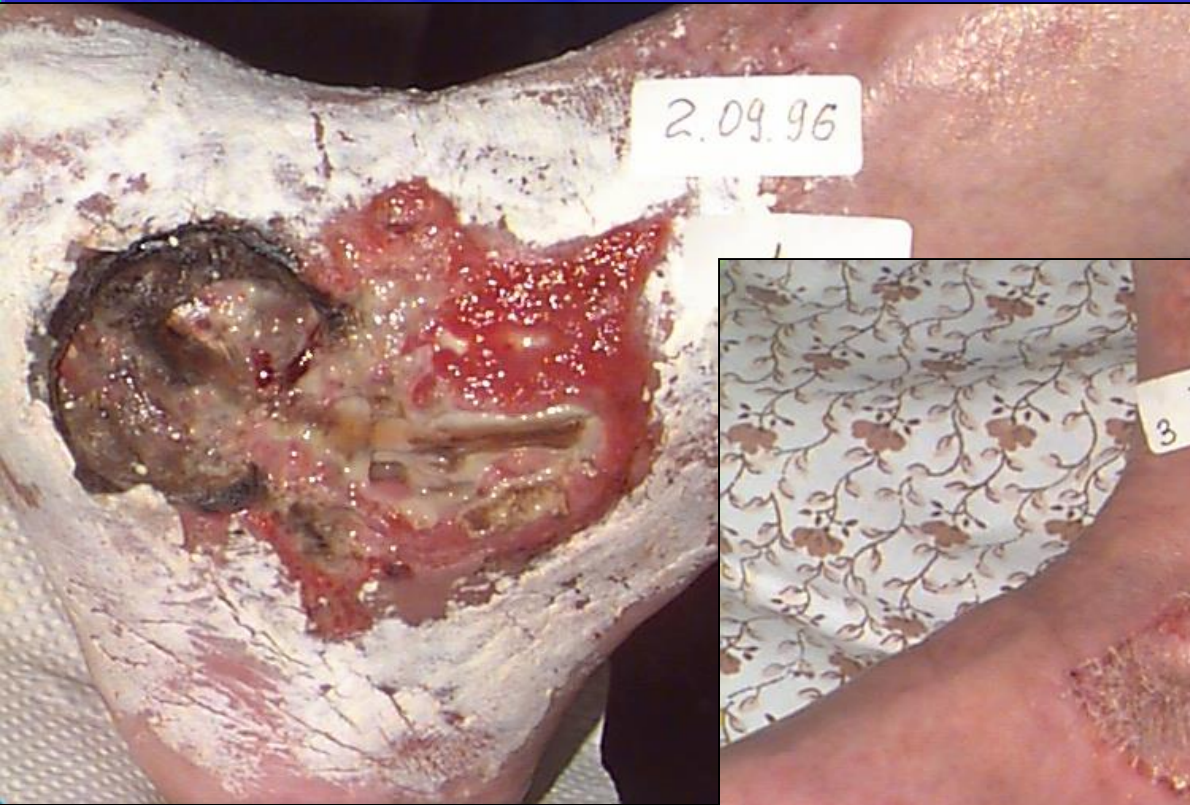


Fathi, Mawsouf 2007



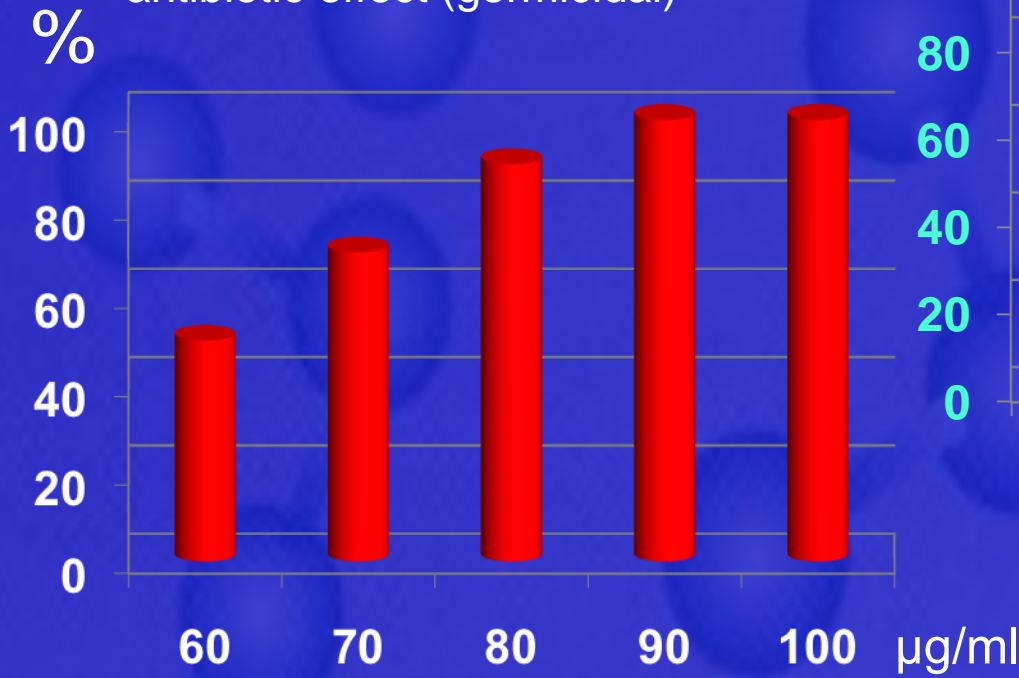
Fathi, Mawsouf 2007

Pat. m. 68 J. Peripheral arterial disorders,
Diabetes mellitus

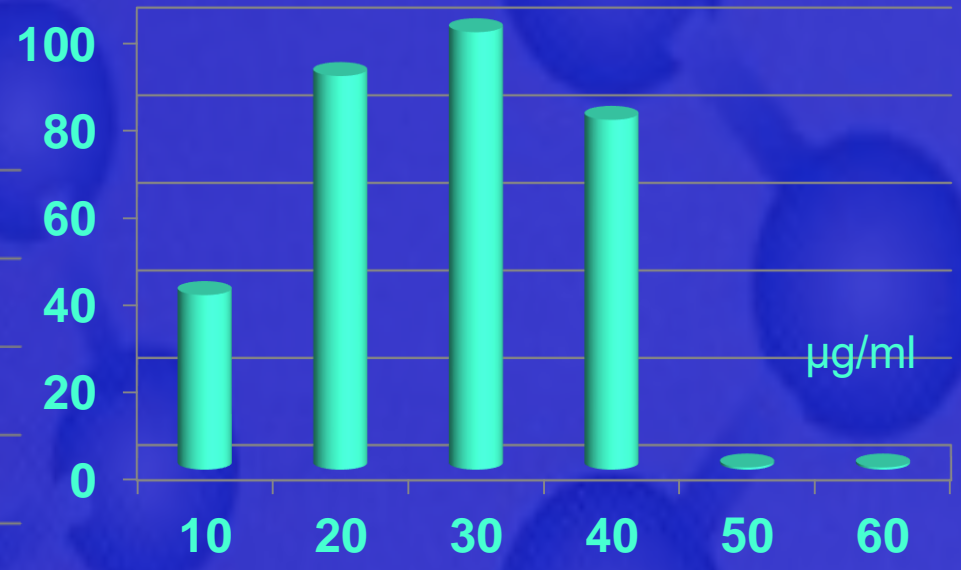


Concentration ($\mu\text{g/ml}$) – Efficacy (%) of short time topically administered Medical Ozone

Wound cleansing
antibiotic effect (germicidal)



%



Wound healing
systemic effect

I. INTRODUCTION

Ozone in Medicine: The Low-Dose Ozone Concept—Guidelines and Treatment Strategies

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The low-dose ozone concept with its moderate oxidative stress represents an ideal hormesis strategy. Dose-response and concentration-effect relationships in the context with specific applications allow one to fix concentration ranges with therapeutical benefit. Based on the well-known reaction mechanisms of ozone, its biochemical and pharmacological effects, international guidelines have to be defined concerning physiological and ozone resistant materials, indications, applications and the effective concentration and dosage range in dependence on the specific indications. Following the international regulations of ozone concentrations outdoors and indoors, as working site concentrations by WHO (World Health Organization) and in conformance with the Medical Device Directives (MDD) for quality assurance and control, some European Medical Societies for the Use of Ozone have set up a draft for the essential requirements for the treatment procedures, including: (a) production of Medical Ozone: reactivity of O₃ and ozone-resistant materials; (b) ozone-free surroundings (WHO regulations) by the integration of effective catalytic systems; (c) ozone-resistant and physiologically indifferent materials used in disposables for MAH, intra-articular, intramuscular and other topical injections (for rectal insufflation, topical treatment as transcutaneous gas bath and/or disinfection); (d) medical device directives for safety of the patient; (e) ozone measurement as requirement for concentrations and dosages; and, (f) concentration, dosages, and treatment frequency are listed in dependence on the indication and disease as well as on the underlying mechanism of action.

Keywords Ozone, Ozone Therapy, Guidelines, Hormesis, Treatment Concepts, Concentration, Dose

INTRODUCTION

When used in specific diseases and conditions, medical ozone produces the same or similar therapy results worldwide. Improper application in the form of erratic methods and doses is the most frequent cause of ineffectiveness and adverse effects—and is always the cause of violent controversies.

For this reason, the medical societies for ozone application have set up treatment protocols as basis for standards and guidelines, revised and published as a result of the most recent research and 30 years of experience (Beck et al. 1998; Knoch et al. 2009). They have been used in the standardization of applications, indications, concentrations, doses, and frequency of treatment as based on the mechanism of action and the pharmacology of ozone.

1. In its pharmacological effect, medical ozone follows the principle of hormesis: low concentrations (or doses) show a high efficacy, which decreases with increasing concentration, finally reversing into a questionable and even toxic effect (Figure 1). The effect/

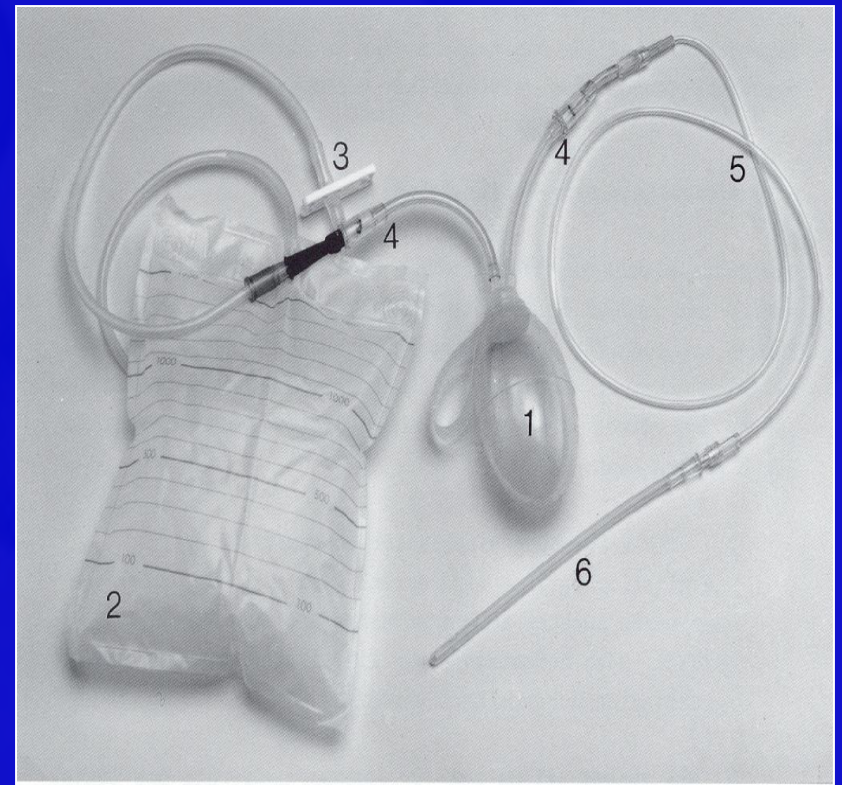
CTA-Lower Limbs Arteries

- Rt Peroneal & Ant Tibial As:
 - Nearly totally occluded all length.

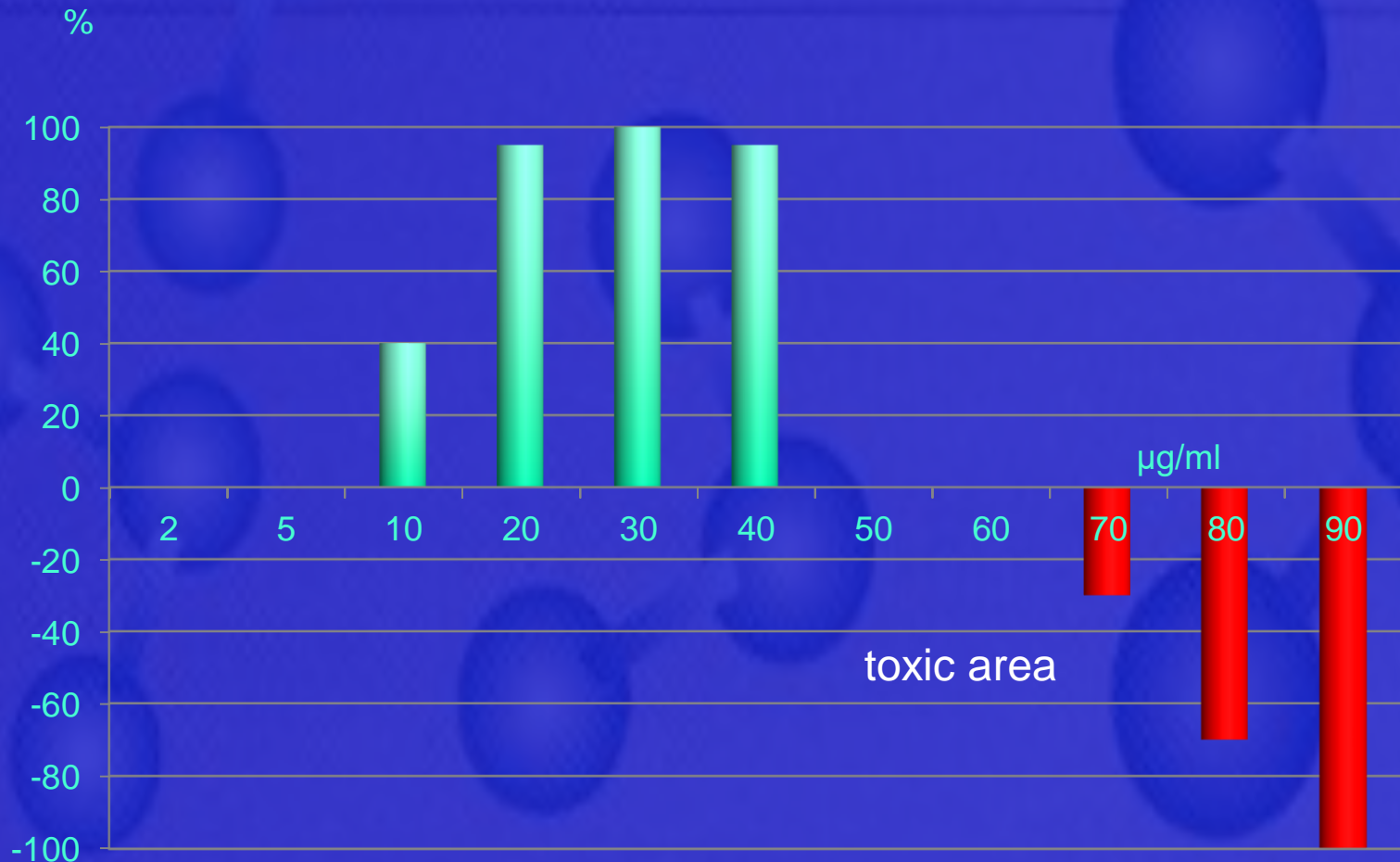
- Rt Dorsalis Pedis A:
 - Distally reconstituted in parts.



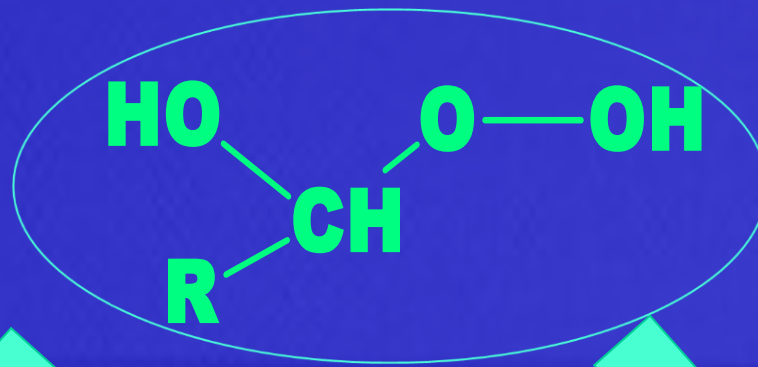
Major Auto Hemo-Therapy and Rectal Insufflation as Systemic Ozone Applications



Concentration ($\mu\text{g/ml}$) – Efficacy (%) of systemically administered Medical Ozone



Cellular Ozone effects
in MAH and rectal
insufflation following
the Low-Dose
Concept



„Ozone peroxide“
as second
messenger ROS

Cystein/GSH

Red Blood Cell
↑G-6P-DH

GSH
Nrf2



White Blood Cell
NFκB

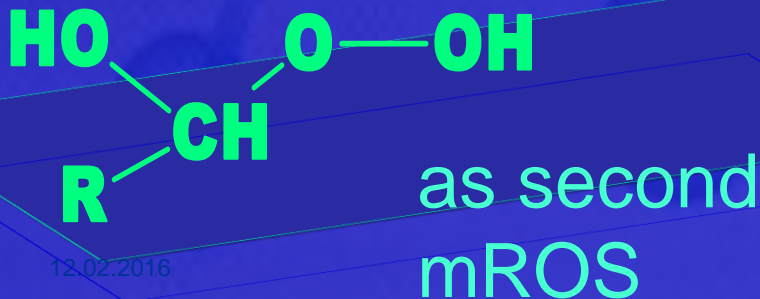
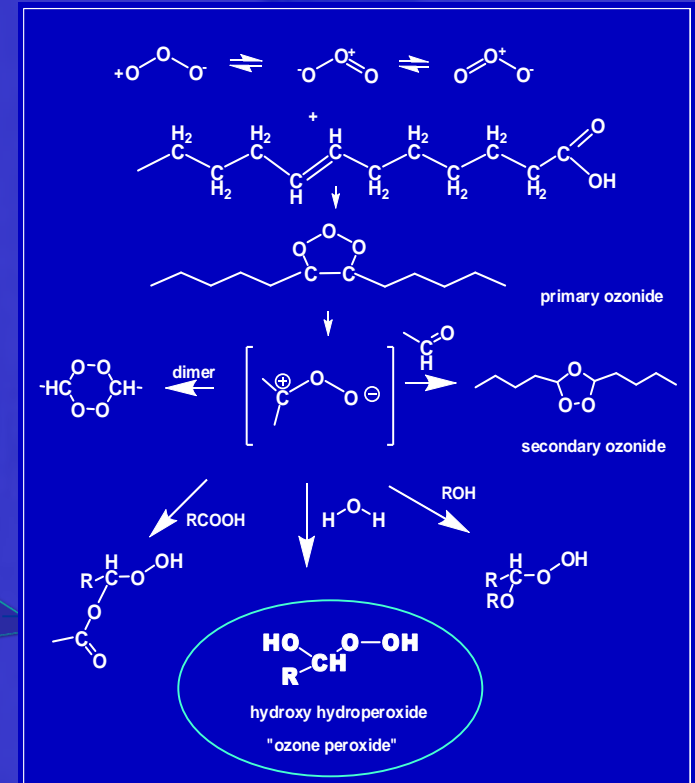
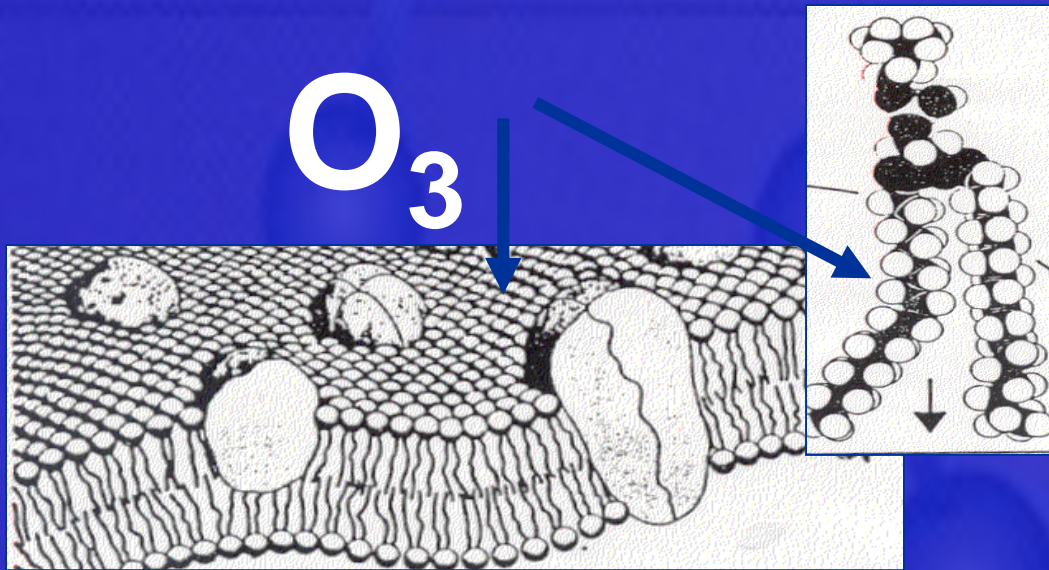


↑ Oxygen release
Oxygen
availability

Immunomodulation

Cellular
redox
regulation

Ozonolysis: Ozone + unsaturated fatty acid with isolated double bond (Crigée Mechanism)



Complementary
therapeutical
concepts

Proinflammatory factors

repair
mechanisms

Information to CD4
helper cells as key
regulators of the
biological response

TH2 >>> TH1

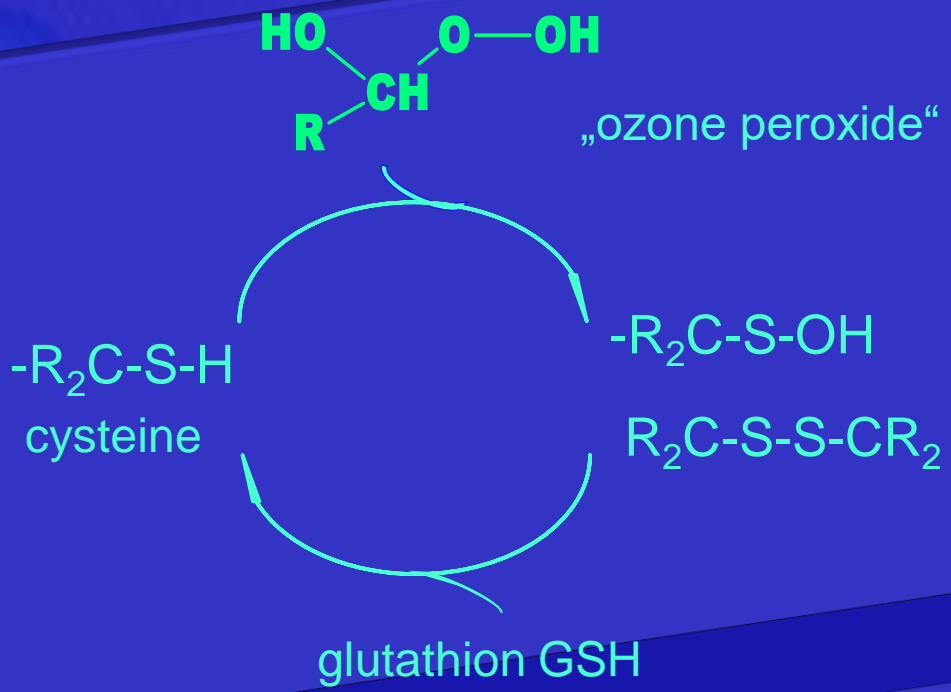
proinflammatory
signal transduction

IL-4, IL-10, IL-13 → IL-1, IL-6,
TNF-α, VEGF,
TGF-β ...

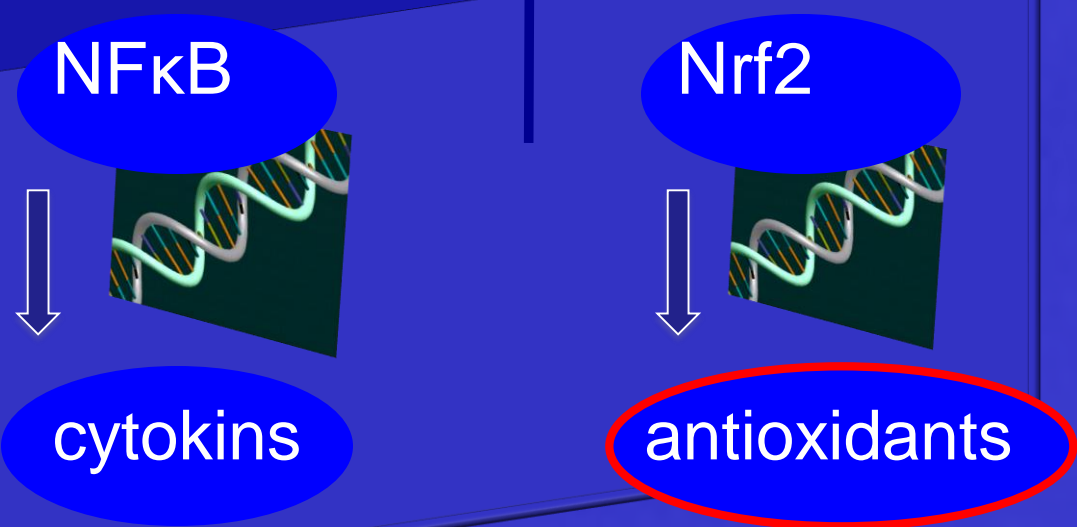
tumor promotion,
rheumatoid arthritis,
angiopathia, diabetes 2
age related diseases,

NO•

O₂•



„Ozone peroxides“
 as
 second messenger
 species: smROS



Signal transduction
 via
 nuclear factor NFkB
 during stress and
 inflammation,

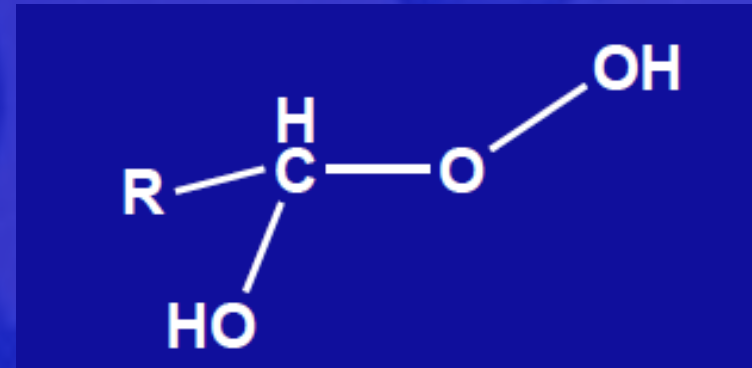
 via Nrf2 for regulation
 of the
 antioxidant system



Hydroperoxide:

long chain, middle position,
+ R• radical chain reactions forming
free radicals, MDA ...

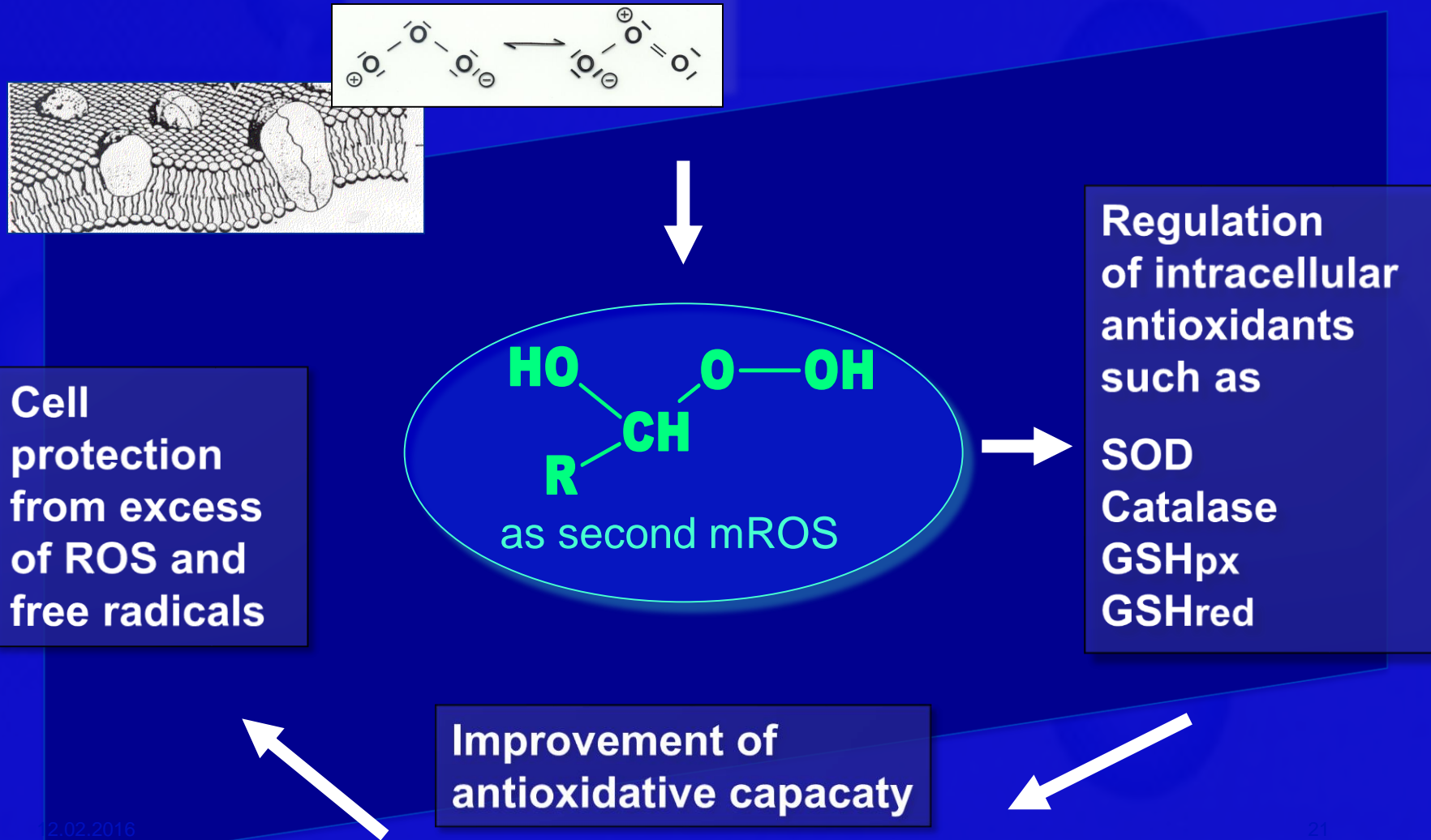
Measure for oxidative stress



„Ozone Peroxide“ Hydroxy hydroperoxide:

short chain, end position,
no radical chain reactions;
reaction with SH-

„Ozone-Peroxide“ LOP as second messenger „Reactive Oxygen Species“: mROS





$\bullet\text{O}_2^-$ H_2O_2
 RCROOH
 MDA

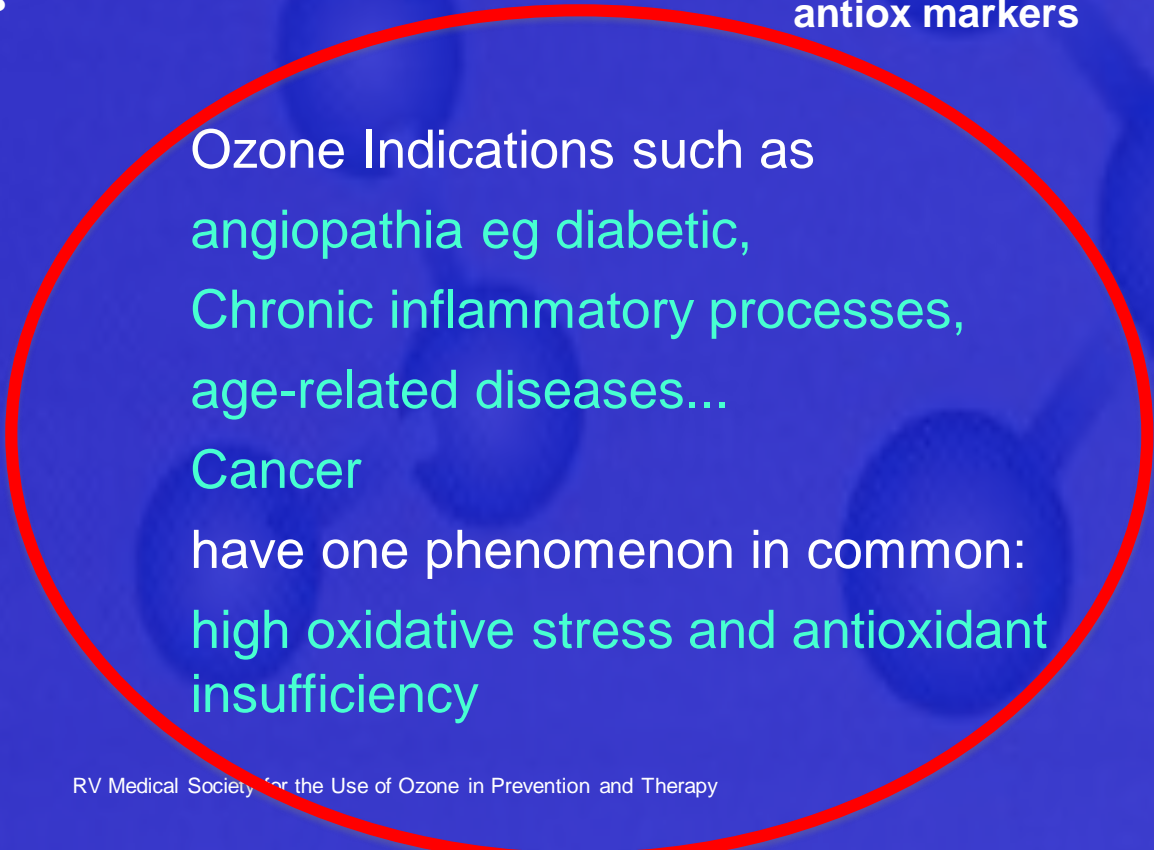
ox stress markers



SOD
 CAT ...

antiox markers

Biomarkers of chronic oxidative stress situation



Oxidant – Antioxidant Balance

OXIDANTS ROS



Antioxidants

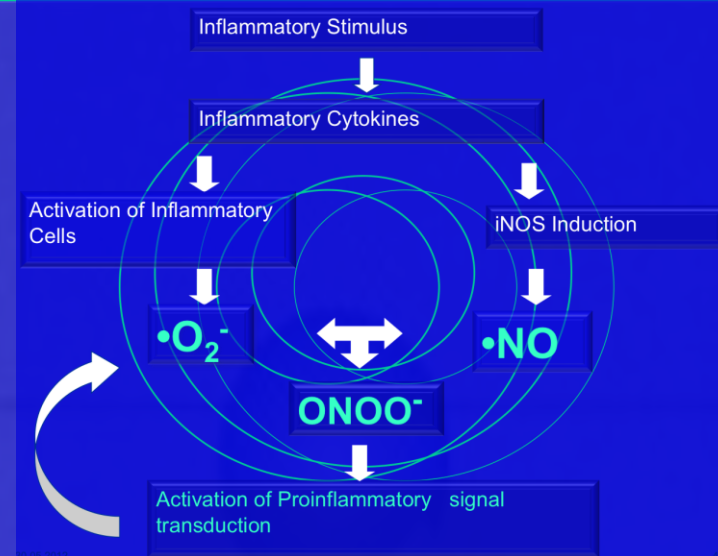
Superoxide dismutase
Catalase, GSH-px.....

Ozone related indications accompanied by oxidative dys-stress



$\bullet\text{O}_2^-$ superoxide
 H_2O_2 hydrogen peroxide
 RCROO hydro peroxide

Superoxide
 dismutase SOD
 Catalase CAT ...



Ozone Indications such as angiopathia eg diabetic, Chronic inflammatory processes, age-related diseases...

Cancer

have one phenomenon in common:

high oxidative stress and antioxidant insufficiency



$\bullet\text{O}_2^-$ H_2O_2
 RCROOH
 MDA



O_3



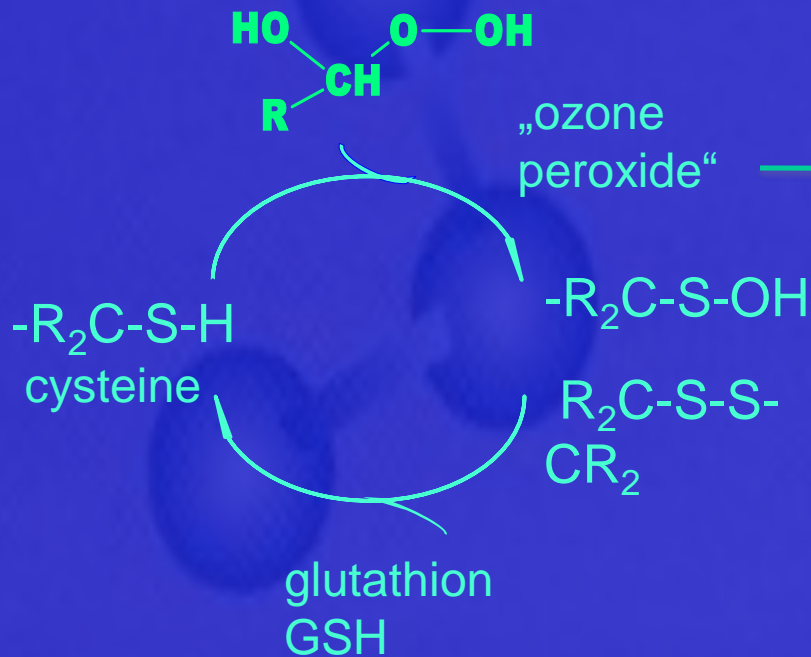
SOD
 CAT ...



O_3

ox stress markers

antiox markers



no SOD
 no CAT
 demand!!!

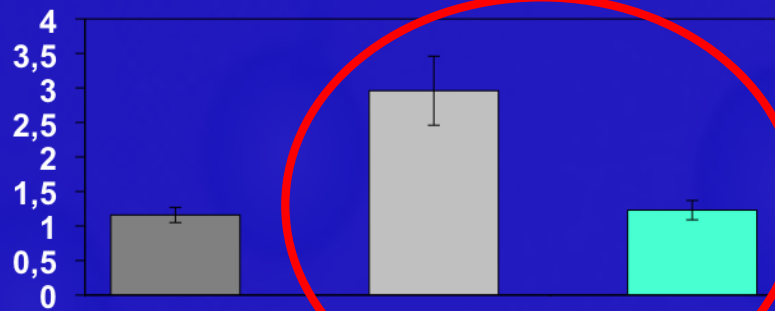
low dose ozone
 concept

Influence on
 chronic oxidative
 stress situation by
 ozone 02.2016

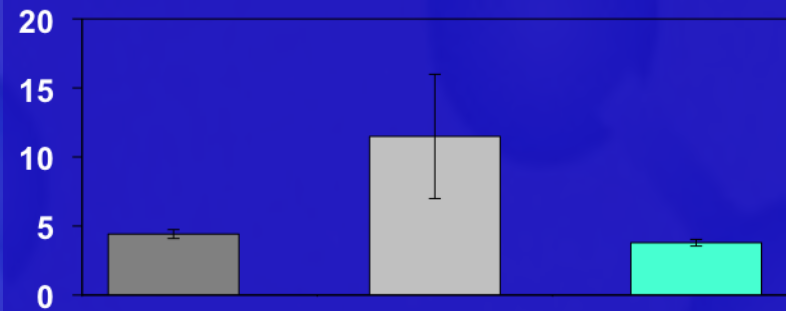
Plasma markers of injury: reference, DH patients before and after ozone treatment

(León 2008)

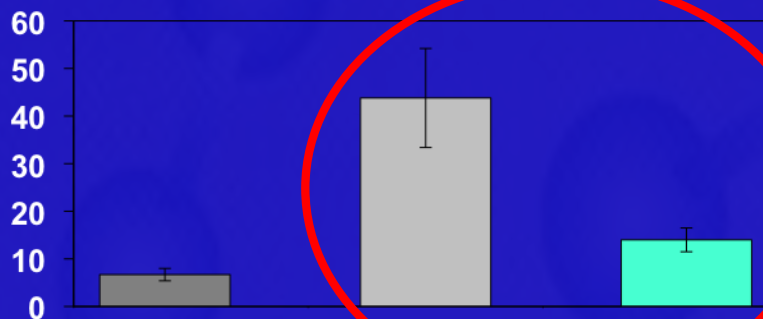
MDA in μmol



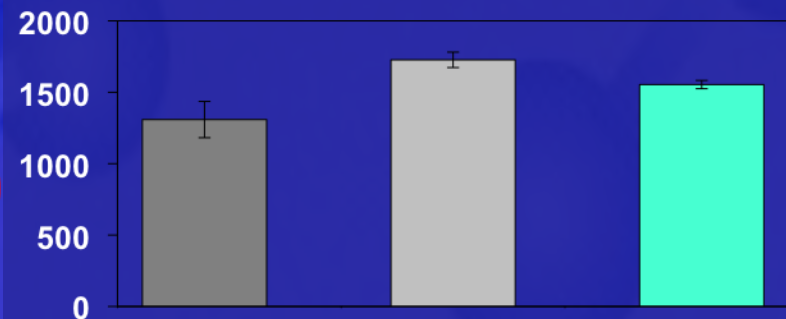
PP in μmol



SOD in u/l/min



GSH in μmol



12.02.2016



reference



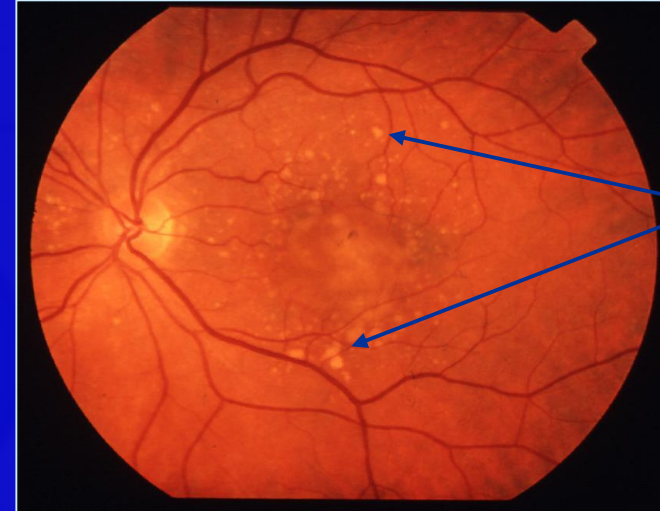
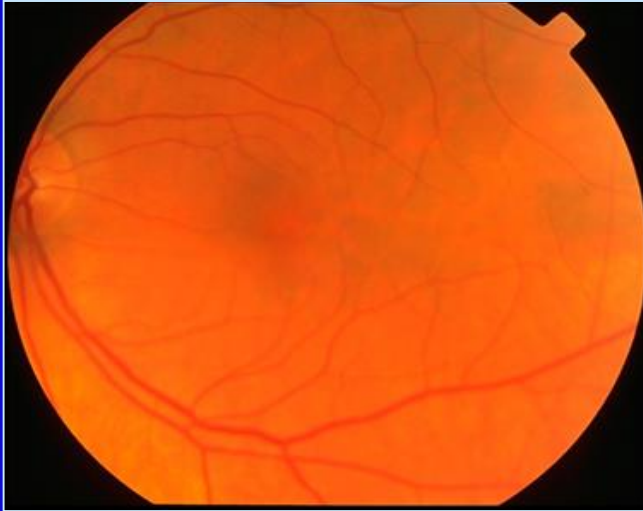
DH patients start

iv OZONOSAN



DH patients 20 treatments

Progression of AMD



druse

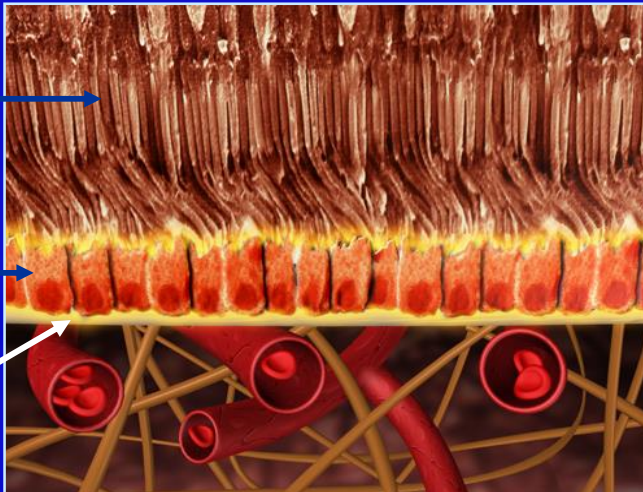
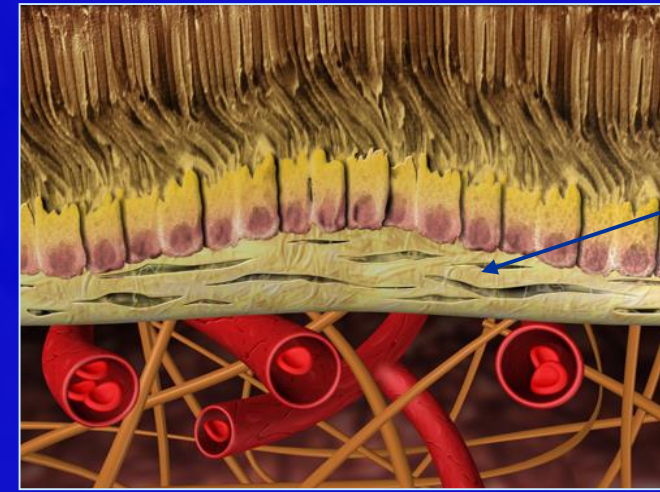


photo-receptors

RPE

Bruch's membrane

healthy retinal tissue

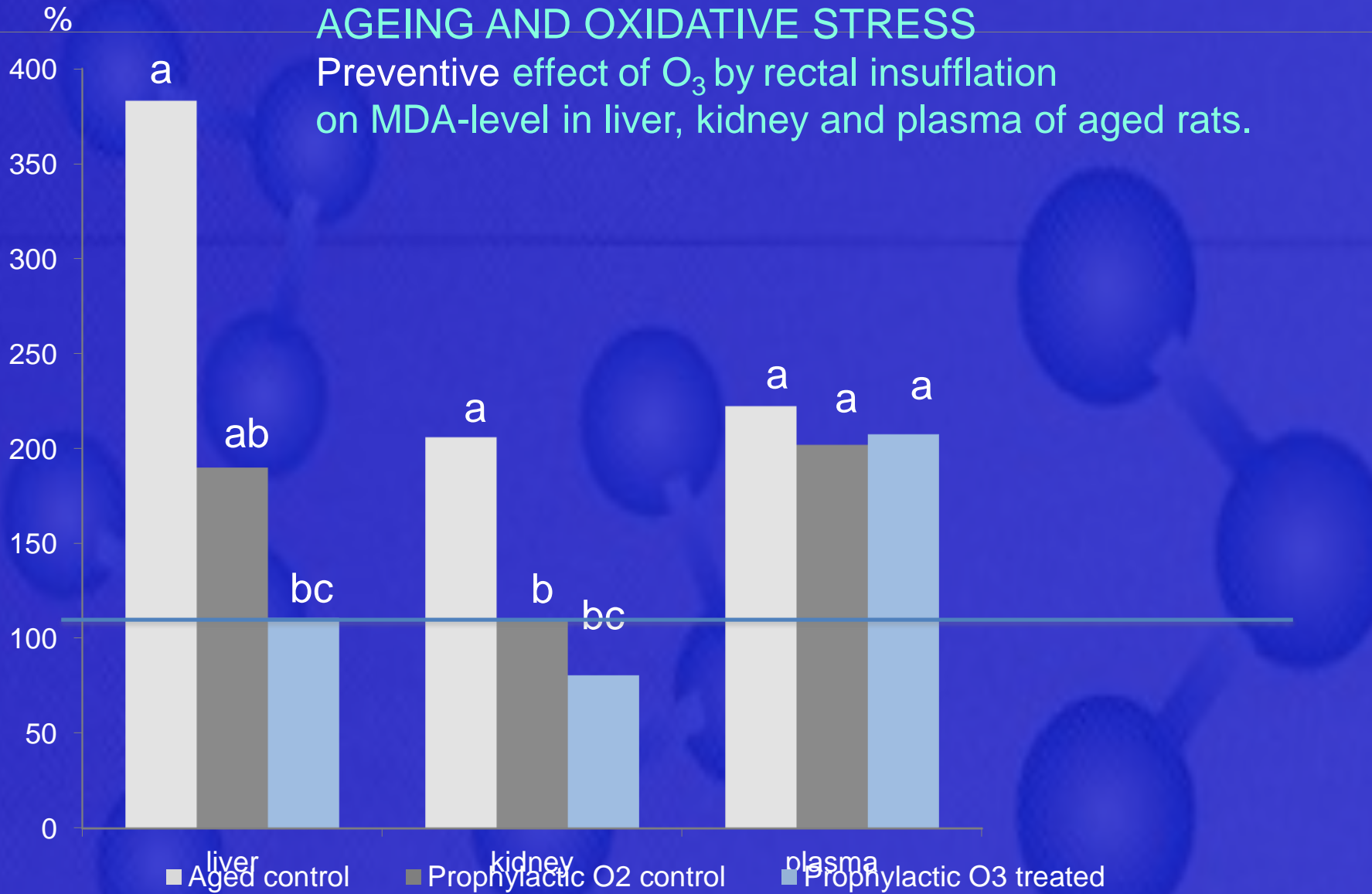


druse

swelling of Bruch's membrane (drusen)

AGEING AND OXIDATIVE STRESS

Preventive effect of O₃ by rectal insufflation on MDA-level in liver, kidney and plasma of aged rats.



Values are expressed as percentage of adult control (%mean \pm %SE)

a: significant difference from adult control at P < 0.05.

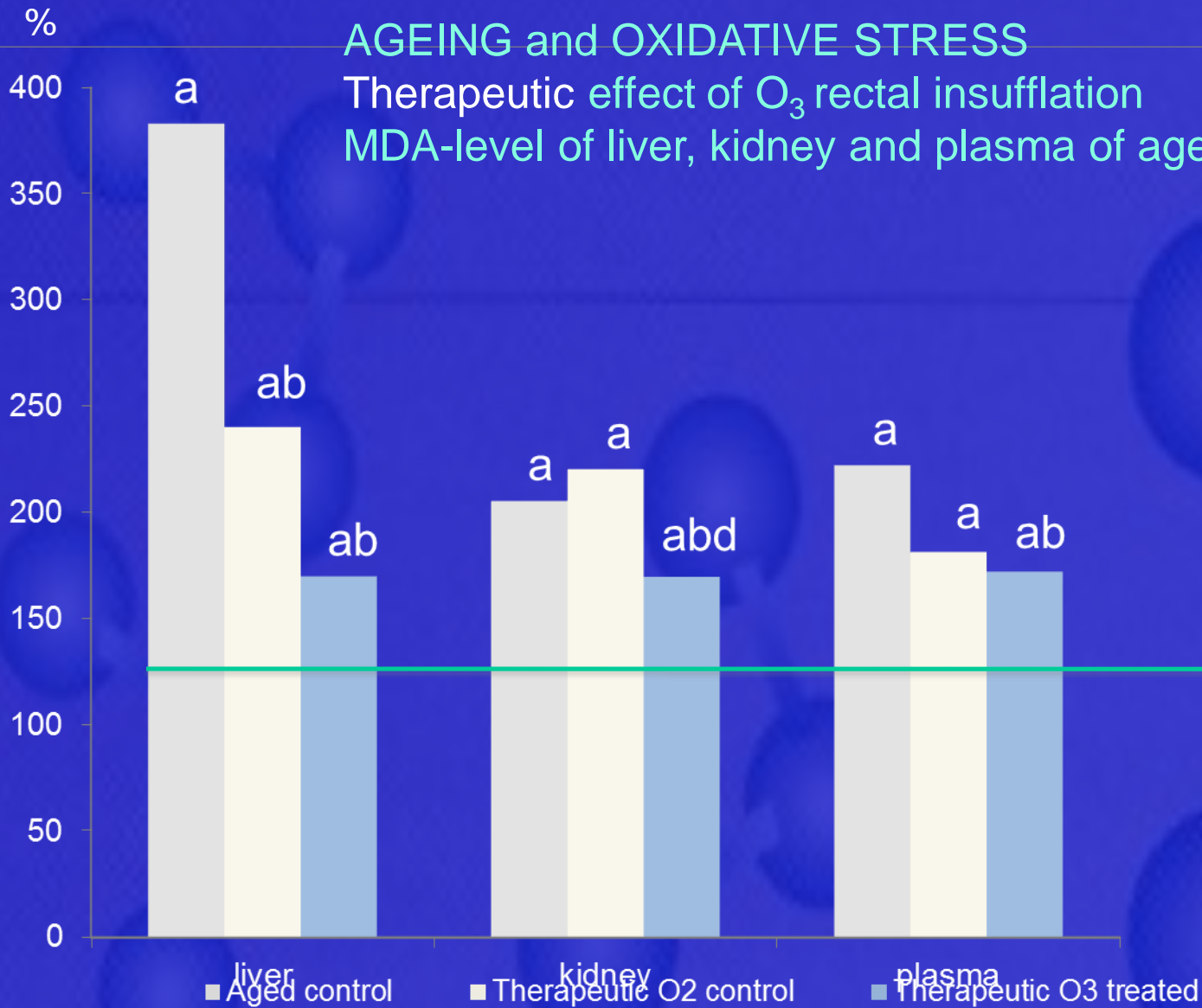
b: significant difference from aged control at P < 0.05.

c: significant difference from prophylactic O₂-control at P < 0.05.

AGEING and OXIDATIVE STRESS

Therapeutic effect of O₃ rectal insufflation

MDA-level of liver, kidney and plasma of aged rats.



Values are expressed as percentage of adult control (%mean \pm %SE)

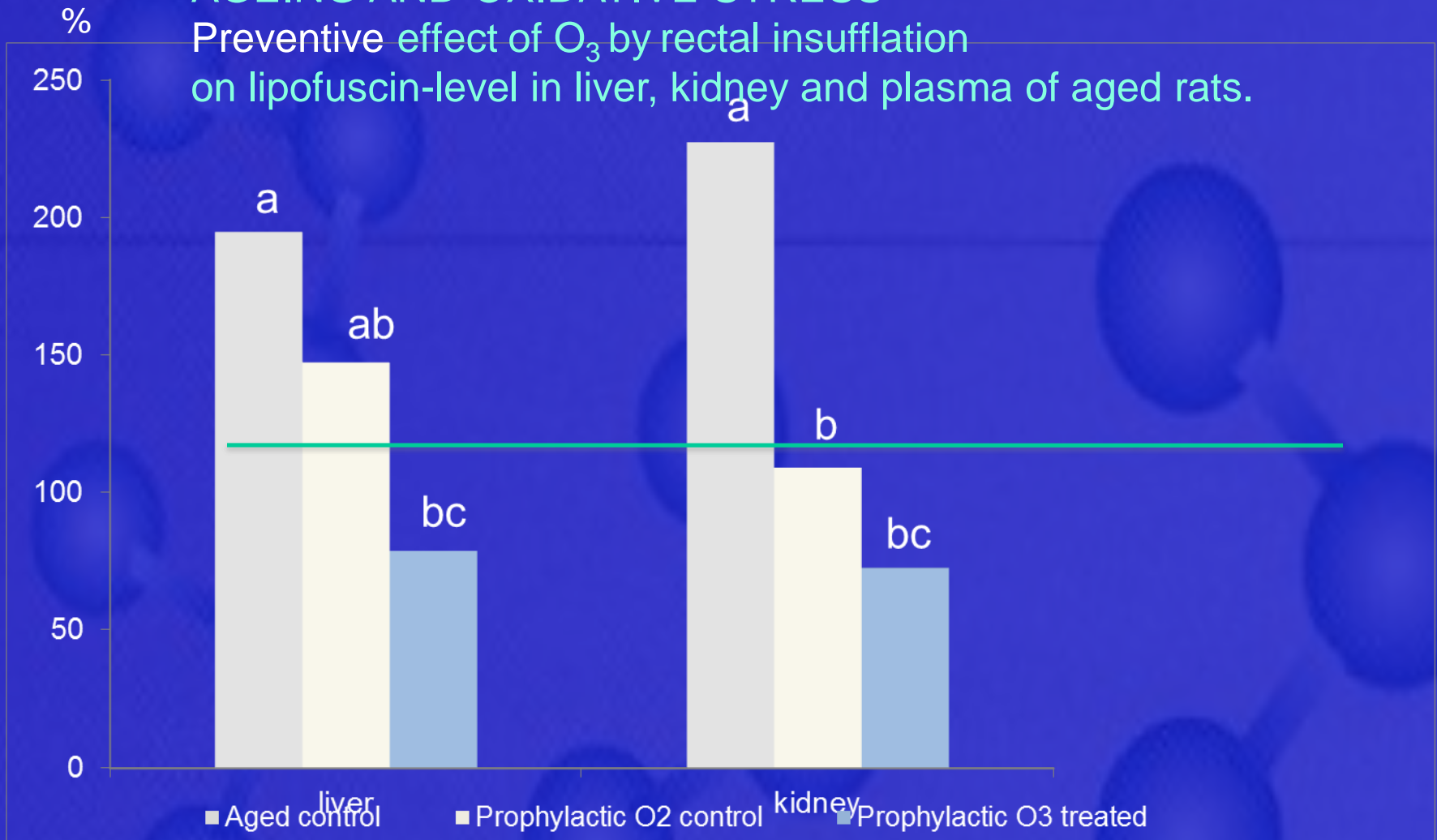
a: significant difference from adult control at $P < 0.05$.

b: significant difference from aged control at $P < 0.05$.

d: significant difference from therapeutic O₂-control at $P < 0.05$.

AGEING AND OXIDATIVE STRESS

Preventive effect of O₃ by rectal insufflation on lipofuscin-level in liver, kidney and plasma of aged rats.



Values are expressed as percentage of adult control (%mean \pm %SE)

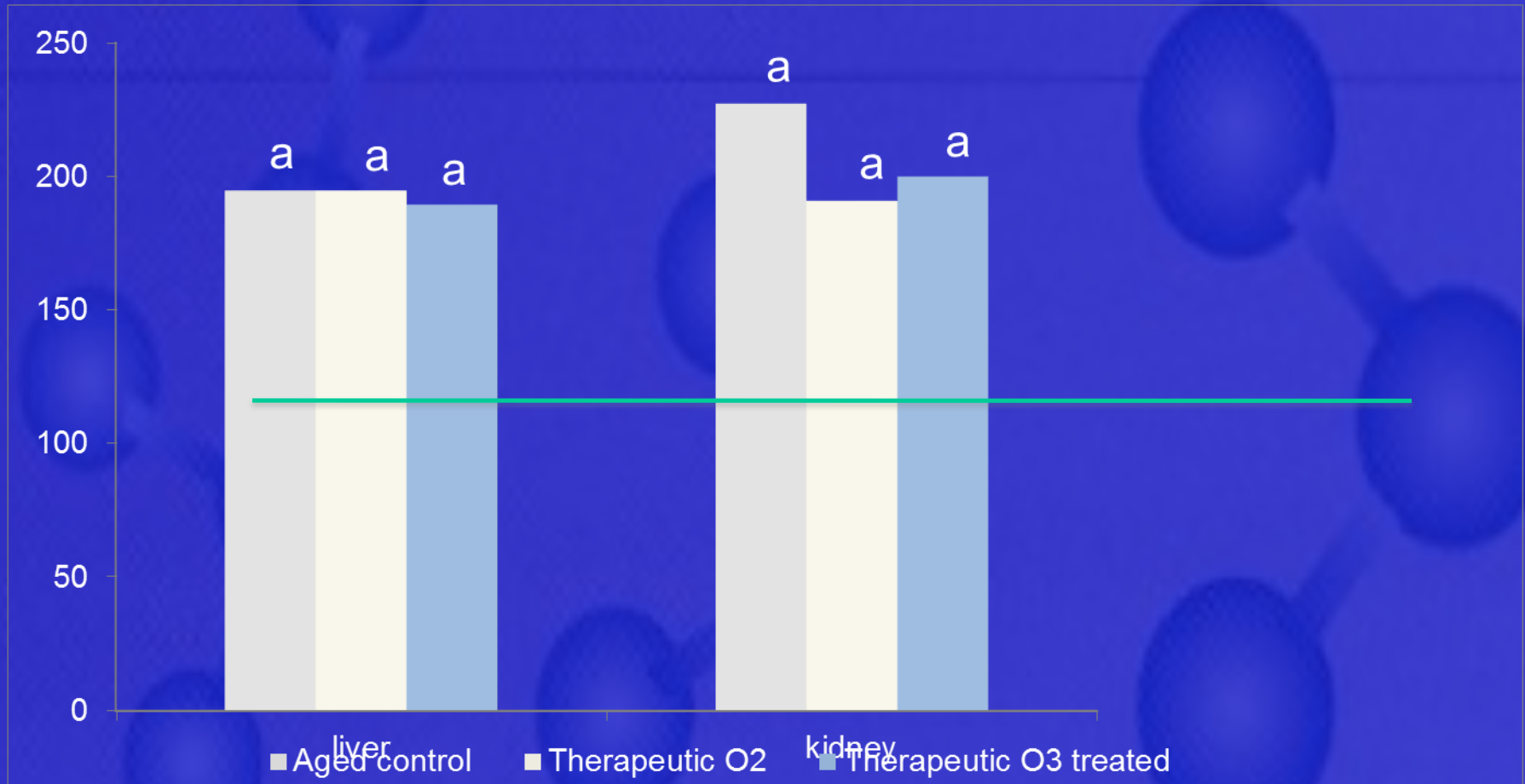
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CTA-Lower Limbs Arteries

- **Rt Post Tibial A:**
 - Patent all through its length apart from multifocal stenotic segments
 - Deep planter branches are fairly opacified

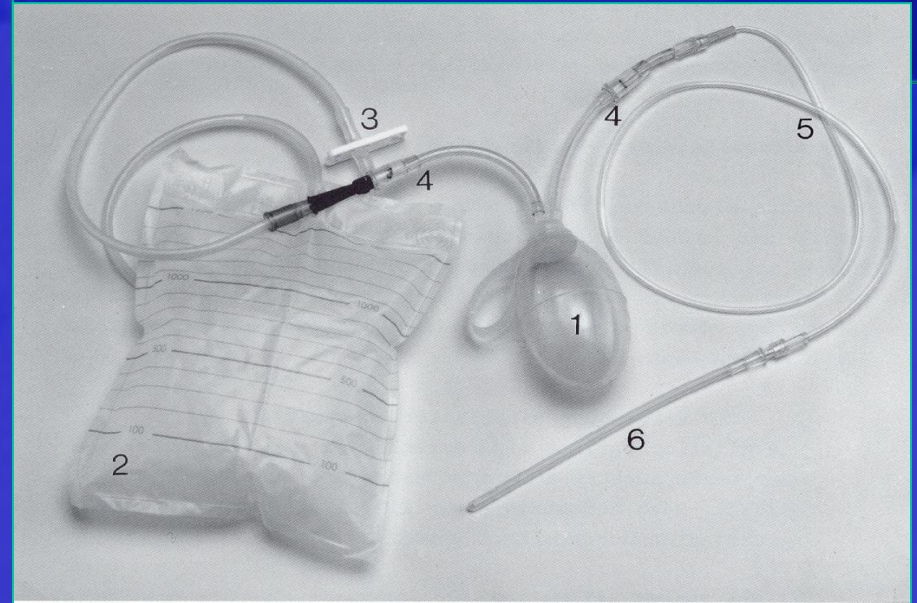
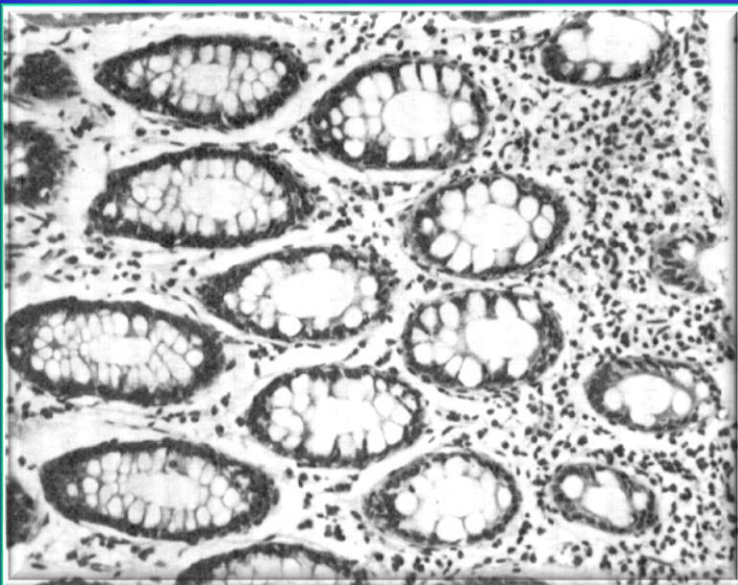
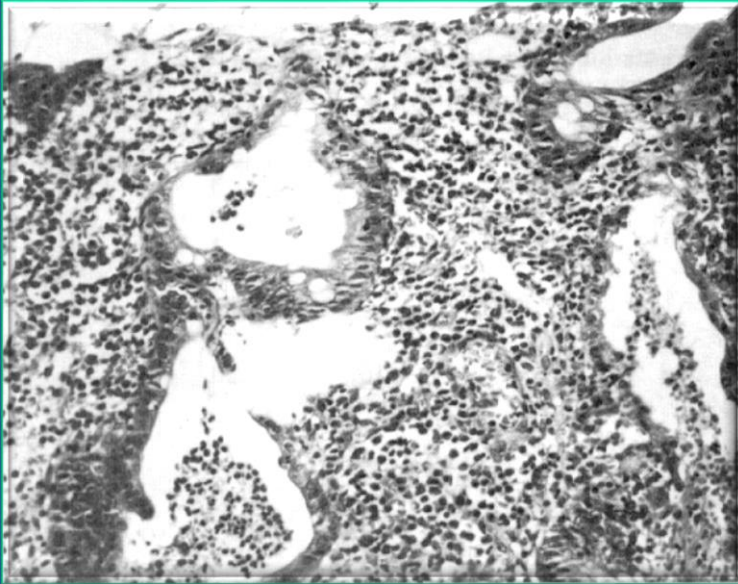


Fathi, Mawsouf 2007

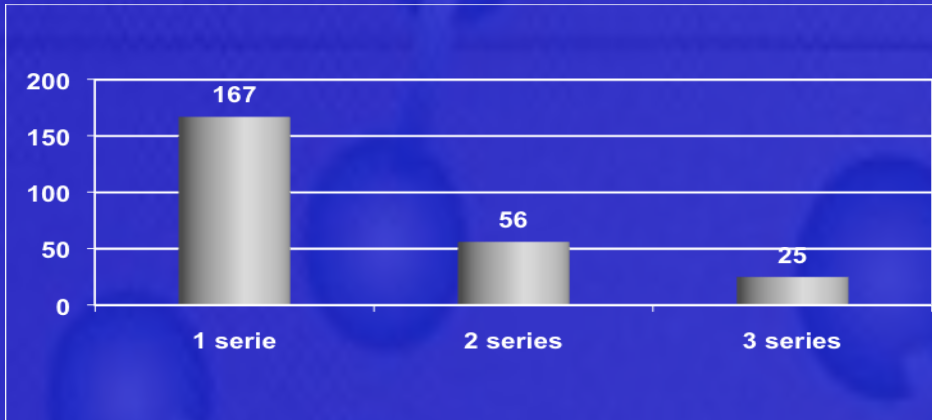
Floride Stage of Proctocolitis

Pat. female, 45 yrs. Destruction of epithelium, cryptal abscesses, massive leucocytic infiltration, partially under inclusion of the tunica propria (350x)

Control biopsy after 4 weeks: Manifest reversal of inflammation. Intact epithelium after rectal ozone insufflation with 20-30 $\mu\text{g}/\text{ml}$ and 300 to 500 ml in volume.



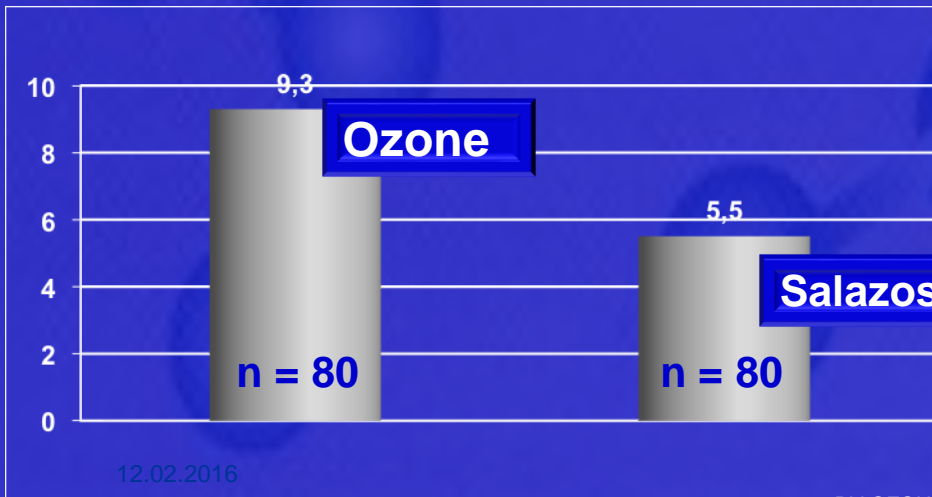
Colitis stage I



Number of patients

$n = 248$

1 serie = 10 treatments



time of remittance

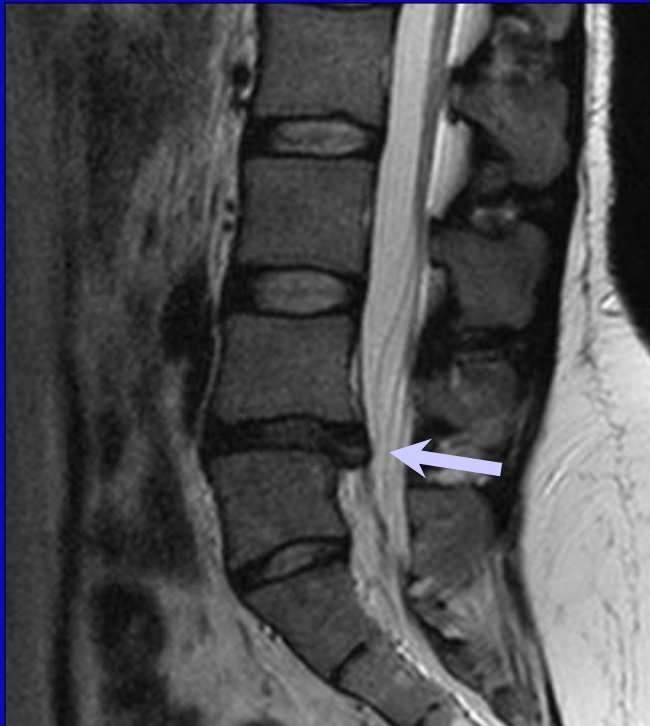
in months

Salazosulfapyridin group

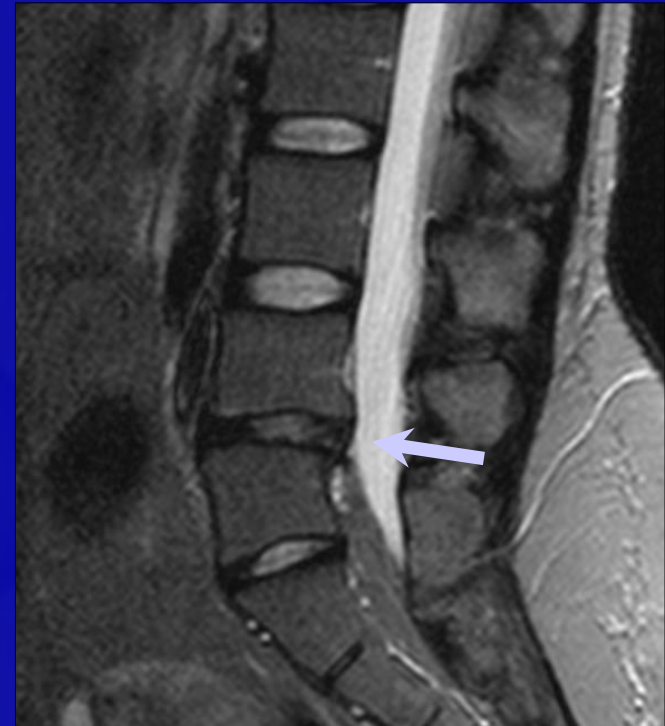
(Knoch et al.1995)

Treatment success following 6 months

before O₂/O₃-injection



6 months after O₂/O₃-injection



A Metaanalysis of the Effectiveness and Safety of Ozone Treatments for Herniated Lumbar Discs

Jim Steppan, PhD, Thomas Meaders, BS, Mario Muto, MD, and Kieran J. Murphy, MD, FRCPC

PURPOSE: To determine statistically significant effects of oxygen/ozone treatment of herniated discs with respect to pain, function, and complication rate.

MATERIALS AND METHODS: Random-effects metaanalyses were used to estimate outcomes for oxygen/ozone treatment of herniated discs. A literature search provided relevant studies that were weighted by a study quality score. Separate metaanalyses were performed for visual analog scale (VAS), Oswestry Disability Index (ODI), and modified MacNab outcome scales, as well as for complication rate. Institutional review board approval was not required for this retrospective analysis.

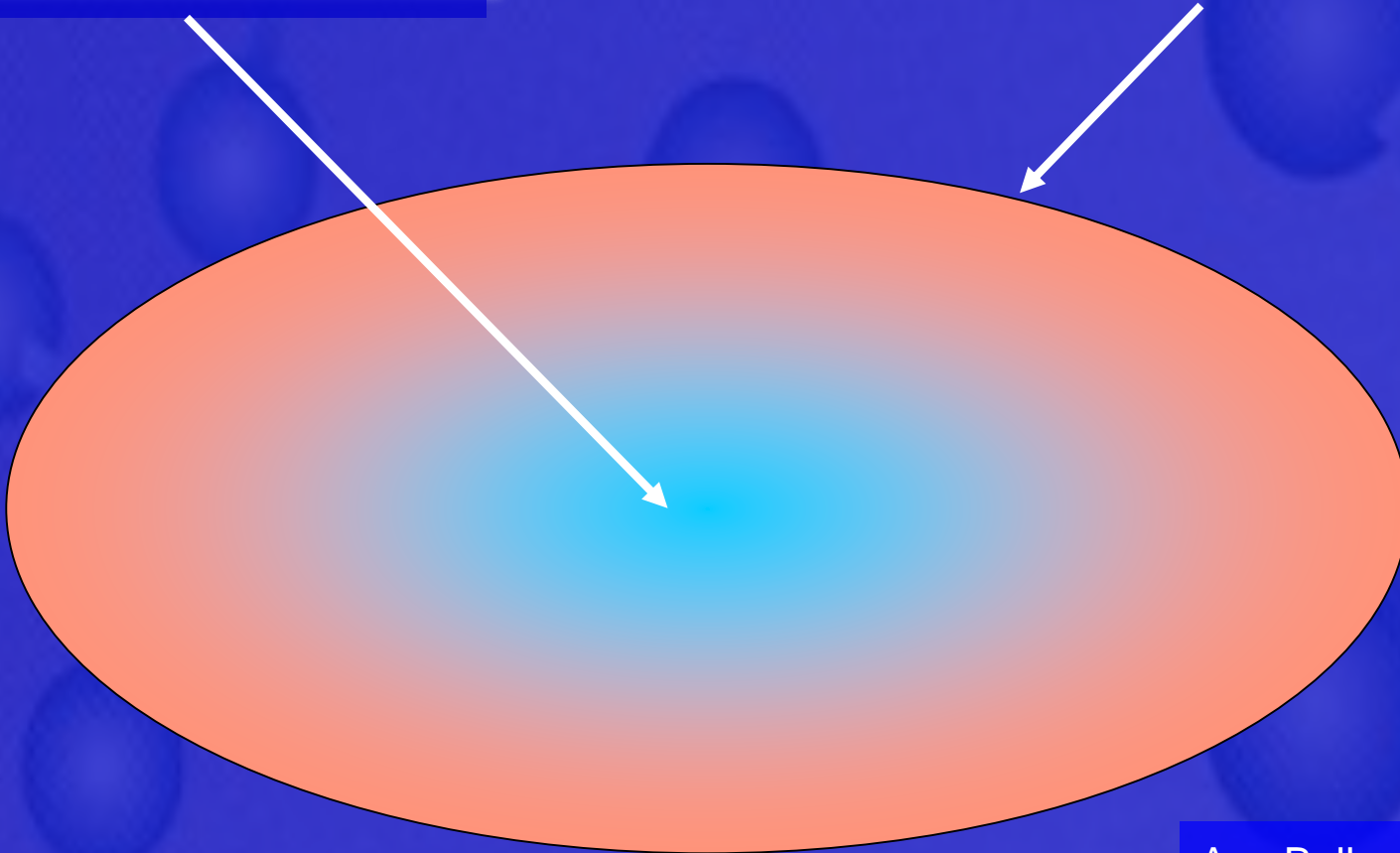
RESULTS: Twelve studies were included in the metaanalyses. The inclusion/exclusion criteria, patient demographics, clinical trial rankings, treatment procedures, outcome measures, and complications are summarized. Metaanalyses were performed on the oxygen/ozone treatment results for almost 8,000 patients from multiple centers. The mean improvement was 3.9 for VAS and 25.7 for ODI. The likelihood of showing improvement on the modified MacNab scale was 79.7%. The means for the VAS and ODI outcomes are well above the minimum clinically important difference and the minimum (significant) detectable change. The likelihood of complications was 0.064%.

CONCLUSIONS: Oxygen/ozone treatment of herniated discs is an effective and extremely safe procedure. The estimated improvement in pain and function is impressive in view of the broad inclusion criteria, which included patients ranging in age from 13 to 94 years with all types of disc herniations. Pain and function outcomes are similar to the outcomes for lumbar discs treated with surgical discectomy, but the complication rate is much lower (<0.1%) and the recovery time is significantly shorter.

pO₂ Gradient in Myogelosis

pO₂ = 5 mm Hg

pO₂ = 38,5 mm Hg (n = 20)
chronically hypertonic muscle
(29,5 in healthy patients n = 10)

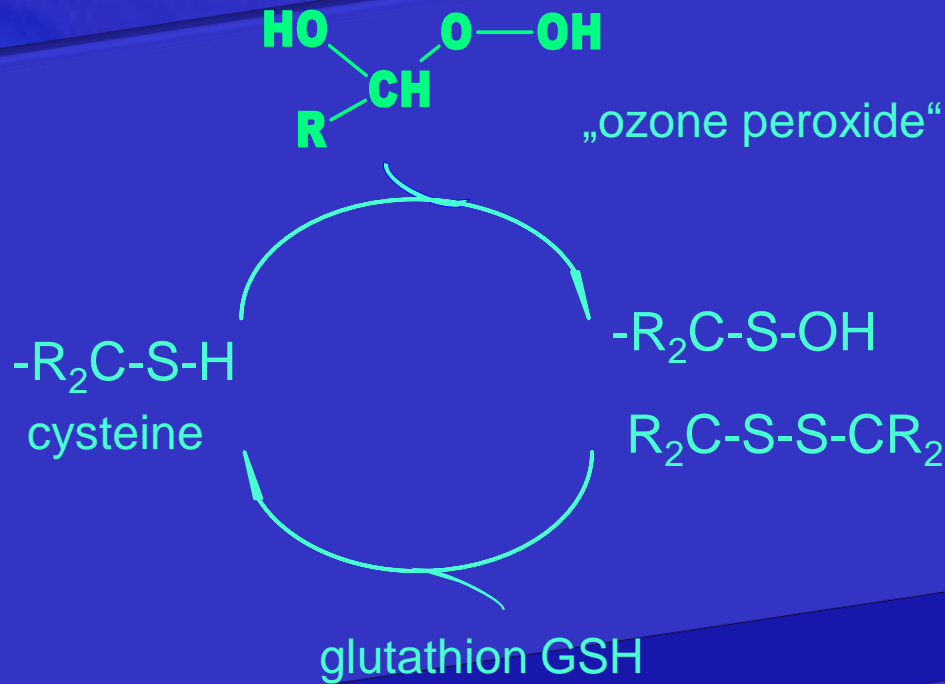


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J Vasc Interv Radiol 2010; 21:534–548

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„Ozone peroxides“

as
second messenger
species: smROS

NFkB



cytokins

Nrf2



antioxidants

Signal transduction
via
nuclear factor NFkB
during stress and
inflammation,

via Nrf2 for regulation
of the
antioxidant system

OZONE AS A COMPLEMENTARY STRATEGY to therapeutical concepts in chronic inflammatory conditions ????

Inhibition
of pro inflammatory
targets

Boost of
antiinflammatory
pathways

Reprogramming /
depleting immune
cells

NF-κB -
proinflammatory
pathways

**Arachidonic acid
metabolism**

**Inflammatory
cytokines**
VEGF antagonists
TNFα-, Il-6, IL-1 (-R)
antagonists.....

Autophagocytosis

T-cells
CSF1 (-R), IL-4 (-R)
antagonists.....
B cell depletion
vaccines.....

Acc. to Coussens et al. Science 2013

**Blocking immune
cell recruitment-**
CSF1 (-R)
antagonists....

**Inflammasome
inhibitors**
...reprogramming
of type 2
macrophages
TH2>>>TH1
reprogramming
...IL-4, IL-13, IL-10
antagonists

OZONE AS A COMPLEMENTARY STRATEGY to therapeutical concepts in chronic inflammatory conditions ????

Inhibition
of pro inflammatory
targets

NF-κB -

proinflammatory
pathways

Arachidonic acid metabolism

Inflammatory cytokines

VEGF antagonists
TNFα-, Il-6, IL-1 (-R)
antagonists.....

Boost of
antiinflammatory
pathways

Autophagocytosis

T-cells

CSF1 (-R), IL-4 (-R)
antagonists.....
B cell depletion
vaccines.....

Acc. to Coussens et al. Science 2013

**Reprogramming /
depleting immune
cells**

Blocking immune cell recruitment-

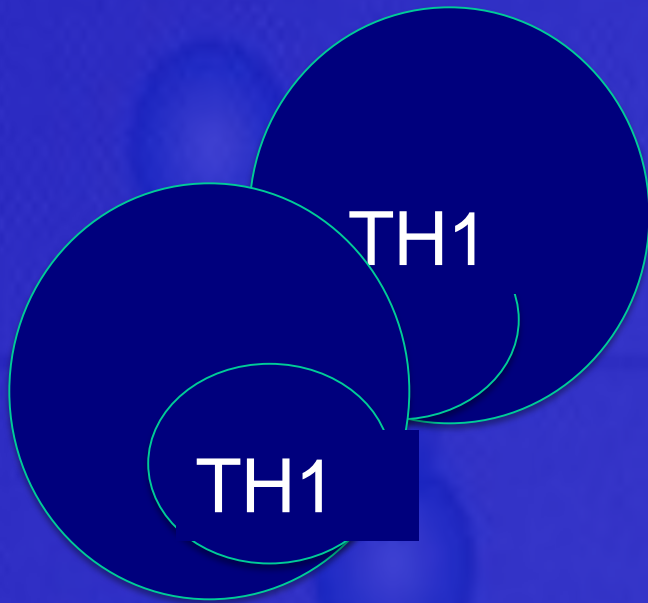
CSF1 (-R)
antagonists....

Inflammasome inhibitors

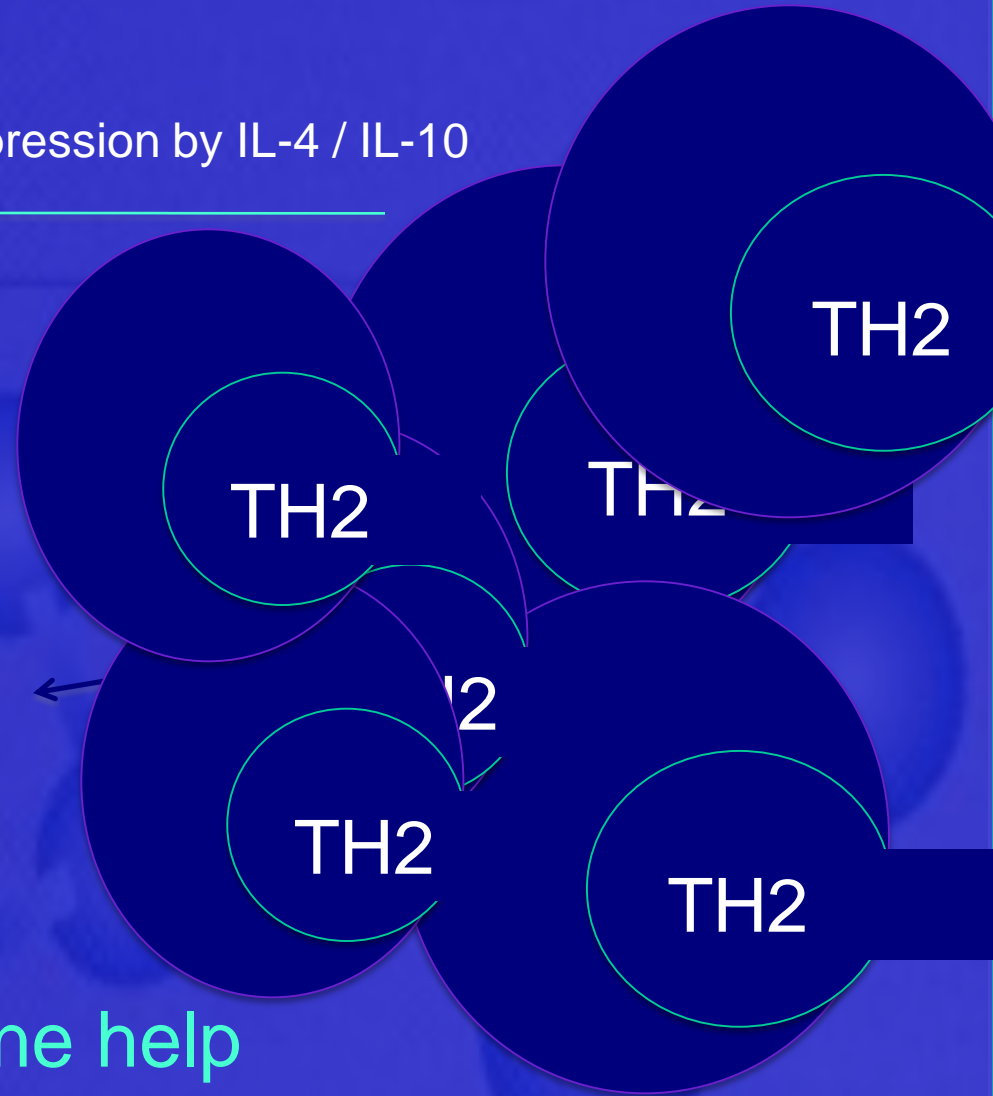
...reprogramming
of type 2
macrophages

TH2>>>TH1 reprogramming

...IL-4, IL-13, IL-10
antagonists



supression by IL-4 / IL-10



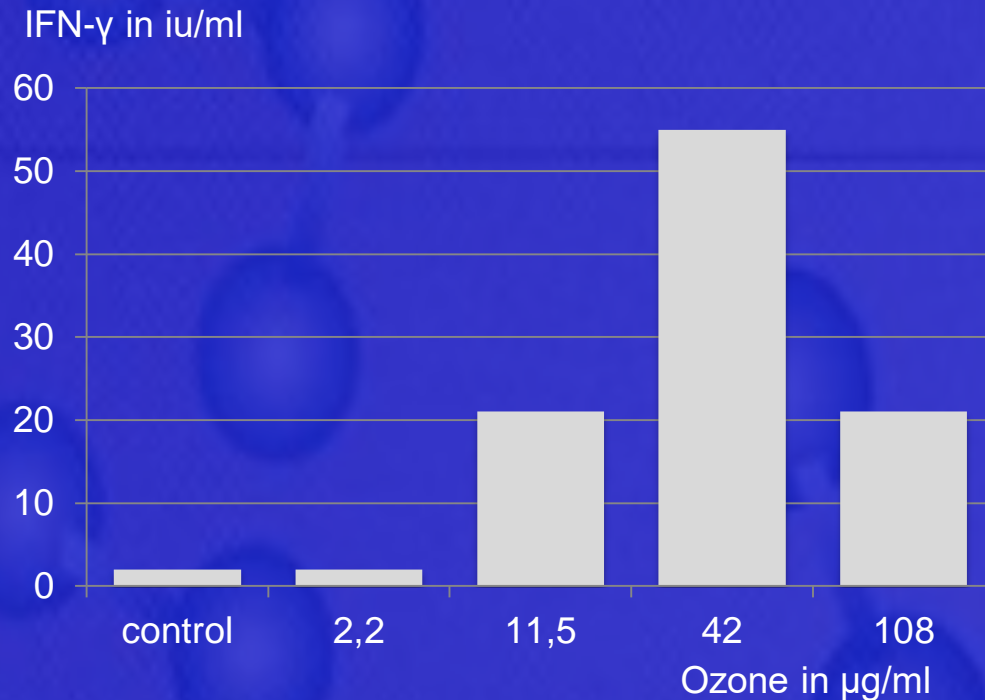
IL-4 / IL-10 /
IL-13 / TNF- α



VEGF / EGF/
TGF- β

IL-1 / IL-6 ...

Can ozone help
to reprogram immune cells?

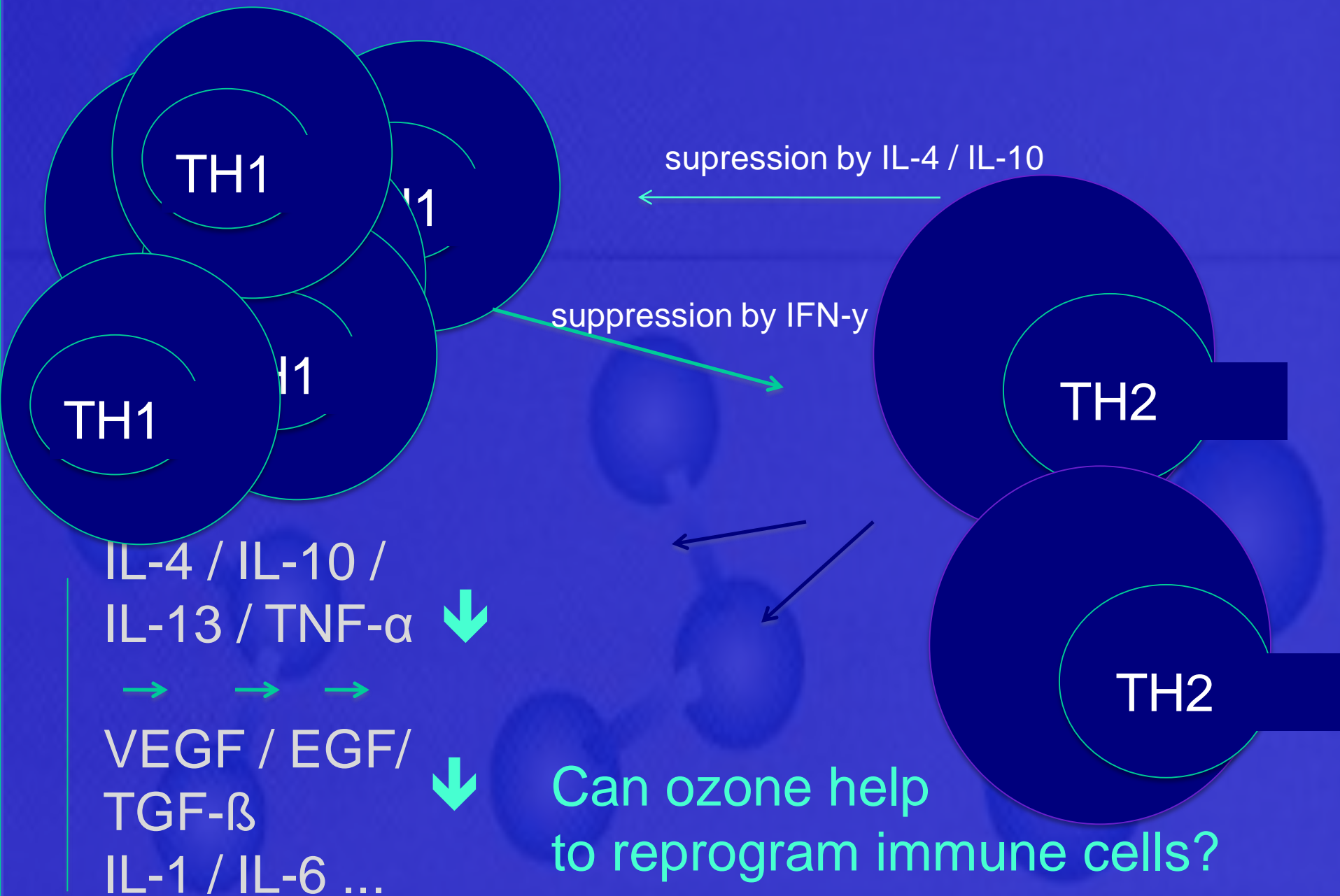


Interferon- γ

in whole blood at different ozone concentrations after 72 hours of incubation

(Bocci 1990)

IFN- γ , mobilized by TH1 /TC1 (effector cells), may partly reverse immunosuppression, antiangiogenic activities, enhancing tumoricidal properties of macrophages.



Complementary
therapeutical
concepts

Proinflammatory factors

repair
mechanisms

Information to CD4
helper cells as key
regulators of the
biological response

TH2 >>> TH1

proinflammatory
signal transduction

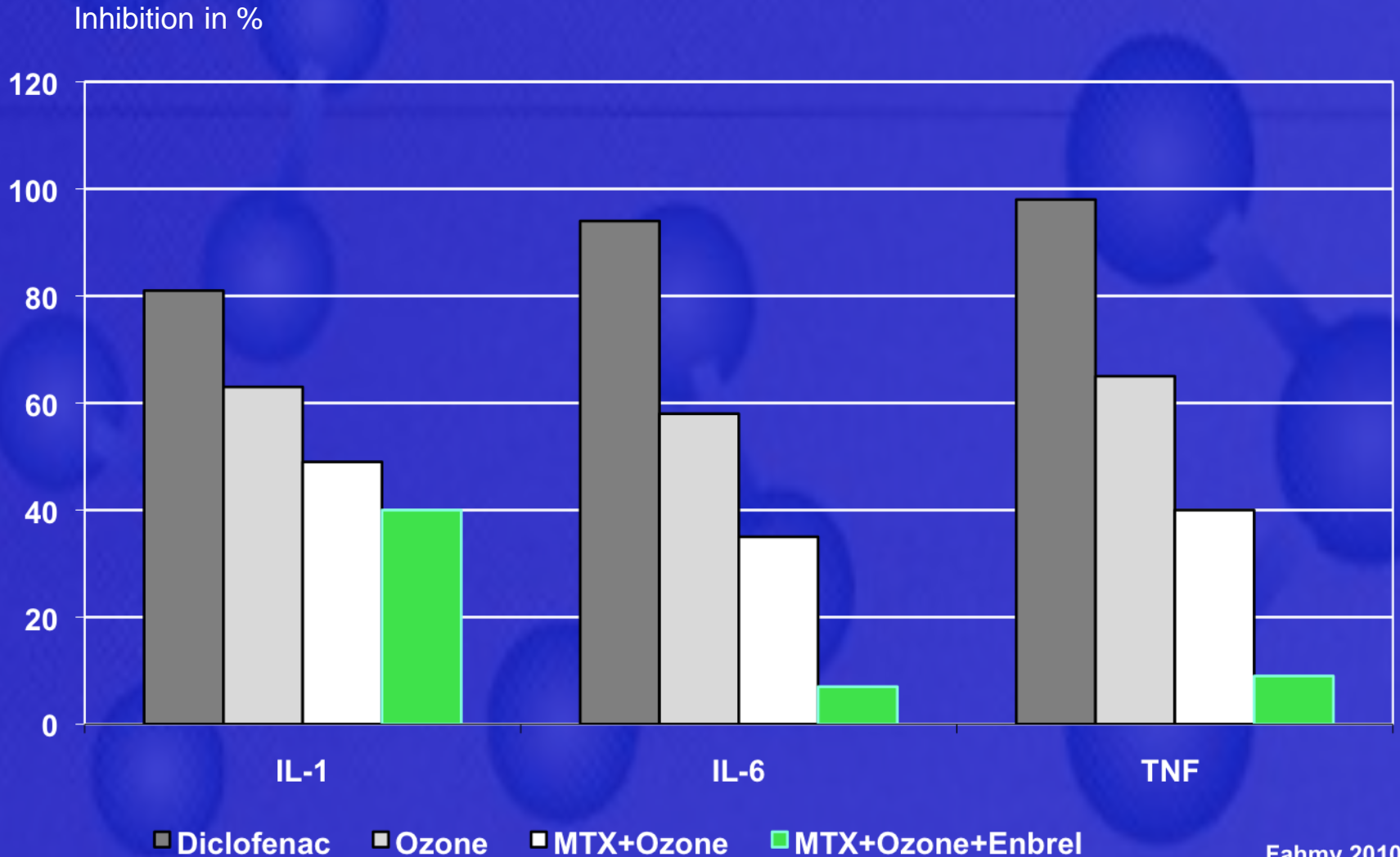
IL-4, IL-10, IL-
13 → IL-1, IL-6,
TNF- α , VEGF,
TGF- β s ...

tumor promotion,
rheumatoid arthritis,
angiopathia, diabetes 2
age related diseases,

NO•

O₂•

The effect on cytokines 180 days RA



Main Indications of Medical Ozone 1

Main Indications	Underlying Mechanisms of Action
Angiopathia, diabetic angiopathia in particular, Arterial circulatory disorders	<ul style="list-style-type: none">▪ Improvement of oxygen release by activation of RBC metabolism,▪ Regulation of angiogenesis,▪ Regulation of ROS, antioxidants and radical scavengers
Chronic forms of Hepatitis B and C, Herpes zoster	<ul style="list-style-type: none">▪ Release of cytokins such as interferons and interleukins by activation of immunocompetent cells,▪ Increase of antioxidant capacity

Main Indications of Medical Ozone 2

Main Indications	Underlying Mechanisms of Action
Supportive therapy in cancer patients, Prevention of side effects of chemotherapy and/or radiation	<ul style="list-style-type: none">▪ Improvement of oxygen availability▪ Regulation of angiogenesis,▪ Increase of antioxidant capacity by activation of biological antioxidants▪ Modulation of the immune system
Supportive therapy in rheumatoid arthritis and Inflammatory conditions <ul style="list-style-type: none">▪ gonarthrosis▪ traumatic knee disorders	<ul style="list-style-type: none">▪ Modulation of the immune system, by activation of immunocompetent cells →interferons andinterleukins..▪ Release of TGF-β▪ Increase of antioxidant capacity

Main Indications of Medical Ozone 3

Main Indications	Underlying Mechanisms of Action
External ulcers and skin lesions	<ul style="list-style-type: none">▪ Disinfection▪ wound cleansing▪ improved wound healing (mechanisms see above)
Dental medicine <ul style="list-style-type: none">▪ following tooth extraction▪ buccal infections▪ aphtae▪ parodontosis	<ul style="list-style-type: none">▪ Disinfection▪ wound cleansing▪ improved wound healing (mechanisms see above)

Major Auto Hemotherapy as Standardized Form of Systemic Ozone Application

50 (100) ml blood + 50 ml (100) Ozone-oxygen-mixture following the guidelines for ozone therapy

Ozone-conc. per ml gas	10 – 20 µg/ml gas	30 - 40 µg/ml gas
Ozone conc. per ml blood (biologically relevant)	10 – 20 µg/ml blood	30 - 40 µg/ml blood
Total ozone amount per 50ml blood	500 - 1000 µg per treatment	1500 - 2000 µg per treatment

