Preclinical toxicity study using direct intravenous ozone

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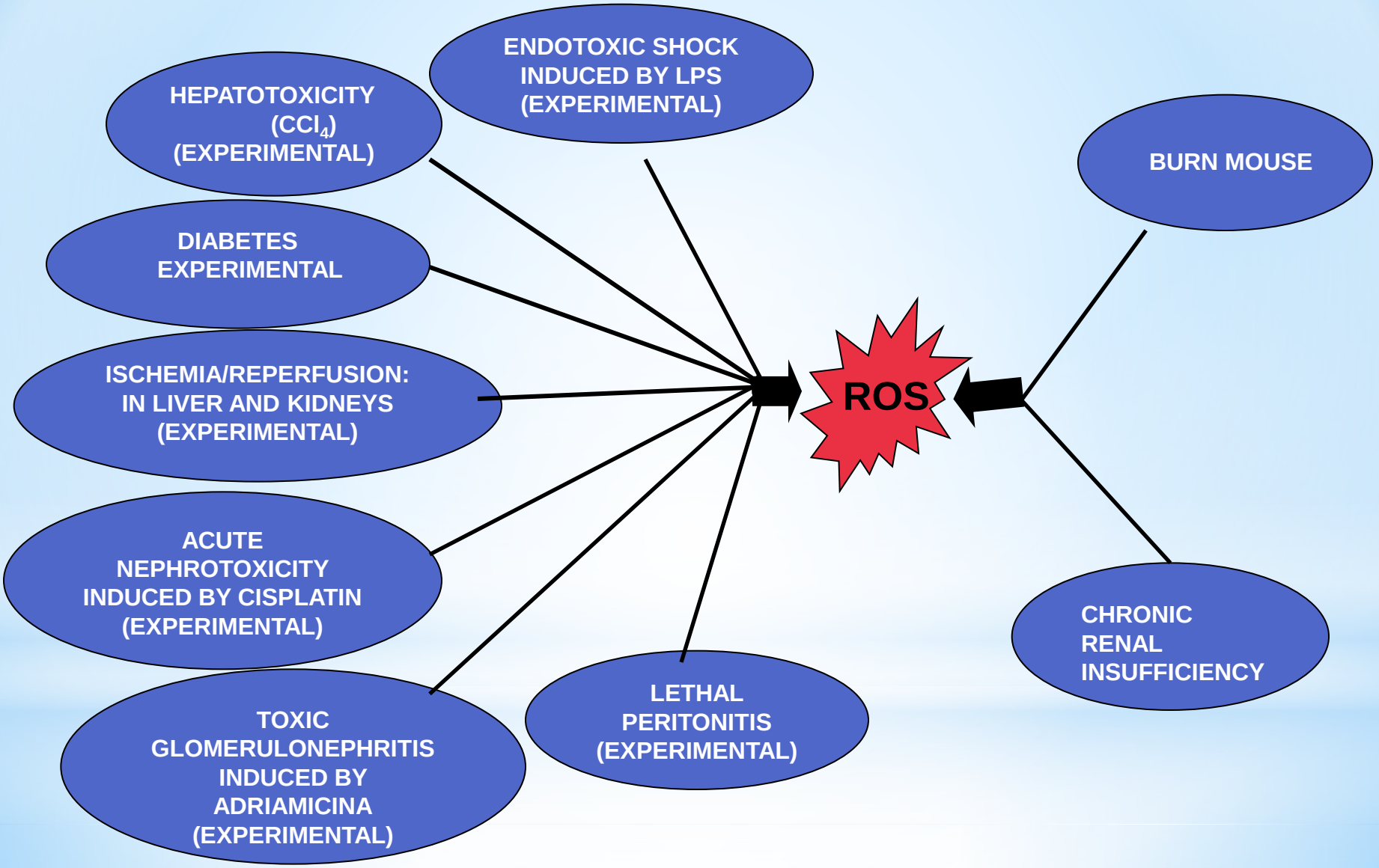
The Ideal Therapeutics

- **High therapeutic efficacy**
- **Survival Benefits**
- **Good control of symptoms**
- **No secondary effects in a short time**
- **Without toxicity in large period of time**
- **Convenient administration**
- **Advantages of cost /effect rate**

SPECIFIC THERAPY

ANIMAL MODELS



DEMOSTRATED PROTECTION MEDIATED BY O_3/O_2

GENERAL OBJECTIVE

Determine the Toxicity Effects of Direct Intravenous Ozone Therapy in Preclinical Models.

SPECIFIC OBJECTIVES

1. *To evaluate the Acute Toxicity of Direct Intravenous Ozone Therapy in rabbits.*

2. *To evaluate the Reiterated Toxicity of Direct Intravenous Ozone Therapy in rabbits and mice.*

MATERIALS AND METHODS

OZONE GENERATOR
OZOMED MOD 01

1. Acute Toxicity of Direct Intravenous Ozone Therapy.

Rabbits NZ-Cenp, males 1.8-2 Kg

Single doses O_3/O_2 : 6 mg/L, volumes: 1.5, 2.5 and 5 mL/Kg

Experimental Groups: 3 (n=2)

2. Reiterated Toxicity Assay of Direct Intravenous Ozone Therapy

2.1. Rabbits NZ-Cenp, males 1.8-2 Kg

Repeated doses O_3/O_2: 6 and 13 mg/L, vol: 0.5 and 1 mL/Kg

Experimental Groups: 4 (n=2)

Daily Administration (7 days) by marginal ear vein

Evaluation

Clinical and Toxicity Signs, Survival

Hematology and Patology Analyses

MATERIALS AND METHODS

OZONE GENERATOR
OZOMED MOD 01

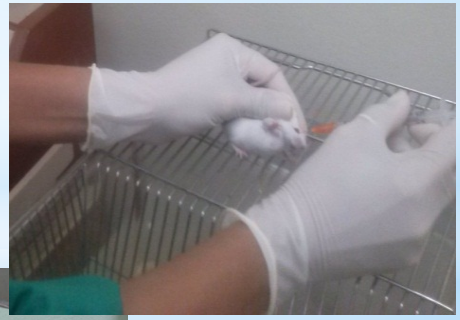
2. Reiterated Toxicity Assay of Direct Intravenous Ozone Therapy

2.2. Mice: Balb/c- Cenp, males 18-20 g

Repeated doses O_3/O_2: 6, 13 y 20 mg/L
volumes 2.5 y 5 mL/Kg

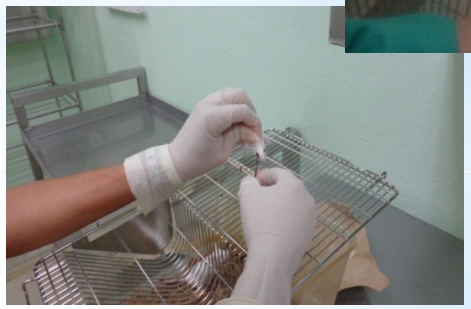
Experimental Groups: 6 (n=10)

Daily Administration (7 days) by retro-orbital plexus



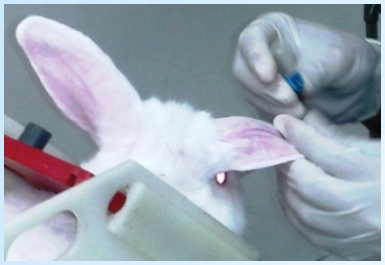
Evaluation

Clinical and Toxicity Signs, Survival
Hematology and Patology Analyses



Results: Acute Toxicity of Direct Intravenous Ozone Therapy.

Single Dose: 6 µg/mL; volumes: 1.5, 2.5 y 5 mL/Kg



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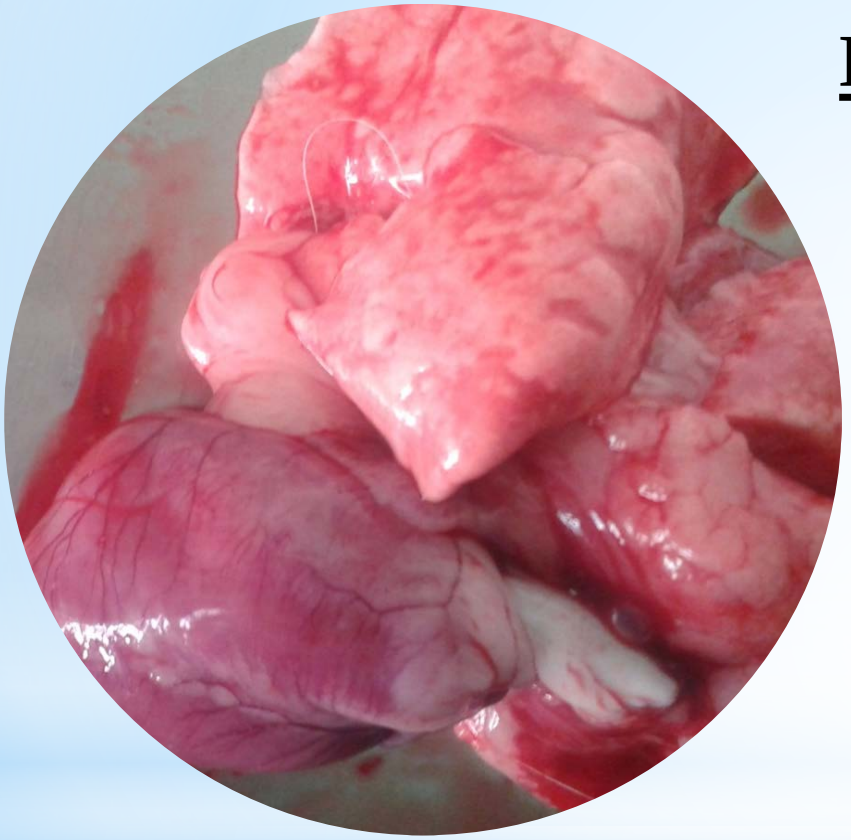
Toxicity Signs

Pupil dilation
Dyspnea (normal value *RR*= 30-60)
Convulsions
Tremors
Cyanotic mucous

sudden death in three doses



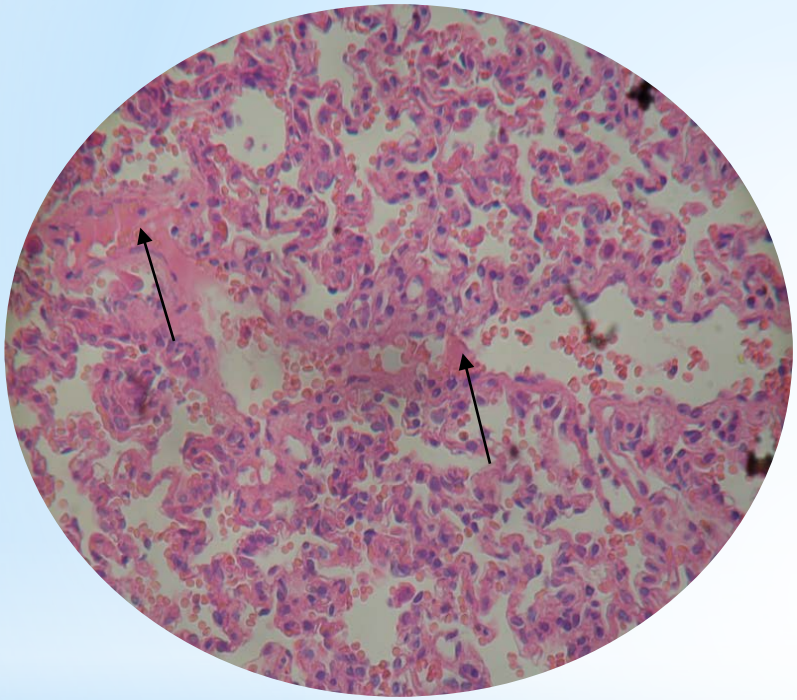
Macroscopic Study of Organs

LUNGS OF EMPHYSEMATOUS APPEARANCE

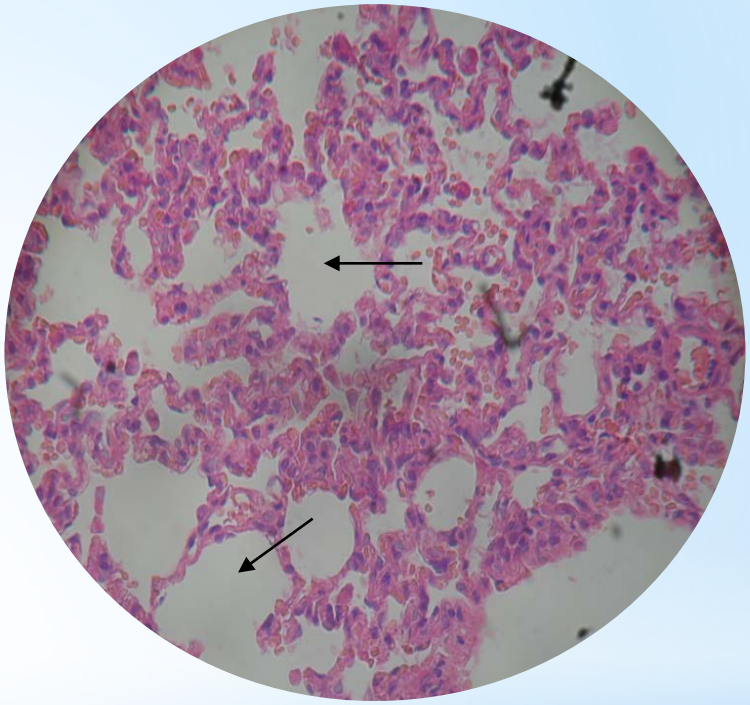
BLOODLESS HEART
CONGESTIVE HEART VALVES

SEVERE HEPATIC DAMAGE
CONGESTION IN LIVER

Histopathological Study of Lungs

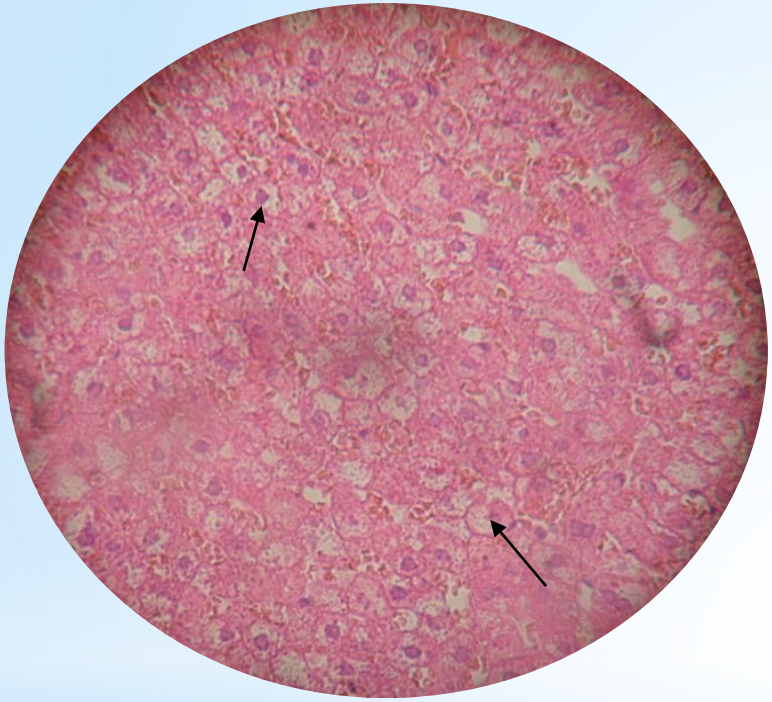


HEMORRHAGICAL FOCUS AMONG ALVEOLAR SPACES AND CONGESTION OF BLOOD VESSELS

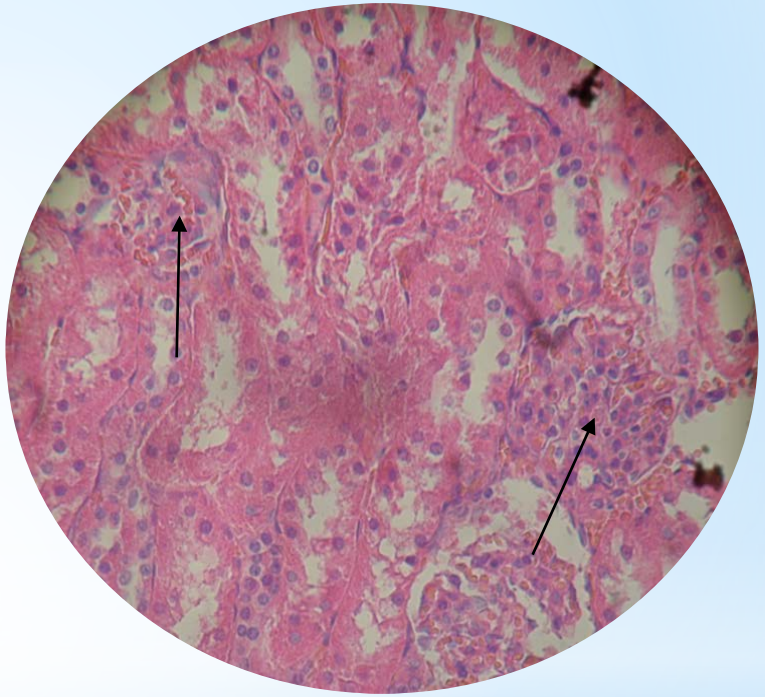


EXPANSION OF ALVEOLAR SPACES AND HEMORRHAGE

Histopathological Study



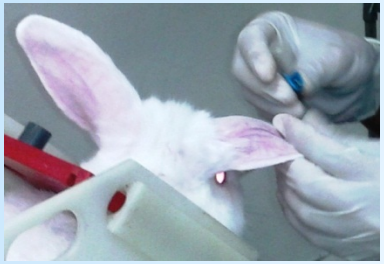
HEPATIC CELLS WITH CLEARING AND GRANULAR CYTOPLASM (HIDROPIC DEGENERATION) CONGESTION AMONG INTERSTICIALS SPACES



HEMORRHAGICAL FOCUS TO RENAL GLOMERULE LEVEL

2. Reiterated Toxicity Assay of Direct Intravenous Ozone Therapy

2.1. Repeated Dose: 6 and 13 µg/mL; volumes: 0.5 and 1 mL/Kg



CLÍNICAL SIGNS

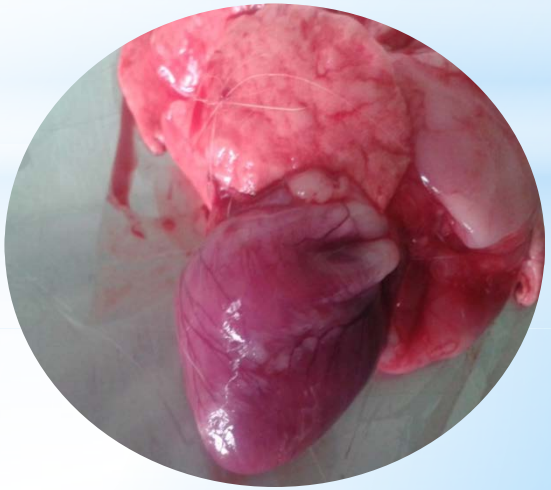
(Normal Values : RR= 30-60, HR= 130-320)

DAYS	Concentration 6 µg/mL	
	0.5 mL/Kg	**1.0 mL/Kg**
1	Pupil dilation Dyspnea (RR=92) HR= 120	Pupil dilation, tremors Dyspnea (RR=128) HR= 126
2	Pupil dilation Dyspnea (RR=120) HR= 144	Pupil dilation, tremors Dyspnea (RR=152) HR= 140
3	Pupil dilation Dyspnea (RR=128) HR= 140	Pupil dilation, tremors Dyspnea (RR=150) HR= 142

DAYS	Concentration 13 µg/mL	
	0.5 mL/Kg	**1.0 mL/Kg**
1	Pupil dilation Dyspnea (RR=136) HR= 128	Pupil dilation, tremors Dyspnea (RR=144) HR= 140
2	Pupil dilation Dyspnea (RR=144) HR= 140	Pupil dilation, tremors Dyspnea (RR=152) HR= 144
3	Pupil dilation Dyspnea (RR=128) HR= 140	Pupil dilation, tremors Dyspnea (RR=170), cyanotic mucous. *sudden death*

(Normal Values : RR= 30-60, HR=130-320)

- **Lungs of emphysematous appearance**
- **Heart valves dilation**
- **Congestive Liver**



Reiterated Toxicity Assay of Direct Intravenous Ozone Therapy in Rabbits NZ-Cenp

HEMATOLOGY ANALYSES

DAY 0 (Before Treatment)	Control	Ozone/Oxygen (IV)		
		6 µg/mL		13 µg/mL
		0.5 mL/Kg	1 mL/Kg	0.5 mL/Kg
Hb (g/dl)	12.0±0.84	12.6±0.76	13.3±0.95	12.0±0.65
HTC (%)	43.7±2.68	42.2±2.18	44.1±2.32	40.3±1.93
LEUC ($x10^3/\mu L$)	8.0±1.42	8.5±1.23	9.0±1.64	7.7±1.12
DAY 7				
Hb (g/dl)	11.2±0.82	12.9±0.89	12.6±0.91	11.4±0.72
HTC (%)	41.4±2.15	43.1±2.34	42.8±1.98	38.0±1.85
LEUC ($x10^3/\mu L$)	5.7±1.34	6.5±1.54	7.3±1.42	6.0±1.55

2. Reiterated Toxicity Assay of Direct Intravenous Ozone Therapy

2.2. Repeated Doses: 6, 13 and 20 µg/mL. Daily for 7 days.
Volumes: 2.5 mL/Kg (50 µL) and 5 mL/Kg (100 µL).
Mice: Balb/c (n=10).

SEVERE TOXICITY SIGNS

Hindquarters paralysis
Pupil dilation
Tremors
Dyspnea
Relaxing Esfínter
Global mortality: 70 % among first and third application

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Reiterated Toxicity Assay of Direct Intravenous Ozone Therapy in Mice Balb/c-Cenp

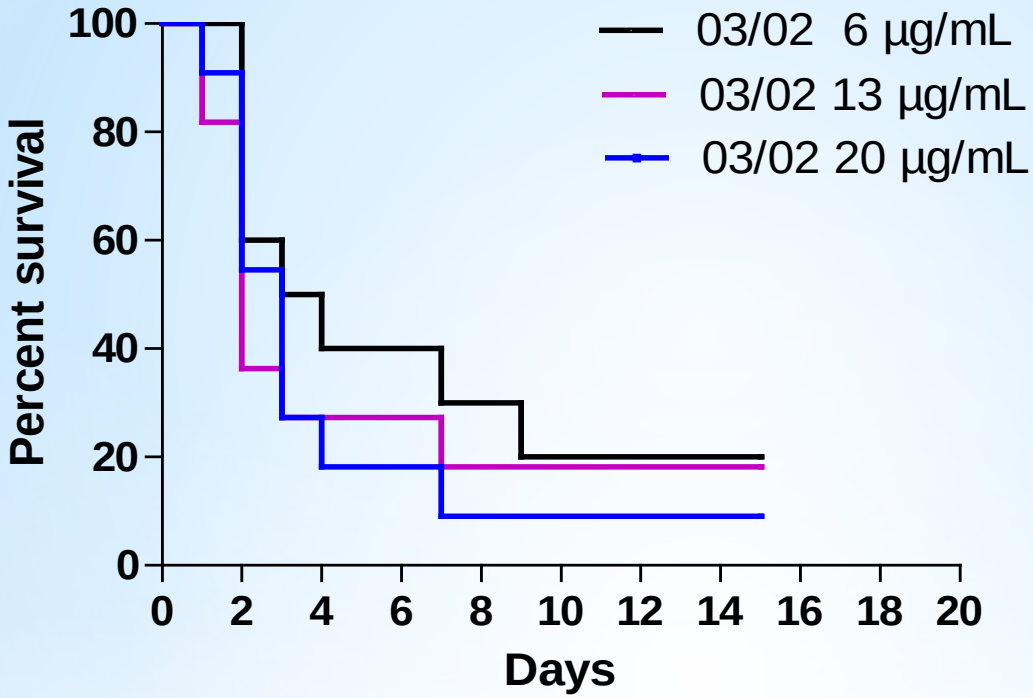

Table: Mortality Percent

Conc/Days	4	7	15
6 µg/mL	60 %	70 %	80 %
13 µg/mL	73 %	82 %	82 %
20 µg/mL	82 %	90 %	90 %

	2.5 mL/Kg (50 µL)	5 mL/Kg (100 µL)
6 µg/mL	Pupil dilation, tremors Dyspnea Hindquarters paralysis Toxicity reversible signs Mortalty: 30 %	Pupil dilation Dyspnea, Tremors Hindquarters paralysis Mortality: 80 % Lungs emphysematous Kidneys: congestive, change of color and volume increase.
13 µg/mL	Pupil dilation Dyspnea, Tremors Hindquarters paralysis Mortality: 40 % Lungs congestive	Pupil dilation Dyspnea, Tremors, convulsions Hindquarters paralysis Mortality: 82 % Congestion on Heart, liver and lungs (Emphysematous)
20 µg/mL	Pupil dilation Dyspnea, Tremors Hindquarters paralysis Mortality: 50 % Lungs congestive	Pupil dilation Dyspnea, Tremors, convulsions Hindquarters paralysis Mortality: 90 % Hemorrhagy on Heart and Liver, possible necrosis zone.

DÍA 0 (Before treatment)	Control	Ozone/Oxygen (IV) 2.5 mL/Kg		
		6 µg/mL	13 µg/mL	20 µg/mL
Hb (g/dl)	14.4±0.34	14.8±0.56	14.9±0.65	14.7±0.78
HTC (%)	48.7±1.38	42.2±2.18	40.3±1.93	48.6±1.33
LEUC (x10^3/µL)	4.3±0.83	5.2±0.23	4.5±0.12	5.9±0.92
	5 mL/Kg			
Hb (g/dl)	14.7±0.22	14.8±0.56	14.9±0.65	14.8±0.56
HTC (%)	49.4±1.25	42.2±2.18	40.3±1.93	49.2±1.72
LEUC (x10^3/µL)	4.5±0.81	5.0±1.11	4.7±0.90	6.1±0.48
DÍA 7		2.5 mL/Kg		
Hb (g/dl)	14.9±0.42	14.7±0.30	14.5±0.31	14.6±0.40
HTC (%)	50.7±0.66	48.5±1.08	48.2±0.96	50.5±1.05
LEUC (x10^3/µL)	5.0±0.82	4.6±0.71	4.5±0.63	5.3±0.72
	5 mL/Kg			
Hb (g/dl)	15.0±0.55	14.8±0.35	15±0.23	14.7±0.15
HTC (%)	52.4±0.88	49.5±1.18	50±0.91	49.1±0.78
LEUC (x10^3/µL)	5.1±0.63	4.9±0.42	6.6±0.33	7.0±0.41

CONCLUSIONS

1. **Single dose administration of Direct Intravenous O_3/O_2 at 6 µg/mL, caused severe multiorgan damages and sudden death of rabbits, in the three dose levels evaluated.**

2. **Repeated dose administration of Direct Intravenous O_3/O_2 in rabbits, mostly produced severe toxicity signs at major dose, mortality at the third application and severe congestion in lung, heart and liver. The lower doses caused toxicity and reversible damage during the first three administrations.**

CONCLUSIONES

3. **Repeated dose administration of Direct Intravenous O_3/O_2 in mice, produced severe toxicity signs, larger and more intense at major doses, with 70 % of global mortality among the first and third administration. Toxicity signs were transitoried and reversible in other 30 % during first three administrations, at lower doses. There was a global suvival of 15 % at the end of the study.**

4. **The preclinical results obtained provide evidence about safety of direct intravenous ozone, that is highly risky, because of the severe adverse effects and the mortality that can lead.**


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OZONE THERAPY
IT COULD BE A LIFE INJECTION!!!
THANKS!!!