Ozone Therapy Global Journal vol. 11, nº 1. pp. 163-177, 2021
Official Journal of Aepromo (Spanish Association of Medical Professionals in Ozone Therapy). Madrid, Spain
Publicación Oficial de Aepromo (Asociación Española de Profesionales Médicos en Ozonoterapia). Madrid, España
ISSN: 2174-3215

Case Report

Ozone Therapy As Co-Adjuvant Treatment In Canine Chronic Renal Insufficiency. A Case Report

Alain Lecat
Veterinary Clinic La Licorne, La Souterraine France

Keywords

ozone,
ozone therapy,
sporotrichosis,
feline.

Abstract

Chronic renal insufficiency or chronic kidney disease is a commonly encountered pathology in veterinary medicine, especially in the elder patient. The purpose of this study was the evaluation of the use of ozone therapy in a canine chronic renal insufficiency patient and the assessment of result related to the main blood parameters and the clinical evolution. Concomitant use of conventional treatment was not obviated. The breed of the patient was a female Griffon Nivernais who presented a severe renal failure. Ozone therapy was applied in treatment cycles by ozonated saline solution and minor autohemotherapy. The concomitant use of ozone therapy improved the quality of life and contributed to a radical change in blood parameters.

.

.

Suggestion on how to quote this paper:
Lecat, Alain (2021). Ozone Therapy As Co-Adjuvant Treatment In Canine Chronic Renal Insufficiency. A Case Report. Reporte de caso. *Ozone Therapy Global Journal* Vol. 11, nº 1, pp 163-177

Autho mail Alain Lecat, Veterinary Clinic La Licorne 10, Boulevard Jean Moulin – 23300 La Souterraine France. E.mail : lecat.alain@gmail.com

Introduction

Chronic renal insufficiency (CRI) is caused by the damage of the different structures of the kidney organ. Intrinsic kidney damage can occur at several levels: Filters of the kidney (the glomeruli); Blood vessels of the kidney (vasculae); Small tubes that connect glomeruli with the area that collects newly made urine (tubuli); Space adjacent to the glomeruli, vasculae and tubuli (interstitial space).

Kidney damage can be due to several causes: Inherited (malformation); Result of immune system disorders; Result of renal adaptation to systemic disease; Result of toxic/allergen exposure.

In healthy animals, the kidneys control the composition of the extracellular fluids; they control the excretion and have an endocrine function. When blood flows through the kidneys, they act as a complex filter that removes from blood wastes that are generated from break down of food, old cells, toxins or poisons and many drugs that are given for treatment of other diseases. The wastes are removed with water as urine. Waste products that can be measured in the blood include creatinine and urea nitrogen but there are many other waste products that are not measured by blood tests. The kidneys also act as a filter to keep "good" substances in the blood. They regulate the amount of water in the blood by excreting extra water and retaining water to prevent dehydration by varying the amount of urine that is produced. The kidneys help regulate blood pressure by saving or eliminating sodium based on how much sodium the pet is eating. Kidneys help regulate calcium and vitamin D which keep bones strong and produce erythropoietin that helps with the creation of new red blood cells. Because the kidneys have so many functions, when they are not working normally, there are many signs that the pet may show.
The basic structures of the kidney are nephrons, the function of which varies according to its locality.

The excretion of phosphates and nitrogenous residues is produced in the glomerulus. Proximal tubuli are responsible for the activation of vitamin D, synthesis of erythropoietin and reabsorption of sodium bicarbonate. The kidneys produce concentrated or diluted urine depending on the animal’s needs.
The distal tubuli secrete potassium and protons.

In most renal diseases, a part of the nephrons is destroyed while the remaining nephrons stay intact and functional. If the number of nephrons that is destroyed is too high, the remaining nephrons will not be able to cope with the functional demands. The consequences of the modified glomerular function are the retention of nitrogenous residues with consequent uraemia and retention of phosphates which will give hyperphosphatemia.

The sick glomeruli will lose their selective permeability which will cause proteinuria.
The consequences of the loss of functionality of the proximal tubuli will be a reduction of erythropoietin production, diminished activation of vitamin D and a diminished reabsorption of bicarbonate. These changes can give in long term non regenerative anaemia, secondary hyperparathyroidism, and metabolic acidosis.

Distal tubuli malfunction will result in hyperpotassaemia or acidosis. The reduction of glomerular perfusion combined with the changes in the processing of water and sodium will contribute to the instauration of systemic hypertension.

The effects of CRI on the organism are many; they can be digestive, urinary, ophthalmologic, muscular, cardiovascular, and endocrine.
The general symptoms of CRI will mainly be anorexia, weight loss and depression. Intestinal symptoms will be vomiting and diarrhoea; urinary symptoms will reflect as polyuria-polydipsia and nocturia. Muscular fatigue and myopathies can occur. Symptoms related to arterial hypertension can appear such as cardiac problems and ophthalmic lesions (retina haemorrhage). Coagulation disorders can appear with intestinal haemorrhage and ulcers.
The diagnosis of CRI will be based on multiples criteria: history of the animal, clinical data, biology, ultrasound, and histopathology. The main tool in veterinary medicine is the evaluation of blood parameters, which are augmented urea and creatinine.

The treatment of CRI consists mainly in two actions which are control of the uraemia and the limitation of the progression of the disease. 1) Uraemia control by fluid therapy: the purpose is to control dehydration, the ionic imbalance, the energy balance. 2) Limitation of the progression of the disease: a) Hypo proteinic and hypo phosphoric diet. b) Control of arterial hypertension. c) Limitation of proteinuria.

To control hypertension, mainly Angiotensin Converting Enzyme inhibitors (ACE inhibitors) are used (Benazepril, Enalapril, Lisinopril, Ramipril and Imidapril). The prognosis of CRI depends on the percentage of nephrons involved in the disease. The International Renal Interest Society (IRIS) proposes a classification based upon blood creatinine levels: Grade 1: < 1,4 mg/dL; Grade 2: 1,4 – 2,8 mg/dL; Grade 3: 2,9 – 5,0 mg/dL; Grade 4: > 5,0 mg/dL

Survival rates according to CRI grades are: Grade 2: survival rate is about 3,2 years; Grade 3: survival rate is about 2,1 years; Grade 4: survival rate is about 3,5 months.

The diminished excretion of toxins, the perturbated water balance, the necrosis of different kidney structures and the associated blood hypertension will unequivocally lead to a persistent state of chronic stress for the patient which will result in an altered redox environment.

The purpose of this case study is to evaluate the use of systemic Ozone Therapy as an integrative approach in the treatment of CRI. Ozonated Saline Solution (O3SS) combined with Minor Autohemotherapy was chosen according to the Madrid Declaration of Ozone Therapy, 3rd Edition, March 2020, ISCO3. The use of O3SS was preferred to Major Autohemotherapy (MAH) because of the physical particularities of Saline Solution since it acts as a plasma expander. This means that a greater quantity of blood will react with the ozone and a major perfusion of the kidneys can be expected. This also permits the easier conjoint administration of the saline perfusion that was performed the same day. The Minor Autohemotherapy (MiAHT) was used as an autovaccine to stimulate the immune system and to reinforce the patient suffering from chronic debilitating pathology, in this case CRI.

Case presentation

Breed: Griffon Nivernais, Female not spayed, 17,8 kg and 8 years old. The animal had a hunting activity. The animal was fed with low quality protein diet daily and once a month raw protein. Clinical evaluation: anorexia,weight loss, dehydration.

Diagnosis: Severe CRI by blood test: BUN: 62.00 mg/dL (normal values between 7.00 – 25.00 mg/dL). Creatinine: 2.90 mg/dL (normal values between 0.30 – 1.40 mg/dL); IRIS grade 3. Lateral and ventro-dorsal radiography was performed to evaluate changes in the kidneys (Fig 1).

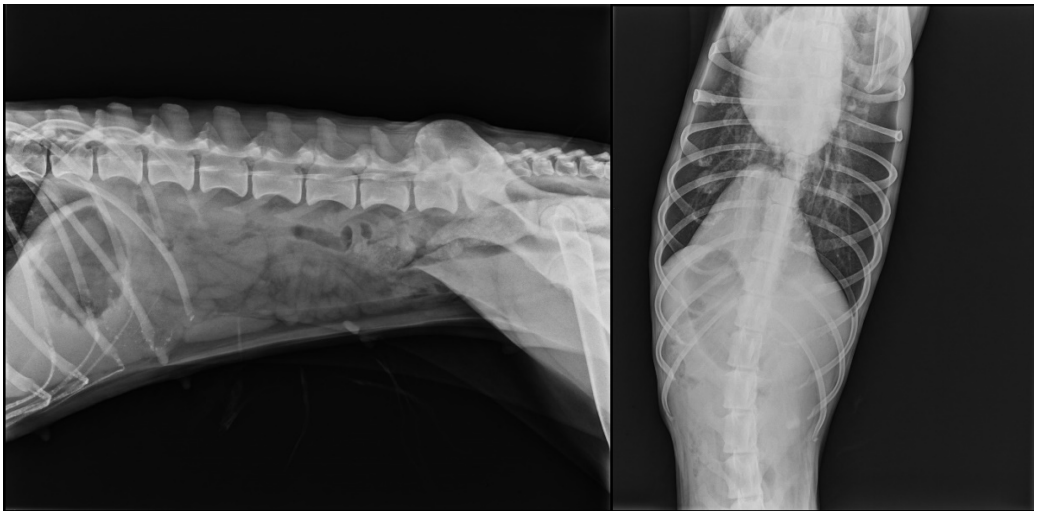

Figure 1. Lateral and ventro-dorsal radiograpy after treatment.

Treatment. The dog has been set on conventional therapy: regular fluid therapy (8 daily sessions), ACE-inhibitors (Enacard®) for life, and a renal diet which is low in protein, but high quality and low in phosphor, Control of daily water intake.

Systemic Ozone Therapy was installed by means of O3SS and MiAHT.

Materials: ozone generator Ozonobaric P (SEDECAL®, Spain), certified under directive 93/42/EEC MDD class IIb, perfusion pump, disposables free of phtalates: 5-10 mL syringes, disposable needles, three-way stop cock, tubes, 250 ml sterile glass bottle, ozone catalysator, sterile saline solution.

According to the pathology, the systemic administration route was preferred, both intravenous infusion of O3SS and MiAHT.

Protocol: The protocol was based on the Madrid Declaration for Ozone Therapy, 3rd edition, March 2020, ISCO3. The O3SS was performed with a minimum of 72-h interval between the sessions, during one day perfusion of normal saline solution. In total eight (8) sessions of O3SS were performed. An average dosage of ozone was used: 2 µg/kg. The dose received by the patient: 2 µg/kg x 20 kg = 40 µg. The ozone was dissolved in 100 mL of saline solution; 40 µg/100 mL = the concentration of ozone dissolved in saline = 0.4 µg/ml.

The concentration of ozone from the generator outlet 0.4 µg x 4 = 1.6 µg/NmL, which has been extrapolated to 2 µg/NmL. The flow rate of the generator was set at 12 L/h and a first ozonisation of 15 min was performed followed by 6 min of ozonisation at the same parameters while the perfusion was done.

The MiAHT was performed on a weekly basis. A total of 4 sessions was performed. Blood was taken from the patient at a ratio of 1 mL/kg: 2 – 3 mL. The ozonisation of the blood was augmented gradually, starting with a concentration of 15 µg/NmL and reaching 25 µg/NmL at the last session. The blood was injected intramuscular into the erector spinae muscle at the Shu points of the kidney meridian (Bladder 23), approximatively 1 mL per point.

A one-month follow-up of the patient was performed. Clinical parameters such as appetite, weight control, body score was followed. Blood parameters, especially BUN and creatinine were compared.

Results

Appetite: The patient's appetite augmented during the protocol with a little regression at mid stage of the protocol. The owner commented that it was a general phenomenon because he observed it with his other animals.

Body condition score: The WSAVA - 9 scale body condition score was used.
The patient was initially scale 1: ribs, lumbar vertebrae, pelvic bones, and all bony prominences are visible from a distance. There was no discernible body fat and there was obvious loss of muscle mass.
At the middle stage of the protocol, two weeks later, the patient reached a body score of 2: she gained muscle mass although ribs and lumbar vertebrae were easily palpable. No palpable fat. Some evidence of other bony prominences.
At the end of the protocol, the body score was 3: Ribs easily palpated and visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones prominent. Obvious waist and abdominal tuck.

Body weight: The patient had an initial weight of 17.80 kg. During the treatment cycle there was a considerable gain of weight. However, a temporary stop of weight gain occurred after the fourth treatment because of a temporary loss of appetite. Apparently, this was not related to the treatment since the owner related similar observation with his other animals.
At the end of the treatment, the patient had a weight of approximatively19,.6 kg which makes a gain of 2,.6 kg between the beginning and the end of the treatment.
The weight evolution is shown in Fig. 2.

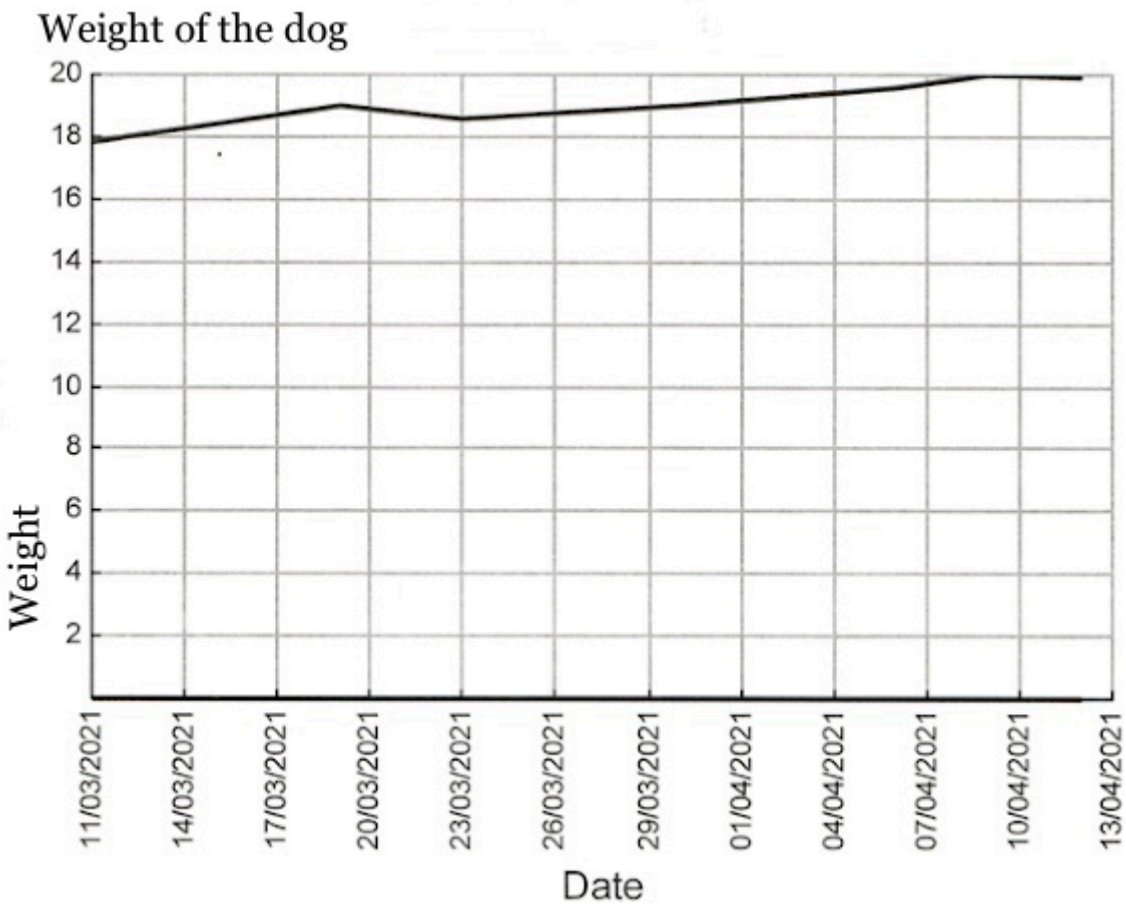

Fig. 2 Weight evolution.

Blood analysis: Blood analysis was performed 3 times (Table 1-3): before, at the middle stage, and three days after the end of the protocol. Specially, the blood values, BUN and Creatinine were compared. The initial BUN and Creatinine were respectively 62.00 mg/dL (normal range: 7.00 – 2.,00 mg/dL) and 2.9 mg/dL (normal range: 0.30 – 1.40 mg/dL) (Tab. 1). At middle stage, the BUN and Creatinine values were respectively 26.00 mg/dL and 1.90 mg/dL (Tab. 2). Three days after the end of the protocol, at one month from the beginning of the protocol; the BUN and Creatinine values were respectively 22.00 mg/dL and 1.60 mg/dL (Tab. 3).

Table 1. Blood analysis at baseline.

Patient Iris (2283), Espèce animale: Chien, Race: griffon nivernais

VetScan VS2 Comprehensive Diagnostic					11/03/2021 11:52:00
ID patient: 2283			N° échantillon:		
Remarque: HEM 0 LIP 0 ICT 0					
ALB	3,40	g/dL	2,50	4,40	
ALP	24,00	U/L	20,00	150,00	
ALT	19,00	U/L	10,00	118,00	
AMY	885,00	U/L	200,00	1 200,00	
TBIL	0,30	mg/dL	0,10	0,60	
BUN	62,00	mg/dL *	7,00	25,00	
CA	11,50	mg/dL	8,60	11,80	
PHOS	6,30	mg/dL	2,90	6,60	
CRE	2,90	mg/dL *	0,30	1,40	
GLU	96,00	mg/dL	60,00	110,00	
NA+	147,00	mmol/L	138,00	160,00	
K+	3,90	mmol/L	3,70	5,80	
TP	6,90	g/dL	5,40	8,20	
GLOB	3,50	g/dL	2,30	5,20	

Legend: ALB (Albumin); ALP (Alkaline Phosphatase); ALT (Alanine aminotransferase); AMY (Amylase); TBIL (Total Bilirubin); BUN (Blood Urea Nitrogen); CA (Calcium); PHOS (Phosphor); CRE (Creatinine); GLU (Glucose); NA+ (Sodium); K+ (Potassium); TP (Total Protein); GLOB (Globulines).

Table 2. Intermediary blood analysis.

Patient Iris (2283), Espèce animale: Chien, Race: griffon nivernais

VetScan VS2
Comprehensive Diagnostic — 30/03/2021 16:25:00

ID patient: 2283 — N° échantillon:

Remarque: HEM 0 LIP 0 ICT 0

ALB	2,50	g/dL		2,50	4,40
ALP	31,00	U/L		20,00	150,00
ALT	15,00	U/L		10,00	118,00
AMY	959,00	U/L		200,00	1 200,00
TBIL	0,30	mg/dL		0,10	0,60
BUN	26,00	mg/dL	*	7,00	25,00
CA	10,60	mg/dL		8,60	11,80
PHOS	6,10	mg/dL		2,90	6,60
CRE	1,90	mg/dL	*	0,30	1,40
GLU	92,00	mg/dL		60,00	110,00
NA+	150,00	mmol/L		138,00	160,00
K+	4,30	mmol/L		3,70	5,80
TP	5,40	g/dL	*	5,40	8,20
GLOB	2,80	g/dL		2,30	5,20

Legend: ALB (Albumin); ALP (Alkaline Phosphatase); ALT (Alanine aminotransferase); AMY (Amylase); TBIL (Total Bilirubin); BUN (Blood Urea Nitrogen); CA (Calcium); PHOS (Phosphor); CRE (Creatinine); GLU (Glucose); NA+ (Sodium); K+ (Potassium); TP (Total Protein); GLOB (Globulines).

Table 3. Final blood analysis.

Patient Iris (2283), Espèce animale: Chien, Race: griffon nivernais

VetScan VS2 — 12/04/2021 14:35:19
Comprehensive Diagnostic
ID patient: 2283 — N° échantillon:
Remarque: HEM 0 LIP 0 ICT 0

ALB	2,10	g/dL	*	2,50	4,40
ALP	45,00	U/L		20,00	150,00
ALT	9,00	U/L	*	10,00	118,00
AMY	890,00	U/L		200,00	1 200,00
TBIL	0,30	mg/dL		0,10	0,60
BUN	22,00	mg/dL		7,00	25,00
CA	10,60	mg/dL		8,60	11,80
PHOS	5,80	mg/dL		2,90	6,60
CRE	1,60	mg/dL	*	0,30	1,40
GLU	98,00	mg/dL		60,00	110,00
NA+	147,00	mmol/L		138,00	160,00
K+	4,50	mmol/L		3,70	5,80
TP	5,70	g/dL		5,40	8,20
GLOB	3,50	g/dL		2,30	5,20

Legend: ALB (Albumin); ALP (Alkaline Phosphatase); ALT (Alanine aminotransferase); AMY (Amylase); TBIL (Total Bilirubin); BUN (Blood Urea Nitrogen); CA (Calcium); PHOS (Phosphor); CRE (Creatinine); GLU (Glucose); NA+ (Sodium); K+ (Potassium); TP (Total Protein); GLOB (Globulines).

Discussion

The interest that Ozone Therapy may represent as complementary treatment in CRI are multiple. The systemic application of ozone will have different effects. When ozone reacts in biological fluids or blood, several mechanisms occur: 1) Direct oxidant capacity: a) Germicidal effect by direct reaction of ozone on the microbial cell wall. b) Oxidation of pain and inflammatory mediators and blockage of cytokine receptors. 2) Indirect effect: the contact of ozone with blood or biological fluids will result in the production of organic peroxides, H_2O_2, ozonides and aldehydes along with other oxidative products. These molecules will generate an endogenous stress response mechanism that can rebalance the redox environment.

When ozone reacts with the blood, in the acute phase a large amount of the ozone is neutralised by the present antioxidants but also several reactive oxygen species (ROS) are formed which are capable to trigger biochemical pathways. The ROS have a noticeably short survival time, they are neutralised in less than a minute.

Another part of the ozone will react in a later phase and form lipid peroxidation products such as alkyl peroxy radicals (ROO), hydroperoxides (R-OOH), low molecular weight aldehydes (malonildialdehyde), alquenales (4-droxi-2,3-trasnonenal) and H_2O_2.

H_2O_2 and LOP are responsible for the late therapeutic and biological effects of ozone. H_2O_2 is an intracellular signalling compound. It is capable to activate thyroxine kinase, that in its turn will phosphorylate the nuclear transcription factor NFκB with the subsequent synthesis of proteins. H_2O_2 acts on mononuclear blood cells, platelets, endothelial cells, and erythrocytes.

When ozone reacts with the erythrocytes, it acts first with the PUFA's (Poly Unsaturated Fatty Acids), present in the cell membrane. The peroxides that are formed will penetrate partially into the cell. Accumulation of intracellular peroxides is prevented by the intracellular glutathione system, vitamin E and vitamin C. The oxidated glutathione will be reduced by the influence of NADPH which is formed in the pentose phosphate pathway; this will strengthen the glycolysis and glucose metabolism. The NADPH will be oxidized to NAD. As result of the activation of enzymatic reactions, an augmentation of 1,3-diphosphoglycerate (1,3-DPG) and finally an increase of 2,3-DPG and an increase in the number of hydrogen ions will occur. The increase of 2,3-DPG concentration plays a key role in the healing effect of ozone: HbO2 + 2,3-DPG -> Hb2,3-DPG + O2.

The release of oxygen from the oxidized haemoglobin is facilitated by the increase of the 2,3-DPG concentration. This means that in ischaemic tissues more oxygen will be released. The increase of hydrogen ions will have complementary deoxygenating effect due to the buffering capacity of the oxidized haemoglobin; this is also called the "Bohr effect".

Since the metabolic processes (pentose phosphate pathway, Krebs cycle …) are activated, there will be an augmentation of ATP production. This results in a restored activity of intramembranous transport pumps; they normalize the concentrations of intracellular potassium and extracellular sodium and this will restore the electric potential of the cell and its charge, his adhesion and cell aggregation properties. Also, the formation of peroxides in the lipid layers of the cell membrane will decrease the viscosity. These changes will result in an improvement of the rheological properties of the blood.

The interaction of ozone with the endothelial cells of the vascular wall will activate NO-synthetase. This produces an augmentation of the nitric oxide radical that will act as a factor of endothelial relaxation which will result in vasodilation and better tissue perfusion.

In normal cells there is a balance between oxidant and antioxidant activity. This balance can be disturbed by excessive production of ROS or deficiencies of the body's own antioxidant defence systems or those provided by diet. All this can lead to an oxidative state. Prolonged oxidant activity will establish oxidative stress. This oxidative stress causes disruption of cellular signalling and control systems. Homeostasis of the cellular redox system is controlled through signalling proteins and transcription factors such as NFκB, Nrf2, P53, … The response depends on the severity of the oxidant/antioxidant imbalance, tolerance mechanisms, genetic factors, microenvironment, and other regulatory factors. The final response can be various: adaptation, protection, stress, or cell death (destruction). A clear link exists between the two transduction factors, NFκB (Nuclear Factor κ Beta) and Nrf2 (Erythroid Nuclear Factor type 2). An imbalance between the NFκB and Nrf2 pathways are associated with many diseases among which are neurodegenerative, autoimmune, metabolic and cancer. The most fundamental biological effects of ozone in its systemic interaction are the normalization of the balance of lipid peroxidation product levels and the antioxidant defence system. Ozone acts as an oxidative stress modulator. Ozone, indirectly generating H^2O^2, activates both signalling NFkB and Nrf2 pathways. It can activate the NFκB pathway which will generate the formation of pro-inflammatory cytokines such as TNFα (Tumor Necrosis Factor alfa), INFγ (Interferon gamma), IL8 (Interleukin 8). It can also activate the Nrf2 pathway which will have a protective role and generate enzymes such as Hemo oxygenase 1(HO1) , Superoxide Dismutase (SOD), Catalase (CAT). The response of these pathways depends on the concentration of ozone and the cellular redox environment, both depend on the patient, so it is important to have a way to evaluate the oxidative stress in the patient. A hypothesis is emitted that, in the case of CRI, Creatinine could be used as a parameter to measure the oxidative stress in the patient since Creatinine is a reliable indicator of the kidney function. A compensatory increase of the activity of antioxidant enzymes (HO1, SOD, CAT) will occur in patients with oxidative stress. This phenomenon is called "oxidative pre-conditioning".

Ozone has an anti-inflammatory effect since it can oxidise compounds that contain double bonds, like arachidonic acid and prostaglandins which contribute to the development and maintenance of inflammatory processes. In addition, ozone restores metabolic processes in the affected tissues at the point of inflammation, diminish hypoxia, correct pH, and electrolyte balance.

Ozone has a similar effect as corticosteroids without their adverse effects. In pathological states, ozone enters oxygen into the inflamed area and oxidises algo genic mediators that are formed by the damaged tissue.

Especially in the liver and the kidney, ozone exerts a detoxifying effect by activating the processes of glucose, fatty acids and glycerol utilisation and intensifying the processes of utilization of glucose, glucose-6-phosphate, lactate, pyruvate, and the oxidative phosphorylation reaction. This normalizes ATP values. In the liver, an optimization of the hepatic microsomal system and strengthening of the hepatic filtration takes place.
Due to the positive action on the metabolism of both liver and kidneys, ozone improves the many liver functions such as antitoxic functions and protects the kidney and liver of dystrophic changes.

The ozone in the blood has an effect of immunological regulation by inducing cytokine synthesis. The effects on the immune system depend on the dosage of the ozone.
Therapeutic concentrations of ozone will stimulate the synthesis of different classes of cytokines in monocytes, macrophages such as interferon, TNF, interleukin 6 and Interleukin 8. The physiological activity of phagocytic cells is changed which will manifest as accelerated homeostasis, the activation of variation capacity of phagocytes and a decrease of circulating immunocomplexes. During ozone administration with therapeutic doses, a release of certain cytokine antagonists is observed but also cytokine production as Interleukin 10 (IL10) and Transforming Growth Factor β1 (TGFβ1) which will protect against autoreactive cytotoxicity.
High doses of ozone on the contrary will have an inhibiting effect on cytokine synthesis and will induce a decrease in the level of antibodies which can be indicated in autoimmune disorders.

Ozone influences the synthesis of hormonal mediators, particularly growth hormone (somatotropin) and β-endorphins. It readjusts the Renine-Angiotensine-Aldesteron system which will contribute to lower the blood pressure in the case of blood hypertension. These hormonal changes are brief and reversible.

Ozone has regulatory action on metabolic indicators. A modulation from initially pathological values towards normal values takes place, among them are glucose, creatinine, haemoglobin, haematocrit, total protein, lactate dehydrogenase, cholesterol, triglycerides, lipoproteins, liver enzymes, bilirubin, uric acid, lactic acid, and calcium. This effect is probably due to the rebalancing of the redox system.

Conclusions

Systemic ozone has a real interest in the treatment of CRI since by its action the rheological properties of the blood are changed which result in augmented kidney tissue oxygenation and perfusion. Kidney tissue will be protected and revitalised. Ozone contributes to the effective control of the blood pressure. The administered ACE inhibitors will be more effective. It has been clearly demonstrated that Ozone therapy contributes actively to the restoration of normal blood parameters. A clear clinical improvement of health was observed with weight gain, more appetite and muscle onset. Ozone therapy can efficiently improve chronic pathology and change the prognosis and life span of the patient.

References

1. Guide Pratique de Médecine Interne chien, chat et nac, Fabrice Hébert – Christophe Bulliot, 5e édition, édition MED'COM, p. 170 – 172.
2. Therapeutica Veterinaria de Pequeños Animales, Kirk & Bonagura XI, Interamicana McGraw-Hill, p. 930 – 970.
3. Sesam Atlas van de Fysiologie, Stefan Silbernagl – Agamemnon Despopoulos, Thieme Verlag 1981, p. 118 – 139.
4. Uro-néphrologie du chien et du chat, Christelle Maurey – Cédric Dufayet, Abrégés Vétérinaires, PCMAC, Masson-AFVAC, 2005.
5. Kirk and Bistner's Handbook of Veterinary Procedures & Emergency Treatment, 6th edition, p. 120 – 126.
6. Chronic Kidney Disease and Failure - VetMedhttps://www.vetmed.wsu.edu/outreach/Pet-Health https://www.vetmed.ucdavis.edu/hospital/animal-health-topics/chronic-renal-disease
7. Body Condition Score – WSAVA https://wsava.org/wp-content/uploads/2020/01/Body-Condition-Score-Dog.pdf
8. A Guide to Body Score Conditioning in Dogs – PetSci https://petsci.co.uk/body-score-for-dogs
9. Interpretation of Labaratory Results for Small Animal Clinicians, B.M.Bush, Blackwell Scientific Publications,1991
10. Ozone, a New Medical Drug, Velio Bocci, 2nd edition, Springer, p. 190 – 193.
11. Clinical Ozone Therapy Manual, Dr. Adriana Schwarz, Medizeus – Soluciones médicas, S.L. 2020.
12. 6th Diploma Online "Ozone Therapy and Concentrated Growth Factors activated by Ozone", AEPROMO 2020-2021.
13. Declaration of Madrid, 3rd edition, March 2020, ISCO3
14. Ozonobaric P Operation Manual, Technical publication OM-0504R10_EN_SED, Sedecal Healthcare.
15. Ozone Therapy in Practice, Oleg V. Maslennikov – Claudia N. Kontorshchikova, Irna A. Gribkova, Nizhny Novgorod, Russia 2008.