Blood ozonization as a complementary treatment in infective chronic wound healing: a single center experience

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Background

Chronic wounds are often characterized by microbial complications, delayed healing and spread of MDR bacteria.

Skin diseases exhibit complex etiologies (diabetes, venous/arterial insufficiency, rheumatic diseases) that cannot be always effectively addressed using currently available therapeutic agents.

Therefore, wound management is a major challenge that require new therapeutic solutions.

In this scenario, systemic ozone therapy could represent a complementary treatment, as O2-O3 has many demonstrated mechanisms of action (Figure 1).

Figure 1
Major ozone mechanism of action/biochemical modulation

1. increased red blood cell 2,3 diglycerophosphate (greater hemoglobin oxygen release)
2. improved rheological properties of red blood cells and endothelial nitric oxide production
3. greater arterial/venous partial pressure difference, indicating greater mitochondrial consumption of oxygen (improved mitochondrial function with energy production)
4. immune system modulation and reduction of inflammation
5. improvement of antioxidant status, antioxidant enzymes (including superoxide dismutase), and glutathione in cells
6. bacterial inactivation
7. enhanced antibiotic efficiency due to preconditioning effect

Table 1: Patients characteristic and cytokines trend

Patients (gender, age)	Comorbities	Type of ulcer	Pathogen	O3-AHT	Surgery/ Treatment	Antibiotics	IL-1β (0-0.2)	IL-6 (<7)	IL-8 (6.7-16.2)	TNF-α (7.8-12.2)	IL-2R (440-1435)	IL-10 (1.8-3.8)	IP-10 (37.2-222)	IFN-γ (0-1.0)
Pt.1 (M, 54)	SWT HPT	V	MSSA + *P. aeruginosa*	43	//	Amicacin	0.0	//	11	8.2	2238	1.6	82	0.7
						Pip/Taz + Dalba	0.0	//	11.1	11.7	1693	0.9	133	0.9
							0.0	//	11.8	11.2	2301	2.8	134	0.9
							0.0	//	12.6	9.4	1798	1.2	123	0.8
							0.0	9.0	9.3	9.6	1751	1.4	109	0.6
			E. cloacae				0.0	6.0	10.1	8.9	1640	1.7	101	1.7
						Bactrim →	0.0	9.0	38.9	10.1	2018	1.1	114	0.7
							0.0	2.0	15.9	8.1	1957	0.7	84.6	0.5
							0.0	6.0	10.3	7.6	1518	0.9	88.7	0.5
Pt.2 (M, 88)	HPT DM AF, CHF CKD	V	MSSA + *K. oxytoca*	16	//	Amoxi/Clav →	0.1	11	29	14	1347	3.1	220	0.8
							0.1	7.0	23.7	13.3	1745	2.8	208	0.6
							0.0	9.0	19.6	11.9	1466	2.0	138	0.5
							0.0	7.0	24.2	13.1	1404	3.1	165	0.6
Pt.3 (F, 79)	HPT, CHF, IHD DM CKD	M	*P. aeruginosa* *K. pneumoniae* *E. coli*	22		Cipro →	0.1	8.0	30.2	11.5	2354	4.6	75.8	0.7
							0.2	7.0	35.5	13.4	2504	3.4	80	0.7
							0.0	7.0	29.2	12.6	2761	2.7	101	0.6
					SD + MSCR	Pip/Taz →	0.0	12.0	52.1	10.8	2331	3.8	103	0.8
							0.0	7.0	33.0	11.9	2187	2.7	109	0.3
Pt.4 (F, 83)	HPT, CHF, AF	V	MSSA *A. baumannii*	19		Dalba →	0.0		50.1	21.5	3209	3.1	2306	0.9
						Moxi →	0.1	8.0	39.5	23.1	3221	4.0	1615	1.1
					SD + PMCI		0.2	26.0	38.2	28.5	3916	3.1	1296	2.3
						Dalba →	0.2	31.0	41.8	38.9	3115	4.8	1886	2.0
Pt.5 (F, 76)	R.A + Sc AF	VA	MSSA	34	//		0.6	//	31.4	7.4	2053	13.5	413	0.5
						Dalba →	1.1	//	33	13.4	2294	28.6	440	0.6
						Dalba →	0.3	352	35.3	10.6	2568	22.8	741	0.9
							0.4	292	34.9	7.8	2030	20.9	761	0.7
						Bactrim →	0.1	431	53.4	8.3	2178	23.6	1032	0.4
							0.3	149	39.4	8.6	2149	20.4	742	0.4
							0.3	846	38.5	10.3	2351	22.1	781	0.7
						Bactrim →	0.5	637	44.2	12.0	2288	23.7	830	0.7
Pt.6 (F, 87)	CKD AF, CHF	V	MSSA *P. aeruginosa*	8	FBM	Cipro + Dalba →	0.0	19	49.4	16.4	3471	8.0	488	0.9
							0.0	31	39.2	19.9	3535	5.7	383	0.9
Pt.7 (F, 84)	HPT, CHF, AF CKD COA	A	*P. aeruginosa* MSSA	23		Amicacin →	0.3	//	54.5	17.4	4181	3.8	430	2.7
							0.0	9.0	39.9	21.9	4228	3.6	723	2.4
					SD + PMCI		0.0	14.0	44.9	7.8	2998	3.4	134	0.4
						Pip/Taz →	0.4	84	82.1	23.3	5890	9.1	715	8.9
							0.2	56	77.2	19.7	4043	5.2	373	2.6
Pt.8 (M, 70)	COA	A	*P. aeruginosa* MRSA	22	//	Levo + Dalba →	0.0	37	46.5	8.5	2521	2.8	123	1.0
							0.0	24	49.9	11.2	3027	2.1	146	1.3
						Dapto + CFZ →	0.2	67	72.2	12.3	3127	4.3	143	2.7
							0.0	12	101	11.0	2352	2.7	183	2.4
							0.0	31	71.5	12.4	2706	2.1	193	1.7
Pt.9 (M, 87)	HPT, CHF Horton	V	MSSA *P. aeruginosa*	26	SD + PMCI	Bactrim + Cipro + Dalba	0.0	//	26.5	13.3	2901	2.2	135	0.3
							0.3	//	50.3	14.0	3153	4.1	325	0.8
							0.0	35	39.9	15.2	2801	2.5	218	0.3
							0.1	56	42.9	13.8	3035	2.7	288	2.0
							//	37	36.7	//	2579	2.0	230	//
							0.0	38	33.5	13.1	2122	1.6	194	1.9
Pt.10 (M, 68)	HPT CKD COA	A	*K. pneumoniae*	30	FBM	Levo + Bactrim	0.1	//	28.1	11.8	2007	3.0	272	1.0
							0.1	8.0	22.4	8.3	2151	3.3	201	1.2
						Bactrim →	0.0	25	29.3	12.5	2960	3.1	340	5.9
							0.2	13	40	11	2892	3.7	213	0.8
							0.0	15	53.8	8.4	2956	2.9	286	0.8
							0.0	16	27.6	11.5	1846	2.0	247	0.9
							0.0	10	28.5	10.1	1791	3.2	295	2.7
Pt.11 (F, 86)	AF	V	*P. aeruginosa*	7			0.2	11	35.9	16	2181	96.1	287	0.6
						Amicacina →	0.0	33	42.2	16.6	3438	93.8	270	0.7
Pt.12 (F, 90)	HypoTh	M	*P. aeruginosa*	8			0.0	16	29.9	27.5	3661	6.7	1348	2.1
							0.0	11.0	29.7	23	2782	4.2	741	10.3

Abbreviation:
M: male - F: female
SWT: Sturge Weber Traunaunay syndrome - HPT: hypertension – DM: diabetes mellitus – AF: atrial fibrillation – CHF: chronic heart failure – IHD: ischemic heart disease - CKD: chronic kidney disease – COA: chronic obliterative arteriopathy – HypoTh: Hypothyroidism
V: venous - A: arterial - M: mixed – VA: vasculitis
MSSA: Methicillin Sensible *Staphylococcus aureus* – MRSA: Methicillin Resistant *Staphylococcus aureus*
SD: surgical debridement - FBM: fotobiomodulation – MSCR: mesenchymal stem cell regenerative treatment – PMCI: peripheral mononuclear cells implantation
Pip/Taz: Piperacillin/Tazobactam - Dalba: Dalbavancin – Amoxi/Clav: Amoxicillin/Clavulanic Acid – Moxi: Moxifloxacin – Cipro: Ciprofloxacin – Levo: Levofloxacin – Dapto: Daptomycin – CFZ: Ceftazidime
Yellow label: Out of range Cytokines level

Materials and Methods

Starting from Nov. 2023 male and non-pregnant female patients ≥18 years of age, with an infective chronic wound (≥3 months to heal), are eligible for O3 auto-hemo-transfusion (O3-AHT) treatment.

O3-AHT (from min. 2 to max. 4 procedures/month for each patient depending on ambulatory availability) is started with the drawing of autologous blood (200/250 ml) and, after O2/O3 mixing (200/250 ml of 96% O2 + 4% O3, with a O3 concentration of 40 µg/mL per mL blood), with its re-infusion.

Each patient undergoes microbiological diagnostic procedure in order to drive proper antibiotic therapy, and is evaluated by vascular surgeon for specific treatment as surgical debridement, photobiomodulation, adipose-derived mesenchymal stem cells wound infusion, peripheral mononuclear cells implantation.

Furthermore for each patient we evaluate the serum cytokines trend every 5 O3-AHT.

Results

We have enrolled 12 patients (Table 1), and performed from min 8 up to max 43 O3-AHT.

The main underlying pathologies are venous and arterial insufficiency; one patient has systemic scleroderma and rheumatic arthritis, one has systemic arterial-venous dysplasia due to Sturge Weber Trenaunay syndrome.

The main isolated pathogens are *S. aureus* and *P. aeruginosa* in 8 cases.

Most of the patients present out of range cytokines levels, with no clear predictable trend accordingly to the expected ozone action: IL-2, IL-8, IFN-g increase v.s. IL-1b, IL-6, TNF-a decrease.

Conclusions

- O3-AHT can be considered a supplementary treatment for infective chronic wound, speeding up the healing process with its anti-oxydant, immuno-modulating and anti-infective properties.
- The next step: understanding a possible correlation between wound healing progress and cytokines trend in *vivo*.