Intravenous Oxygen Insufflation (IOI) changes the IL-1-Ra:IL-1 β ratio in Autologous Conditioned Serum

Brigitte König (✉ brigitte.koenig@medizin.uni-leipzig.de)

Medical Microbiology and Virology <https://orcid.org/0000-0002-5073-3849>

Dirk Wiechert

Praxis Institute

Research

Keywords: Interleukin-1, interleukin-1RA, intravenous oxygen, osteoarthritis, autologous conditioned serum, Sanakin

Posted Date: July 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-355919/v2>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Translational Medicine Communications on March 30th, 2022. See the published version at <https://doi.org/10.1186/s41231-021-00107-z>.

Abstract

Background

Interleukin-1 (IL-1) is still regarded as the main offender that promotes the pro-inflammatory cascade in muscle injuries, tendopathies and especially in osteoarthritis. Thus, if present in high enough concentrations, IL-1 receptor antagonist (IL-1Ra) has the potential to inhibit Interleukin-1. In this regard, autologous conditioned serum with an IL-1Ra/IL-1 ratio of at least >10 might fulfill optimal therapeutic effects. The purpose of this study was to analyze the combination of Regelsberger's intravenous oxygen insufflation (IOI) with autologous conditioned serum (ACS) on IL-1Ra and IL-1 formation.

Methods

Venous blood from 15 patients which underwent intravenous oxygen insufflation (IOI) for routinely preventive purposes was taken before the first, the 6th, and the 9th session of intravenous oxygen insufflation. IL-1 β and IL-1-RA levels were quantified from serum and from autologous conditioned serum (ACS).

Results

Previous intravenous oxygen insufflation treatments significantly reduced IL-1 β levels in autologous conditioned serum from mean 67.85pg/ml (before the first treatment) down to mean 4.08pg/ml (before the 9th treatment). Post conditioning levels of IL-1Ra were not changed to any significant degree (before 1st/before 6th/ before 9th treatment: 1038.37 $\pm$ 140.51 / 900.30 $\pm$ 79.24 / 902.84 $\pm$ 95.68). Thus, the IL-1Ra:IL-1 β ratio was altered on a molecule to molecule basis from a mean of 37.03:1 up to a mean 223.54:1 through the pretreatment.

Conclusion

IOI alters the IL-1Ra:IL-1 β ratio of autologous conditioned serum to a more favorable ratio which might mitigate the inflammatory cascade more efficaciously. Therefore, we suggest to perform intravenous oxygen insufflation on patients before they give blood for preparing ACS.

Background

Osteoarthritis (OA) is the most prevalent arthritis in the world with increasing numbers of people expected to acquire the disease as the population ages. Cartilage degeneration with osteoarthritis (OA) is a debilitating condition that may ultimately require total joint replacement and is believed to involve the activities of interleukin-1 (IL-1), especially due to an imbalance of IL-1 β and IL-1Ra concentration [1]. The same holds true for tendinopathies, muscle injuries, and tunnel widening after reconstruction of the anterior cruciate ligament and other musculoskeletal disorders as well as wound healing disorder e.g. in diabetes.

Therapies commonly used to manage the above-mentioned diseases have limited efficacy and might carry some significant risks. Among the non-operative treatments are bracing, oral analgesics, physical therapy, and injections using corticosteroids, hyaluronic acid, analgesics, local anesthetics [2]. IL-1Ra has thus been considered as a promising disease-modifying OA drug (DMOAD). In this regard, the interleukin-1 receptor antagonist (IL-1Ra) anakinra has been introduced into the treatment of the inflammation and bone destruction of osteoarthritis [3]. The newer products of regenerative medicine, such as autologous conditioned serum,

platelet-rich plasma (PRP) formulations, autologous protein solution, mesenchymal stem cell injections and potential gene therapy with exogenous expression of IL-1Ra address not only the progressively inflammatory environment of the disorder but aim to potentially reversing and correcting the underlying disease process [4–8].

Autologous conditioned serum (ACS) or also called autologous cytokine rich serum (ACRS) was developed in the mid 1990s as an expeditious, practical, and relatively inexpensive means of generating the interleukin-1 receptor antagonist (IL-1Ra), a naturally occurring inhibitor of the cytokine interleukin-1 (IL-1) [9–12]. The safety profile of autologous conditioned serum is proven [13, 14]. Nonetheless, conditioning of serum might not only enhance the induction of IL-1Ra as an anti-inflammatory cytokine; proinflammatory cytokines such as IL-1 β may increase as well. Therefore, treatments which might diminish the proinflammatory potential are desirable. Intravenous oxygen insufflation (IOI) was developed by Dr. Regelsberger and continued by Dr. med. Kreutzer [15, 16]. Meanwhile, more than 1000 therapeutics use this technique in different diseases. Actually, multiple evidence, suggest that IOI modulates a variety of intracellular signal transduction pathways.

The aim of this study was to analyze the effect of IOI on the IL-1Ra:IL-1 β ratio in autologous conditioned serum (ACS) to improve the supportive application of ACS.

Methods

Participants

An informed consent was obtained from a total of 15 asymptomatic patients for taking 10 ml blood of samples at three time points. The oxygen therapy was carried out in all patients for routinely preventive purposes.

Intravenous oxygen insufflation according to Regelsberger (IOI)

Venous oxygen is supplied via a special device such as the Applimed-O2 1000. Applimed-O2 1000 is a class IIb product according to Directive 93/42 / EEC, Annex IX, Rule 5 (Dr. med. H.S. Regelsberger GmbH & Co. KG; Lingen, Germany). The dosage is between 10–60 mL oxygen in increasing doses at an insufflation speed of 1-2 mL / min. The individual dosages for each patient over the time course of 9 days are summarized in Table 1.

Processing of whole blood and production of autologous conditioned serum (ACS)

Before the first IOI treatment, blood samples were taken for analyses of the basal levels of the respective cytokines (no Sanakin™ procedure). For the production of ACS venous blood was taken from all patients before the first, before the 6th and before the 9th treatment with intravenous oxygen insufflation. Each blood sample was conditioned to induce IL-1Ra secretion using the Sanakin™ (ACS) procedure (Scientific BioTech GmbH, Cologne, Germany) according to the companies suggestions. In brief: using aseptic techniques, 10 mL of whole blood from the median cubital vein was harvested into a sterile syringe. Thereafter, the blood was transferred into the Sanakin™-containing medical grade beads, mixed and allowed to incubate for 3 hours at 37°C. After the incubation time, the samples were centrifuged for 5 minutes at 1,300 g. At this time point the ACS is ready for injections e.g. intraarticularly, intradermal or at other parts of the body depending on the symptoms. In this study the serum samples were stored in aliquots at -80°C before performing biomarker assays.

Biomarker assays

The concentrations of cytokines were characterized in the baseline blood and ACS of

each of the 15 patient samples. The measuring IL-1-Ra and IL-1 β were achieved by using the cytokine-specific highly sensitive, commercially available quantitative sandwich enzyme-linked immunoassay technique (R&D Systems, Quantikine ELISA; Minneapolis, MN, USA) according to the manufacturer's instructions. The measurements were carried out using a Mithras LB 940 Multimode Microplate Reader (Berthold Technologies) and were further analyzed using the Magellan7 Software (Tecan).

Statistical analysis

Results obtained were evaluated using one-way ANOVA, with a Tukey post-hoc test. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc). All p-value ≤ 0.05 were considered to be statistically significant.

Results And Discussion

Basal levels of IL-1 β , IL-1-Ra, and IL-1Ra:IL-1 β ratio

Before each treatment, the concentration of IL-1 β and IL-1-Ra were determined in serum (Baseline). In the case of IL-1-Ra, an average concentration of 332.24 ± 53.29 pg/ml was detectable in all samples, while the concentrations of IL-1 β were below the detection limit of 0.5 pg/ml. Calculation of the amount of IL-1-Ra and IL-1 β molecules resulted in a IL-1Ra:IL-1 β ratio of approximately 495:1 (Fig. 1 and Fig. 2). Therefore, our measurements revealed about 6.6-fold higher IL-1Ra:IL-1 β ratio as shown previously. These differences in our observations in comparison to Barreto et al. [14] are due to the low abundance of IL-1 β in the patients of our cohort. Detected IL-1Ra levels, however, were lower as shown by Barreto et al. (332.24 ± 53.29 vs. 549.6 ± 52.6) [14]. Other studies found baseline IL-1-Ra serum levels were between 192.73 pg/ml and 910.91 pg/ml. However, Meijer et al. described IL-1Ra and IL-1 β serum basal levels of only $73 \pm 4.8/3.3 \pm 1.1$ pg/ml [17]. In the study of Magalon et al. IL-1-Ra and IL-1 β , basal serum levels were below the detection limits [13]. Taken together, our data suggest an average basal ratio of IL-1Ra:IL-1 β of about 495:1, whereas the level of IL-1 β was tremendously (undetectable) low (Fig. 1, Fig. 2).

The concentration of IL-1-Ra, IL-1 β , and IL-1Ra:IL-1 β ratio in conditioned autologous serum (ACS)

Further, we determined the levels of both cytokines (IL-1Ra and IL-1 β) in the Sanakin autologous conditioned serum (ACS) and calculated the respective IL-1Ra:IL-1 β ratio (prior 1. IOI). Intriguingly, we found a significant increase in IL-1Ra levels from 332.24 ± 53.29 up to 1038.37 ± 140.51 pg/ml (approximately 3,1-fold). Moreover, IL-1 β levels were also significantly elevated from undetectable levels up to a median of $67,85$ pg/ml ± 21.83 pg/ml. Due to the increase in IL-1 β the overall ratio of IL-1Ra:IL-1 β shifted from 495:1 to 37:1.

In detail, prior first oxygenation (prior OXY1) IL-1Ra levels were between 502.73 pg/ml and 2.449.55 pg/ml. The increase in IL-1Ra ranges from 1.6 to 5.6-fold towards the baseline level. Whereas only one patient displayed an increase lower than 2-fold, six patients were between 2- and 3-fold, three patients between 3- and 4-fold and the remaining five displayed an increase in IL-1Ra level between 4- and 5.6-fold.

Nevertheless, even though the concentration of IL-1Ra and IL-1 β was increased upon ACS the ratio of IL-1Ra:IL-1 β was lower than the ratio of the baseline. Intriguingly, the drop in the ratio of IL-1Ra:IL-1 β of the baseline to the first ACS differs tremendously between single patients and ranges from a 3.2-fold to a 116.3-fold. These data indicate that the success of ACS is highly dependent on the patient. Due to the fact that the ratio of IL-1Ra: IL-1 is higher in serum (baseline) than in ACS one might suggest that straight serum make a better product for treating osteoarthritis than ACS. However, we have to keep in mind that the increase in the absolute number of IL-1Ra molecules exceeds the increase in IL-1 β molecules several times over. Thus, more IL-1 β molecules can be neutralized after IOI.

In the course of the following IOI treatments (6.IOI and 8.IOI), it was observed that the IL-1Ra:IL-1 β ratio in the ACS further increased. The reason for the higher IL-1Ra:IL-1 β ratio was primarily due to a lower IL-1 β level which was lowered from 67.85 ± 21.83 pg/ml (prior 1.IOI) to 22.92 ± 8.51 pg/ml (prior 6.IOI) to a final concentration of 4.08 ± 1.49 pg/ml (after 8.IOI). The concentration of IL-1Ra, in turn, was only slightly lowered.

Taken together, it was observable that ACS increases both IL-1Ra and IL-1 β but in combination with intravenous oxygen insufflation (IOT) the IL-1 β concentration was significantly lowered thus promoting a potential displacement of IL-1 β from cell surface receptors by IL-1Ra (Fig. 1, Fig. 2).

Table 1: Parameter of intravenous oxygen insufflation (IOI) according to Regelsberger [15] for the 15 patients under study using the the Applimed-O2 1000

IOI parameter									
Patient No	1	2	3	4	5	6	7	8	9
1	10ml	20ml	20ml; twice	30ml; twice	40ml; twice	40ml; twice	45ml; twice	50ml; twice	50ml; twice
2	30ml; twice	45ml; twice	55ml; twice	55ml; twice	60ml; twice	70ml ; twice	70ml; twice	80ml; twice	80ml; twice
3	10ml	15ml	20ml	20ml	25ml	25ml; twice	30ml; twice	35ml; twice	40ml; twice
4	10ml	20ml	30ml	40ml; twice	50ml; twice	50ml; twice	60ml; twice	70ml; twice	75ml; twice
5	10ml	20ml	25ml	11ml	35ml; twice	45ml	50ml	55ml	60ml; twice
6	10ml	15ml	20ml	30ml	30ml; twice	40ml	45ml	50ml	50ml
7	30ml; twice	45ml; twice	50ml; twice	50ml; twice	60ml; twice	60ml; twice	70ml; twice	70ml; twice	70ml; twice
8	10ml	20ml	30ml	30ml	30ml	35ml	35ml	35ml	35ml
9	30ml; twice	45ml; twice	60ml; twice	70ml; twice	65ml; twice	70ml; twice	70ml; twice	75ml; twice	80ml; twice
10	5ml	6ml	10ml	15ml	15ml	15ml	20ml	20ml	20ml
11	30ml	30ml	20ml	20ml	20ml	20ml; twice	25ml; twice	25ml; twice	20ml; twice
12	10ml	20ml	30ml	30ml; twice	40 ml; twice	40ml; twice	45ml; twice	50ml; twice	50ml; twice
13	60ml; twice	70ml; twice	70ml; twice	70ml; twice	70ml; twice	75ml; twice	75ml; twice	70ml; twice	75ml; twice
14	10ml	10ml	15ml	25ml	25ml ; twice	30ml; twice	35ml; twice	40ml; twice	50ml; twice
15	30ml; twice	40ml; twice	40ml; twice	40ml; threefold	50ml; threefold	60ml; threefold	70ml; threefold	80ml; threefold	90ml; threefold

Conclusions

Our data have clearly shown that the combination of intravenous oxygen insufflation previous to autologous conditioned serum preparation favors the IL-1Ra:IL-1 β ratio mainly by reducing the IL-1 β concentrations. Thus, IOI could be a promising method to increase the ratio of IL-1Ra :IL-1 and thus prolong the efficacy of anti-inflammatory treatment. At present limited data are available on the detailed combination of cytokines and growth factors in the Sanakin™ autologous conditioned serum (ACS) prepared before and after intravenous oxygen insufflation. Thus, their respective contributions to the clinical effects remain to be unraveled. In this regard, new investigations are necessary to determine the mechanisms by which the effects of ACS

(with/without IOI) are mediated and the quality of the product, by analysis of e.g. radiographs after a more extended follow-up period. Nonetheless, we may assume that ACS especially in combination with other treatments such as intravenous oxygen insufflation, might lead to the enhancement of tissue regeneration and to the reduction of degenerative mechanisms. The main indications for the described combination therapy are sterile inflamed arthritic joints, which react with the formation of effusions (knee, shoulder, vertebral joints, rhizarthrosis, metatarsophalangeal joints), tendopathies (diseases of tendons and ligaments) as they are experienced in many sports as well as golfer elbow, tennis elbow, supination trauma of the hocks, irritation of the Plantaraponeurose, heel spurs but also fresh muscle injuries that have to heal quickly especially in competitive sports. Addressing these symptoms might help to establish beneficial future low-cost and easy-to-carry-out therapies for multiple disorders.

Abbreviations

IOT: Intravenous oxygen insufflation; OA: Osteoarthritis; ACS: autologous conditioned serum; IL-1 β : interleukin-1 β ; IL-1RA. Interleukin-1 receptor antagonist

Declarations

Ethics approval and consent to participate

Approved by the Institutional Review Board (IRB), University of Leipzig, Germany (approval number 348-18-ek)

Consent for publication

Non applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests

DW is using the method of IOI and ACS in his private praxis for therapy purposes.

Funding

There were three sources of funding: 1) BK used project flat rates from various third-party funded projects (project manager BK); 2) from International Society for Oxyvenation Therapy, where DW holds the position of 1st Chairman; 3) from Scientific BioTech GmbH, Kranhaus 1, Im Zollhafen 18, 50678 Köln, Germany

Authors' contributions

BK: design of the work, analysis, interpretation of data

DW: drawing of blood samples and generating the autologous serum for analysis.

All authors read and approved the final manuscript.

Acknowledgements

Non applicable

References

1. Burger D, Dayer JM, Palmer G, Gabay C. Is IL-1 a good therapeutic target in the treatment of arthritis? *Best Pract Res Clin Rheumatol*. 2006 Oct;20(5):879 – 96. doi: 10.1016/j.berh.2006.06.004. PMID: 16980212.
2. Wehling P, Evans C, Wehling J, Maixner W. Effectiveness of intra-articular therapies in osteoarthritis: a literature review. *Ther Adv Musculoskelet Dis*. 2017 Aug;9(8):183–96. doi:10.1177/1759720X17712695.
3. Iqbal I, Fleischmann R. Treatment of osteoarthritis with anakinra. *Curr Rheumatol Rep*. 2007 Apr;9(1):31 – 5. doi: 10.1007/s11926-007-0019-9. PMID: 17437664.
4. Richards MM, Maxwell JS, Weng L, Angelos MG, Golzarian J. Intra-articular treatment of knee osteoarthritis: from anti-inflammatories to products of regenerative medicine. *Phys Sportsmed*. 2016;44(2):101–8. doi:10.1080/00913847.2016.1168272.
5. Cole BJ, Karas V, Hussey K, Pilz K, Fortier LA. Hyaluronic Acid Versus Platelet-Rich Plasma: A Prospective, Double-Blind Randomized Controlled Trial Comparing Clinical Outcomes and Effects on Intra-articular Biology for the Treatment of Knee Osteoarthritis. *Am J Sports Med*. 2017 Feb;45(2):339–346. doi: 10.1177/0363546516665809. Epub 2016 Oct 21. Erratum in: *Am J Sports Med*. 2017 Apr;45(5):NP10. PMID: 28146403.
6. Evans C. Editorial. Arthritis Gene Therapy Using Interleukin-1 Receptor Antagonist. *Arthritis Rheumatol*. 2018 Nov;70(11):1699–701. doi:10.1002/art.40675. PMID: 30035385; PMCID: PMC6203597.
7. Nixon AJ, Grol MW, Lang HM, Ruan MZC, Stone A, Begum L, Chen Y, Dawson B, Gannon F, Plutizki S, Lee BHL, Guse K. Disease-modifying Osteoarthritis Treatment with Interleukin-1 Receptor Antagonist Gene Therapy in Small and Large Animal Models. *Arthritis Rheumatol*. 2018 Jul 25. doi:10.1002/art.40668.
8. Harrell CR, Markovic BS, Fellabaum C, Arsenijevic N, Djonov V, Volarevic V. The role of Interleukin 1 receptor antagonist in mesenchymal stem cell-based tissue repair and regeneration. *Biofactors*. 2020 Mar;46(2):263–75. doi:10.1002/biof.1587. Epub 2019 Nov 22. PMID: 31755595.
9. Barreto A, Braun TR. A method to induce Interleukin-1 Receptor Antagonist Protein from autologous whole blood. *Cytokine*. 2016 May;81:137–41. doi: 10.1016/j.cyto.2016.03.008.
10. Becker C, Heidersdorf S, Drewlo S, de Rodriguez SZ, Krämer J, Willburger RE. Efficacy of epidural perineural injections with autologous conditioned serum for lumbar radicular compression: an investigator-initiated, prospective, double-blind, reference-controlled study. *Spine (Phila Pa 1976)*. 2007 Aug 1;32(17):1803-8. Erratum in: *Spine*. 2007 Nov 15;32(24).
11. Frizziero A, Erika Giannotti F, Oliva MS, Maffulli N. Autologous conditioned serum for the treatment of osteoarthritis and other possible applications in musculoskeletal disorders. *Br Med Bull*. 2013;105:169–84. DOI:10.1093/bmb/lds016.
12. Godek P. Use of Autologous Serum in Treatment of Lumbar Radiculopathy Pain. Pilot Study. *Ortop Traumatol Rehabil*. 2016 Jan-Feb;18(1):11–20. doi:10.5604/15093492.1198829.
13. Zarringam D, Bekkers JEJ, Saris DBF. Long-term Effect of Injection Treatment for Osteoarthritis in the Knee by Orthokin Autologous Conditioned Serum. *Cartilage*. 2018 Apr;9(2):140–145. doi:

14. Barreto A, Braun TR. A new treatment for knee osteoarthritis: Clinical evidence for the efficacy of Arthrokinex™ autologous conditioned serum. *J Orthop.* 2016 Oct 25;14(1):4–9.
15. REGELSBERGER HS. BEDEUTUNG DER DIREKTEN. INTRAVENOESSEN SAUERSTOFF-INSUFFLATION (OXYVENIERUNG) FUER DIE MODERNE THERAPIE UND PROPHYLAXE [SIGNIFICANCE OF THE DIRECT INTRAVENOUS OXYGEN INSUFFLATION IN MODERN THERAPY AND PROPHYLAXIS]. *Acta Neuroveg (Wien).* 1964 Nov 16;27:1–74. German. doi: 10.1007/BF01226937. PMID: 14348501.
16. Kreutzer FJ, Intravenöse, Sauerstofftherapie. (IOT): Oxyvenierungstherapie nach Regelsberger in Theorie und Praxis. Von den Anfängen bis zur Gegenwart. Dr. med. H.S. Regelsberger GmbH & Co. KG, Herausgeber.
17. Meijer H, Reinecke J, Becker C, Tholen G, Wehling P. The production of cytokines in whole blood by physico-chemical induction. *Inflamm Res.* 2003 Oct;52(10):404–7.

Figures

Figure 1

Concentration of IL-1 β (A) and IL-1Ra (B) detected using ELISA in a total of 15 patients. Note that due to low signal intensity in the case of “Baseline” IL-1 β , the concentration was set to 0.5 pg/ml. Shown is the mean $\pm$ SE. Statistical analyses were carried out as described in the method section. IOI = intravenous oxygen insufflation; ACS = autologous conditioned serum.

Figure 2

Ratio of IL-1Ra to IL-1 β detected using ELISA in a total of 15 patients. Shown is the mean $\pm$ SE. Statistical analyses were carried out as described in the method section. IOI = intravenous oxygen insufflation; ACS = autologous conditioned serum.