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Intravenous oxygen insufflation (IOI) changes the IL-1-Ra:IL-1β ratio in autologous conditioned serum

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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-1receptor 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 aim of the study was to analyze whether a pretreatment of patients with the oxygen insufflation according to Regelsberger (IOI) might lead to an increased ratio of IL-RA to IL-1ß in autologous serum prepared after the respective therapy.

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-1β and IL-1-RA levels were quantified from serum and from autologous conditioned serum (ACS) prepared from the drawn venous blood.

Results: Previous intravenous oxygen insufflation treatments significantly reduced IL-1β levels in autologous conditioned serum from mean 67.85 pg/ml (before the first treatment) down to mean 4.08 pg/ml (before the 9th treatment). Post conditioning levels of IL-1Ra were not changed to any significant degree (before 1st/before6th/ before 9th treatment: 1038.37 ± 140.51 / 900.30 ± 79.24 / 902.84 ± 95.68). Thus, the IL-1Ra:IL-1β 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 with oxygen insufflation according to Regelsberger.

Conclusion: Pretreatment of patients with IOI alters the IL-1Ra:IL-1ß 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.

Keywords: Interleukin-1, Interleukin-1RA, Intravenous oxygen, Osteoarthritis, Autologous conditioned serum, Sanakin

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

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interleukin-1 (IL-1), especially due to an imbalance of IL-1β 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 mid1990s 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-1ß may increase as well [15]. Therefore, treatments which might diminish the proinflammatory potential in ACS are desirable. Intravenous oxygen insufflation (IOI) was developed by Dr. Regelsberger and continued by Dr. med. Kreutzer [16, 17]. Actually, multiple clinical evidence suggest that IOI modulates the anti-inflammatory cytokine response.

The aim of this study was to analyze whether pretreatment of patients with IOI might have a favourable outcome on the IL-1Ra:IL-1ß ratio in subsequently prepared autologous conditioned serum (ACS). The proposed working mechanism of ACS is intra-articular inhibition of the resulting proinflammatory cytokine interleukin-1 (IL-1) through the injection of autologous incubated serum containing increased levels of IL-1 receptor antagonist (IL-1ra). Thus, pretreatment of patients with underlying diseases such as osteoarthritis, tendinopathies or muscle injuries with intravenous oxygen insufflation could improve the supportive application of ACS.

Table 1 Baseline characteristics of the subjects

N	15
Age, years	
Males	Mean 55.88 (33–71)
Females	Mean 53.16 (19–72)
Males	9 (60%)
Females	6 (40%)
Chronic medical conditions	
Hashimoto	1 (6.6%)
Hypertension	4 (26.6%)
Ischemic heart disease	1 (6.6%)
Osteoporosis	1 (6.6%)
Psoriasis	1 (6.6%)
Asthma	2 (13.3%)
Hypothyreosis	1 (6.6%)
Diabetes	1 (6.6%)
Allergy	1 (6.6%)
Hyperthyreosis	1 (6.6%)
Colitis ulcerosa	1 (6.6%)

Methods

Subjects

Fifteen adults who attended the clinical practice receiving the intravenous oxygen insufflation (IOI) for preventive purposes, were enrolled. The study was performed between 2015-and 2020 in the clinical praxis of Dr. Wiechert, Bremen, germany. Included patients did not have cardiac or cerebrovascular ischemia histories for the last year prior to inclusion. Exclusion criteria included: previous treatment with IOI for any reason during the last three months, any history of malignancy during the last year, any pathological cognitive decline, severe chronic renal failure (GFR < 30), uncontrolled diabetes mellitus (HbA1C > 8, fasting glucose > 200), immunosuppressants, BMI > 35, active smoking or pulmonary diseases as well as acute infections. The baseline characteristics of the subjects are summarized in Table 1.

Study design

The study protocol was approved by the Institutional Review Board (IRB), University of Leipzig, Germany. An informed consent was obtained from a total of 15 patients for taking additional 10 ml blood at three time points. During therapy with intravenous oxygen insufflation according to Regelsberger blood is drawn at least before each session for routine purposes (monitoring blood count). 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 2.

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 h at 37 °C. After the incubation time, the samples were centrifuged for 5 min 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-1β 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 Sofware, Inc). All p-value ≤ 0.05 were considered to be statistically significant.

Results and discussion

Basal levels of IL-1ß, IL-1-Ra, and IL-1RA:IL-1ß ratio

Before each treatment, the concentration of IL-1β and IL-1-Ra were determined in serum (Baseline). In the case of IL1-Ra, an average concentration of 332.24 ± 53.29 pg/ml was detectable in all samples, while the concentrations of

Table 2 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	10 ml	20 ml	20 ml; twice	30 ml; twice	40 ml; twice	40 ml; twice	45 ml; twice	50 ml; twice	50 ml; twice
2	30 ml; twice	45 ml; twice	55 ml; twice	55 ml; twice	60 ml; twice	70 ml; twice	70 ml; twice	80 ml; twice	80 ml; twice
3	10 ml	15 ml	20 ml	20 ml	25 ml	25 ml; twice	30 ml; twice	35 ml; twice	40 ml; twice
4	10 ml	20 ml	30 ml	40 ml; twice	50 ml; twice	50 ml; twice	60 ml; twice	70 ml; twice	75 ml; twice
5	10 ml	20 ml	25 ml	11 ml	35 ml; twice	45 ml	50 ml	55 ml	60 ml; twice
6	10 ml	15 ml	20 ml	30 ml	30 ml; twice	40 ml	45 ml	50 ml	50 ml
7	30 ml; twice	45 ml; twice	50 ml; twice	50 ml; twice	60 ml; twice	60 ml; twice	70 ml; twice	70 ml; twice	70 ml; twice
8	10 ml	20 ml	30 ml	30 ml	30 ml	35 ml	35 ml	35 ml	35 ml
9	30 ml; twice	45 ml; twice	60 ml; twice	70 ml; twice	65 ml; twice	70 ml; twice	70 ml; twice	75 ml; twice	80 ml; twice
10	5 ml	6 ml	10 ml	15 ml	15 ml	15 ml	20 ml	20 ml	20 ml
11	30 ml	30 ml	20 ml	20 ml	20 ml	20 ml; twice	25 ml; twice	25 ml; twice	20 ml; twice
12	10 ml	20 ml	30 ml	30 ml; twice	40 ml; twice	40 ml; twice	45 ml; twice	50 ml; twice	50 ml; twice
13	60 ml; twice	70 ml; twice	70 ml; twice	70 ml; twice	70 ml; twice	75 ml; twice	75 ml; twice	70 ml; twice	75 ml; twice
14	10 ml	10 ml	15 ml	25 ml	25 ml; twice	30 ml; twice	35 ml; twice	40 ml; twice	50 ml; twice
15	30 ml; twice	40 ml; twice	40 ml; twice	40 ml; threefold	50 ml; threefold	60 ml; threefold	70 ml; threefold	80 ml; threefold	90 ml; threefold

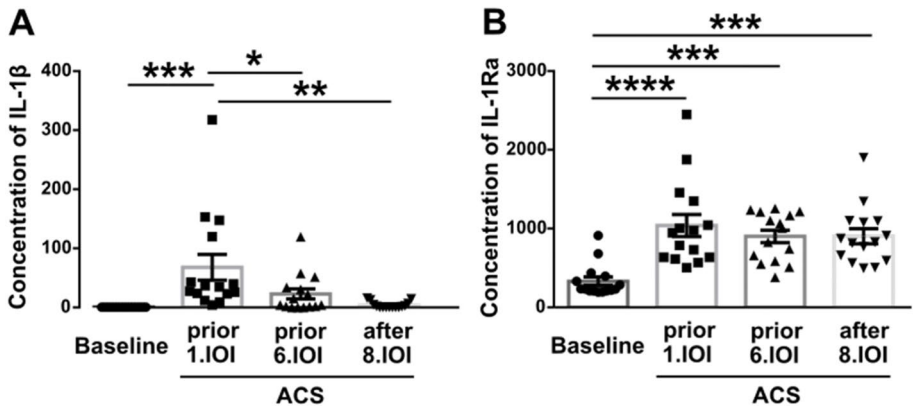

Fig. 1 Concentration of IL-1β (A) and IL-1Ra (B) detected using ELISA in a total of 15 patients. Note that due to low signal intensity in the case of "Baseline" IL-1β, the concentration was set to 0.5 pg/ml. Shown is the mean ± SE. Statistical analyses were carried out as described in the method section.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

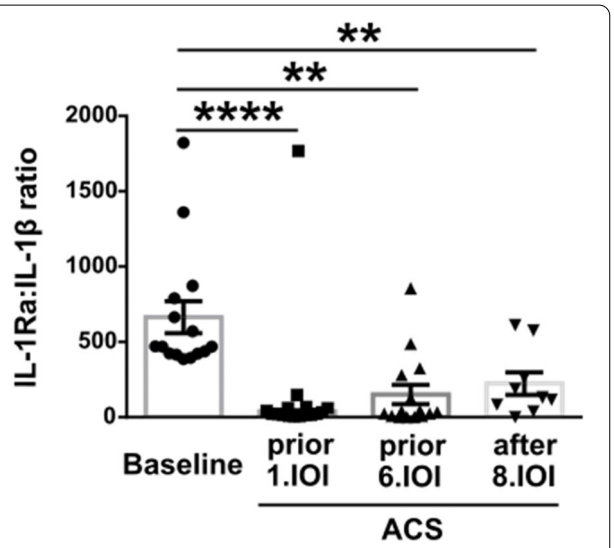

Fig. 2 Ratio of IL-1Ra to IL-1β detected using ELISA in a total of 15 patients. Shown is the mean ± SE. Statistical analyses were carried out as described in the method section. IOI = intravenous oxygen insufflation; ACS = autologous conditioned serum

IL-1β were below the detection limit of 0.5 pg/ml. Calculation of the amount of IL-1-Ra and IL-1β molecules resulted in a IL-1Ra:IL-1β ratio of approximately 495:1 (Figs. 1 and 2). Therefore, our measurements revealed about 6.6-fold higher IL-1Ra:IL-1β ratio as shown previously [13, 14, 18]. These differences in our observations in comparison to Barreto et al. [14] are due to the low abundance of IL-1β 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-1β serum basal levels of only 73 ± 4.8/3.3 ± 1.1 pg/ml [18]. In the study of Magalon et al. IL-1-Ra and IL-1β, basal serum levels were below the detection limits [13]. Taken together, our data suggest an average basal ratio of IL-1Ra:IL-1β of about 495:1, whereas the level of IL-1β was tremendously (undetectable) low (Figs. 1 and 2).

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

Further, we determined the levels of both cytokines (IL-1Ra and IL-1β) in the Sanakin autologous conditioned serum (ACS) at baseline and calculated the respective IL-1Ra:IL-1β ratio (prior 1. IOI). Intriguingly, we found a significant increase in IL-1Ra levels from 332.24 ± 53.29 pg/ml in serum up to 1038.37 ± 140.51 pg/ml (approximately 3,onefold) in the respective Sanakin autologous conditioned serum. Moreover, IL-1β levels were also significantly elevated from undetectable levels in the serum up to a median of 67.85 pg/ml ± 21.83 pg/ml in the ACS. Due to the increase in IL-1β the overall ratio of IL-1Ra:IL-1β 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 twofold, six patients were between 2- and threefold, three patients between 3- and fourfold and the remaining five displayed an increase in IL-1RA level between 4- and 5.6-fold. Nevertheless, the ratio of IL-1Ra:IL-1β was lower than the ratio of the baseline. The drop in the ratio of IL-1Ra:IL-1β 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 that a IL-1RA:IL-1ß ratio > 10 is sufficient to neutralize IL-1ß and the increase in the absolute number of IL-1Ra molecules exceeds the increase in IL-1ß molecules several times over. Moreover, ACS contains elevated levels of additional anti-inflammatory cytokines and growth factors which fulfil beneficial effects beside IL-1RA [15].

In the course of the following IOI treatments (6.IOI and 8.IOI), it was observed that the IL1Ra:IL-1β ratio in the ACS further increased. The reason for the higher IL-1Ra:IL-1β ratio was primarily due to a lower IL-1β level which was lowered from 67.85 ± 21.83 pg/ml (ACS; prior 1.IOI) to 22.92 ± 8.51 pg/ml (ACS, prior 6.IOI) to a final concentration of 4.08 ± 1.49 pg/ml (ACS, 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-1β but in combination with intravenous oxygen insufflation (IOT) the IL-1β concentration was significantly lowered thus promoting a potential displacement of IL-1β from cell surface receptors by IL-1Ra (Figs. 1 and 2).

Conclusions

Our data have clearly shown that the combination of intravenous oxygen insufflation previous to autologous conditioned serum preparation favors the Il-1-Ra:IL-1β ratio mainly by reducing the IL-1β 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-1ß: Interleukin-1ß; IL-1RA: Interleukin-1 receptor antagonist.

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Author's 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.

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Availability of data and materials

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

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.

Competing interests

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

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