ANNUAL REPORT

2011

Ludwig Boltzmann Cluster

Rheumatology, Balneology and Rehabilitation

A CLUSTER OF THE LUDWIG BOLTZMANN SOCIETY

FRAMEWORK OF THE CLUSTER

Mission and Aims

It is the primary aim of the Cluster to provide a unique research platform for disease- and patient-oriented research in rheumatology, balneology and rehabilitation

The Cluster for Rheumatology, Balneology and Rehabilitation was established by the Ludwig Boltzmann Society in 2007 and currently consists of the following Ludwig Boltzmann institutes

- **Institute for Rheumatology and Balneology, Vienna-Oberlaa**
- **Institute for Rehabilitation of Internal Diseases, Saalfelden**
- **Research Unit for Epidemiology of Rheumatic Diseases, Baden**

While high quality research on rheumatic diseases is done at all Austrian Medical Universities, and particularly at the Medical University of Vienna, research activities addressing issues of balneology and rehabilitation are rather limited. Therefore the Cluster´s research programmes are focused on questions related to the application of balneological therapies and rehabilitation programmes in the treatment of rheumatic disorders, particularly osteoarthritis and rheumatoid arthritis.

The **basic and translational research** is primarily aimed at investigating pathogenetic mechanisms of inflammatory rheumatic diseases and elucidating the molecular and cellular mechanisms of balneological treatments.

The **clinical research** is focused on developing novel therapeutic tools for rehabilitation programmes and on analysing the effectiveness and sustainability of cure and rehabilitation programmes.

This unique **multi-disciplinary combination** of basic and clinical research performed in the Cluster allows to addresss simultaneously and in a coordinated manner several important questions in the fields of rheumatology, balneology and rehabilitation.

Finance

The cluster´s finance is shown in the Table below. Total annual revenues amount to 552.618 €, total expenses were 606.091 €, the difference of 53.473 € is compensated by funds from previous years. About two thirds of the budget was used for personnel, 20% for research (reagents and consumables), 5% for administration. Approximately 60% of the budget is provided by the Boltzmann society, the rest is third party funding, the major sponsor are the Austrian Pension Insurance Organization (PVA) with an annual contribution of 130.000 € and the state of Salzburg with an annual contribution of 20.000 €. These funds form the financial basis of the budget of the LBI Saalfelden. A relatively small amount of third party funds is dedicated to specific projects.

Funds from previous years	**252.786,--**
Revenues	
Boltzmann Society	326.913,--
Third Party	179.230,--
Third Party project specific	44.672,--
Other revenues	1.803,--
Total	**552.618,--**
Expenses	
Personnel	441.920,--
Research	123.296,--
Investments	4.721,--
Administration	33.364,--
Other expenses	2.790,--
Total	**606.091,--**

Partners

Major partners are

- Rheumazentrum Wien–Oberlaa GmbH (Center for Rheumatic Diseases, Vienna-Oberlaa)
- Pensionsversicherungsanstalt (Austrian Pension Insurance Organization, PVA)
- Medizinische Universität Wien (Medical University Vienna)

Steering Board (Lenkungsausschuss)

The steering board decides on projects and finance and consists of five members from the fields of rheumatology, balneology and rehabilitation.

Univ.-Prof. Dr. Steffen Gay, Universität Zürich, Chairman
Univ.-Prof. Dr. Josef, Smolen, Medizinische Universität Wien
Univ.-Doz. Dr. Tanja Stamm, Medizinische Universität Wien
Prim. Univ.-Prof. Dr. Hans Bröll, Rheuma Zentrum Wien-Oberlaa
Prim. Dr. Reinhold Hawel, Sonderkrankenanstalt für Erkrankungen des Stütz- und Bewegungsapparates der PVA, Bad Hofgastein

Personnel

Full-time employees.	4
Part-time employees:	12
PhD Student	..1
Contract for work:	2
Total	19

Ao. Univ.Prof. Dr. Günter Steiner: Cluster coordinator since 2010 and leader of the research programme RHEUMA. Major affiliation: Medical University of Vienna, Internal Medicine III, Division of Rheumatology

Univ.Doz. Dr. Werner Kullich: Head of the Ludwig Boltzmann Institute for Rehabilitation of Internal Diseases Saalfelden (LBI Rehab) since 2004; full-time employed since 1983; leader of the Research Programme REHAB.

Prim. Dr. Ernst Wagner: Head of the Ludwig Boltzmann Research Unit for Epidemiology of Rheumatic Diseases; Baden

Ao. Univ.Prof. Dr.Cem Ekmekcioglu: physiologist; employed via contract for work, performing studies on clock gene expression and function in patients with rheumatic diseases; place of work: Institute of Physiology, Medical University of Vienna

Dr. Burkhard Klösch: key researcher at the Ludwig Boltzmann Institute for Rheumatology and Balneology (LBI Rheum); Vienna-Oberlaa; full-time employed; leader of the research programme BALNEO. investigations on the mode of action of hydrogen sulphide and sulphur bath therapy

Dr. Makyieh Tohidast-Akrad: research associate since 1986, full-time employed; head of the immunohistochemistry laboratory of the LBI Rheum branch located at the 2nd Department of Medicine, Hietzing Hospital, Vienna

Dr. Bibiane Steinecker-Frohnwieser: research associate with free employment contract since 2005, regular employment status since October 2009, 16 hours a week, increase to 28 hours a week beginning with September 2011; involved in outcome research projects as well as in basic research studies on cellular and molecular mechanisms of NMR therapy; place of work: LBI Rehab-branch Gröbming

Mag. Nicola Fagerer: research associate since October 2005 with regular employment status from August 2007 till September 2011; 30 hours a week; participation in several projects, such as outcome measurement and radical research; place of work: Saalfelden

Mag. Gabriele Nebel: assistant to Prim. Univ.Prof. Dr. Hans Bröll (steering board member); regular employment contract till December 2011, 5 hours a week; place of work: Vienna-Oberlaa

Dipl.Ing. Norbert Klammer: chemist; study administration and data analysis; employed on a small basis of less than 10 hours a month; place of work: Institute of Physiology, Medical University of Vienna

Melissa Liszt: biomedical analyst; full-time employment from May 2008-April 2011, assistant to Dr. Burkhard Klösch; place of work: Vienna-Oberlaa

Brigitta Schweiger: biomedical analyst; regular employment contract since 1981, 20 hours a week; place of work: Saalfelden

Susanne Humpeler: biomedical analyst; administration of studies; employed on a small basis of less than 10 hours a month; place of work: Institute of Physiology, Medical University of Vienna

Angela Schwaiger: secretary, assistant to Dr. Kullich; regular employment contract since 1995, 30 hours a week; place of work: Saalfelden.

Johanna Leibl: secretary, assistant to Prof. Steiner and Prof. Smolen (member of the steering committee); regular employment contract, 8 hours per week, place of work: Medical University of Vienna, Department of Internal Medicine III.

PhD and diploma students

Mag. Daniela Krehan: PhD student. Topic of her thesis: Effects of hydrogen sulphide on the inflammatory state of fibroblast-like synoviocytes and T-lymphocytes from patients with rheumatoid arthritis or osteoarthritis; employed 30 hours a week; place of work: Vienna-Oberlaa

New employments

Mag. Elisabeth Dietersdorfer: biologist; full-time employed from July 2011, assistant to Dr. Burkhard Klösch; place of work: Vienna-Oberlaa

Mag. Silvia Löbsch: biologist; regular employment from August 2011, 30 hours per week; assistant to Dr. Burkhard Klösch; place of work: Vienna-Oberlaa

Mag. Tatjana Becker; research associate with a free employment contract since September 2011, 16 hours a week, working on the clock gene project; place of work: Institute of Physiology.

BSc. Barbara Stritzinger: biomedical analyst from the FH Urstein, free employment contract since 1st October 2011, 68 hours per month; place of work: Saalfelden.

Highlights of the year

Awards

Best-Paper-Award 2011 for Young First Author Scientists of the International Society of Pteridinology, awarded to Nicola Fagerer for her Article: "Expression of Neopterin and Chemokines in Rheumatoid Arthritis and Cardiovascular Disease" (Pteridines 2011; 22:7-12).

Publications

Hoffmann MH, Skriner K, Herman S, Baumann C, Steiner CW, Ospelt C, Meyer B, Gleiss A, Pfatschbacher J, Niederreiter B, Tuncel J, Zanoni G, Steiner G.
Nucleic acid-stimulated antigen presenting cells trigger T cells to induce disease in a rat transfer model of inflammatory arthritis.
J Autoimmun. 2011; 36:288-300.
IF 8.136

Kloesch B, Liszt M, Broell J, Steiner G.
Dimethyl sulphoxide and dimethyl sulphone are potent inhibitors of IL-6 and IL-8 expression in the human chondrocyte cell line C-28/I2.
Life Sci. 2011; 89 (13-14):473-8.
IF 2.661

RESEARCH PROGRAMMES

The research programmes of the Cluster are addressing questions of clinical and translational research in the fields of rheumatology, balneology and rehabilitation. The focus of clinical research is on the two major rheumatic diseases, rheumatoid arthritis (RA) and osteoarthritis (OA), the focus of translational research is on cellular and molecular mechanisms of balneotherapy, especially sulphur spa therapy and nuclear magnetic resonance therapy because only few studies have so far adressed these issues and scientific evidence for effectiveness of these therapies is scarce and still doubtful.

Research Programme RHEUMA

LBI for Rheumatology and Balneology, Vienna-Oberlaa

Project 1. Involvement of hydrogen sulfide and sulphur-containing compounds in the pathogenesis and therapy of rheumatic disorders

Effects of hydrogen sulfide on the inflammmatory status of fibroblast-like synoviocytes of rheumatoid arthritis and osteoarthritis patients

Background

Sulfur bath therapy has long been in use for the therapy of patients suffering from rheumatic disorders and is still considered helpful for the treatment of diseases such as rheumatoid arthritis (RA) or osteoarthritis (OA). However, scientific investigations dealing with the beneficial as well as adverse effects of this kind of treatment are rare and have sometimes led to controversial results (1-3). Moreover, the underlying molecular mechanisms are poorly understood (4, 5). In vitro, H2S exerts a host of effects on various biological targets, resulting in responses that range from cytotoxicity (6-10) to cytoprotective effects (11). Several studies have demonstrated cytoprotective effects of H2S at micromolar concentrations, which may be related to its ability to neutralize a variety of reactive species including oxyradicals (12), peroxynitrite (13), hypochlorous acid (14) and homocysteine (15). Exposure to higher concentrations (millimolar) of H2S tends to be cytotoxic due to free radical and oxidant generation (8), calcium mobilization (16), glutathione depletion (9), as well as the induction of mitochondrial cell death pathways (7, 17).

Our own data obtained in the past three years suggest that H2S may have anti- as well as pro-inflammatory properties depending on concentration and cell type (18-20). At high concentrations (0.5 – 1.0 mM NaHS), H2S upregulated pro-inflammatory genes (e.g. IL-6, IL-8 and COX-2) in fibroblast-like synoviocytes (FLS) derived from RA and OA patients (20). In contrast, H2S shows pronounced anti-inflammatory properties in monocytes and macrophages (manuscript in preparation).

Results

To explore the effects of H2S, four FLS lines (2 from patients with RA and 2 from patients with OA) were incubated for 20 min with 1.o mM of the H_2S-donor sodium hydrogen sulfide (NaHS). After changing the culture medium, incubation was continued for 12 h. At different time points, cell culture supernatants were collected and IL-6 release was quantified by ELISA.

IL-6 expression of two FLS lines (one from a patient with RA and one from a patient with OA) was significantly increased by H_2S treatment (Fig. 1) while the two other lines remained

unaffected (data not shown). Furthermore, quantitative real-time PCR (qRT-PCR) revealed that in RA-FLS IL-8, COX-2 and MMP-3 were also upregulated by H2S treatment (Fig. 2, left panel), whereas MMP-2 and MMP-14 were negatively regulated by H2S (Fig. 2, right panel).

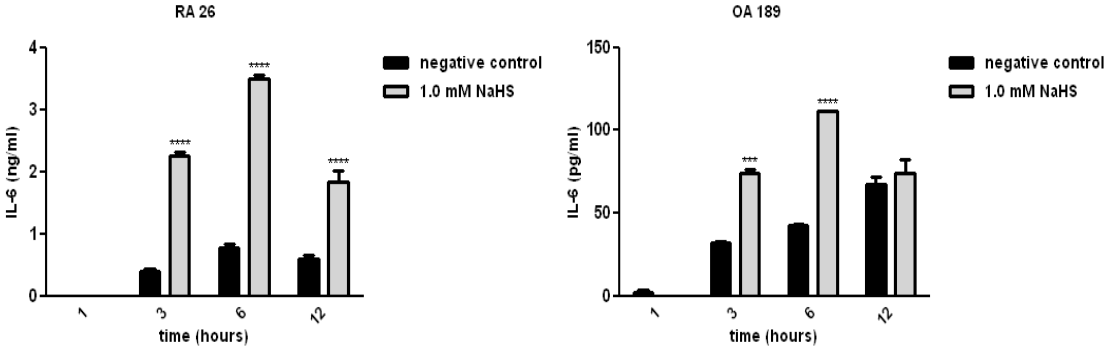

Fig. 1 H2S upregulates IL-6 expression in FLS from a patient with RA (left panel) and a patient with OA (right panel). FLS were incubated for 20 min with 1.o mM of NaHS. After 1, 3, 6 and 12 h, IL-6 secretion was quantified by ELISA. Significant changes are indicated by asteriks: ***p<0.001, ****p<0.0001.

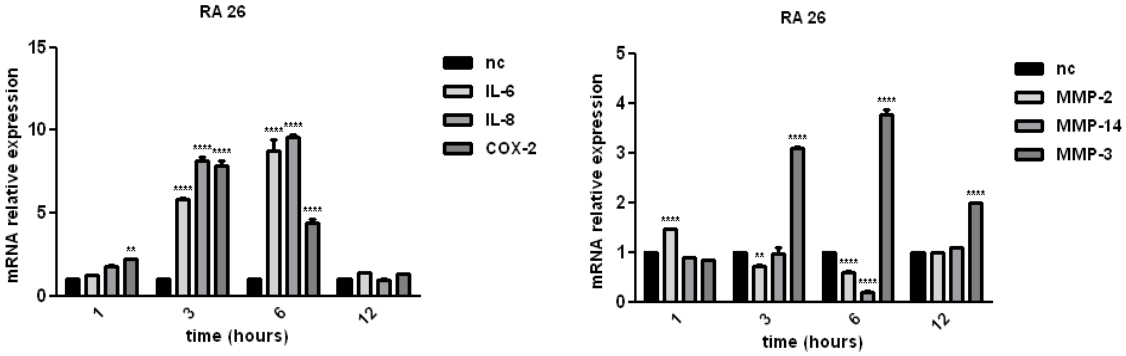

Fig. 2. H2S upregulates mRNA levels of IL-6, IL-8, COX-2 (left panel) and MMP-3 (right panel) in RA-FLS. 1, 3, 6 and 12 h after initial H2S exposure, total RNA was isolated and mRNA levels were quantified by qRT-PCR. Significant changes are indicated by asteriks: **p<0.01,

To clarify the underlying mechanism leading to the induction of expression of pro-inflammatory genes by H2S, phosphorylation of extracellular signal-regulated kinase (ERK1/2) was analyzed by Western blotting in RA and OA-FLS (Fig. 3): already 15 min after initial H2S exposure ERK1/2 was activated in both cell lines.

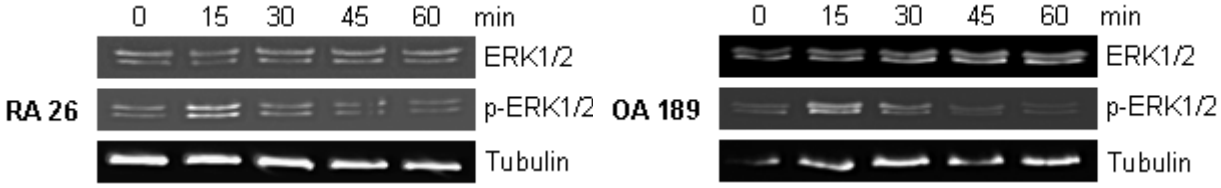

Fig. 3: H2S rapidly induces phosphorylation of ERK1/2 in RA (left panel) and OA-FLS (right panel). FLS were incubated for 60 min with 1.0 mM NaHS. After 0, 15, 30, 45 and 60 min, phosphorylation of ERK1/2 was analyzed by Western blotting.

Furthermore, inhibitors of p38 and MEK1/2 MAPK, SB203580 and U0126, respectively, and of NF-κB (BAY-117082) completely blocked H2S-induced IL-6 expression (data not shown). Taken together, these results demonstrate that high concentrations of H2S can stimulate expression of IL-6 and other pro-inflammatory genes such as IL-8 and COX-2 as well as MMP-3 in FLS from RA and OA patients via activation of p38 and ERK1/2 MAPK and of NF-κB pathway.

Effects of H2S and the sulfur-containing anti-oxidants dimethyl sulfoxide and dimethyl sulfone on IL-6 and IL-8 expression in a human chondrocyte cell line

Background

Next, we were interested to study the effects of H2S on chondrocytes which are centrally involved in cartilage synthesis and metabolism. In addition, we studied the effects of the sulfur containing anti-oxidants dimethyl sulfoxide (DMSO) and dimethyl sulfone (DMS). DMSO is a powerful water miscible solvent that dissolves most waterinsoluble drugs (21). DMSO possesses anti-inflammatory properties (22), as well as the ability to act as a free radical scavenger (23). Thus, its properties have been exploited in the treatment of dermatological, rheumatic, and renal manifestations of amyloidosis. DMSO is capable of inducing or inhibiting cell proliferation, apoptosis and/or differentiation (24). However, limited data are available on the underlying molecular mechanism.

Besides the development of new therapeutic drugs for the treatment of RA and OA, there is also a strong interest in finding new anti-inflammatory agents derived from naturalproducts. DMS is found in small amounts in many foods, including unpasteurized milk, grains, meat, eggs, and fish (25-27). It is also present in popular dietary supplements (28). Reported effects claimed to be associated with DMS include relief of pain, reduction of inflammation, arthritis, allergies and asthma (29-31).

Results

We studied the effects of NaHS, DMSO and DMS on IL-6 and IL-8 expression in C-28/I2 cells, a human chondrocyte cell line, originally derived from a young patient with OA (32, 33). In the case of H2S treatment, cells were left untreated or were incubated for 15 min with different concentrations of NaHS (0.125 and 1.0 mM). After changing the culture medium, incubation was continued for 12 h. In contrast to H2S treatment, cells were incubated with DMSO (0.5 and 1.0 %) or DMS (10 and 100 mM) over a total period of 12 h. At different time points, cell culture supernatants were collected and IL-6 and IL-8 levels were quantified by ELISAs.

As shown in Fig. 1 (left panel), reduced levels of IL-6 were detected from 1 to 6 h after initial H2S exposure. 3 h afterwards, at 0.125 mM NaHS, inhibition of IL-6 expression was about 35 %. After 6 h and 1.0 mM NaHS, IL-6 level was still about 20 % lower than in the untreated control.

As shown in Fig. 1 (right panel), only the higher DMSO concentration (1.0 %) effectively blocked IL-6 expression. Similar inhibitory effects were observed when the cells were treated with DMS (Fig. 1, lower panel). Only the higher DMS concentration (100 mM) was efficiently blocking the secretion of IL-6: after 12 h almost 70 % inhibition was obtained with both substances. Similar results were obtained when cell culture supernatants were analyzed for IL-8 secretion (data not shown).

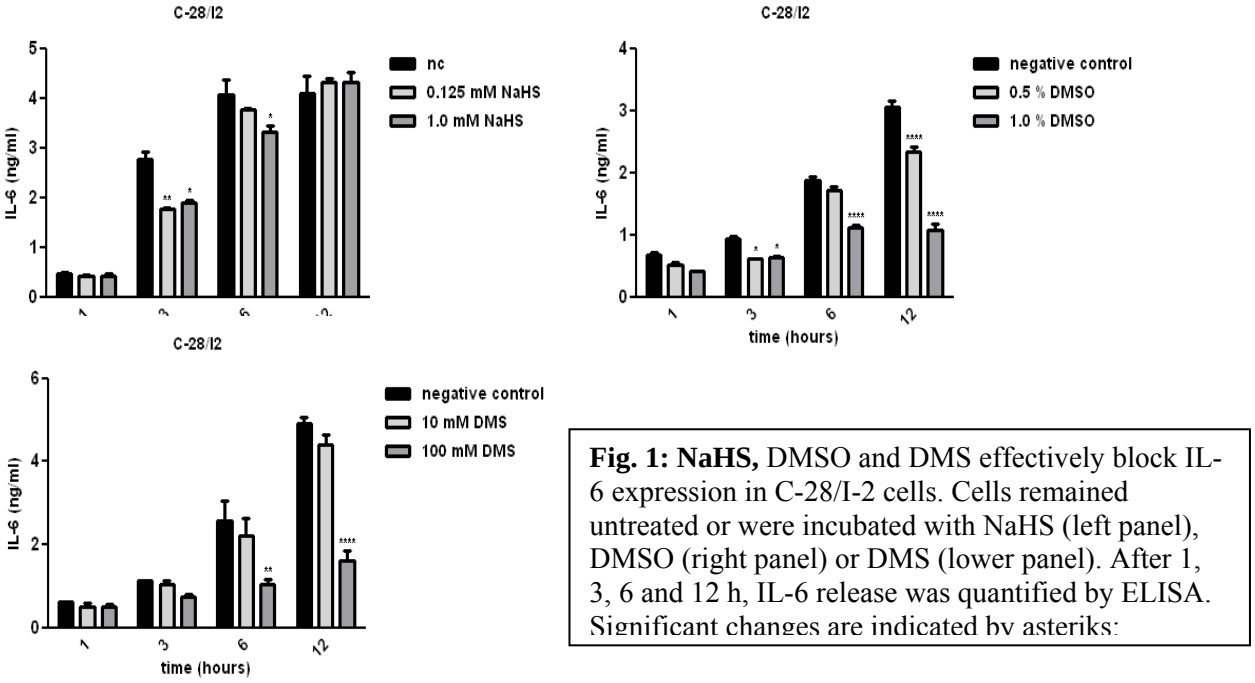

Fig. 1: NaHS, DMSO and DMS effectively block IL-6 expression in C-28/I-2 cells. Cells remained untreated or were incubated with NaHS (left panel), DMSO (right panel) or DMS (lower panel). After 1, 3, 6 and 12 h, IL-6 release was quantified by ELISA. Significant changes are indicated by asteriks:

Furthermore, both DMSO and DMS blocked also IL-1β-induced IL-6 and IL-8 expression. For this experiment, C-28/I2 cells were either treated for 60 min with DMSO or DMS before being stimulated with IL-1β (10 ng/ml) or were incubated with IL-1β and both of the substances concurrently. Blockage of IL-6 secretion was only effective when the cells were incubated with IL-1β in the presence of either DMSO or DMS (data not shown). Similar results were obtained when IL-8 release was monitored (data not shown).

Taken together, these studies show that NaHS and the sulfur containing anti-oxidants DMSO and DMS are potent inhibitors of constitutive as well as IL-1β-induced cytokine expression in a human chondrocyte cell line (34). Identification of the components of the signal transduction pathways that are sensitive to anti-oxidants may eventually open a new territory to more selective treatment of inflammatory disorders.

References

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Project 2. Clock gene expression in rheumatoid arthritis and osteoarthritis

Circadian rhythms are controlled and generated by the biological clock located in the hypothalamic suprachiasmatic nucleus (SCN) (1,2). This "master clock" is synchronized to 24h by various environmental factors, primarily the dark-light-cycle but also by regularly occurring social processes, motor activity and food intake (3,4). Patients with RA show modulated circadian rhythms of inflammatory cytokines and cortisol, which may be associated with a modified expression of clock genes (5). The objective of this project is to study the expression and synchronization of clock genes in synovial specimens and fibroblasts from patients with RA or OA. Furthermore, the effect of TNF-α on clock gene expression is investigated.

The expression of 5 different clock genes and Dbp in synovial tissues of RA and OA patients was studied by immunohistochemistry (IHC). All of the clock genes were found to be expressed in the specimens, especially in the intimal layer, but considerable staining was also detected in the subintimal layer (Fig. 1).

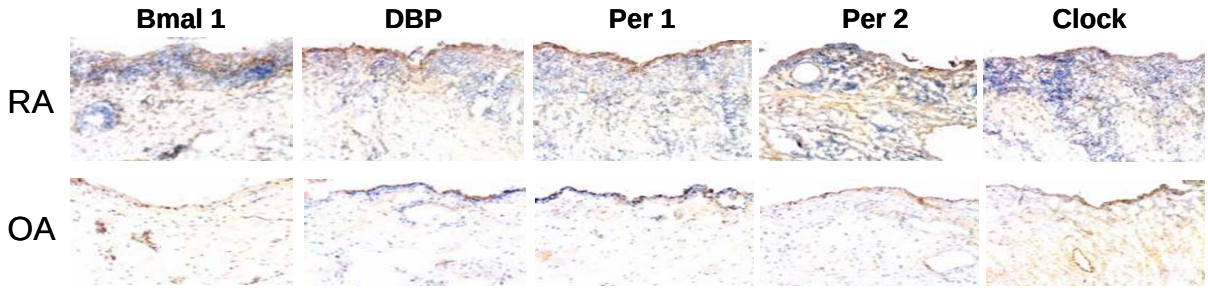

Fig.1. Immunohistochemical analysis of clock gene expression in synovial tissues from patients with rheumatoid arthritis (RA) and osteoarthritis (OA).

Double stainings showed that mainly macrophages (CD68 positive) and fibroblasts (CD90 positive) expressed the five clock genes, while T- (CD3 positive) and B- (CD20 positive) lymphocytes showed lesser staining of all of the clock genes (not shown). RA patients showed higher expression of especially Bmal-1, but also Clock and Dbp. Of special interest was the first time detection of the Dbp protein in human samples, which, to our knowledge, has not been performed before.

In accordance with the results from the IHC, quantitative analyses of clock gene expression by real time PCR showed that expression of Bmal-1, Clock and Dbp were higher in RA patients (especially Bmal-1 and Dbp), although the difference did not reach the level of statistcal significance, whereas Per1, Per2, and Cry-1 were expressed to a similar degree in OA and RA patients (not shown).

Furthermore, the effect of a 2 h serum shock (a standard method to synchronize clock gene expression) on 24h clock gene expression was studied in fibroblast-like synoviocytes (FLS) derived from RA or OA patients. The transcription factor Bmal-1 showed an expression peak at Zeitgeber Time (ZT) = 8 in both RA and OA SFs (Fig. 2), and tended to higher values in RA FLS as compared to OA FLS. Another interesting observation was that the transcript of Clock was significantly higher expressed in RA FLS than in FLS of OA patients with a peak at ZT = 12 whereas the expression in OA FLS exhibited nearly no rhythmicity. Simialr results were obtained whenth ecells were stimulated with TNFα (not shown).

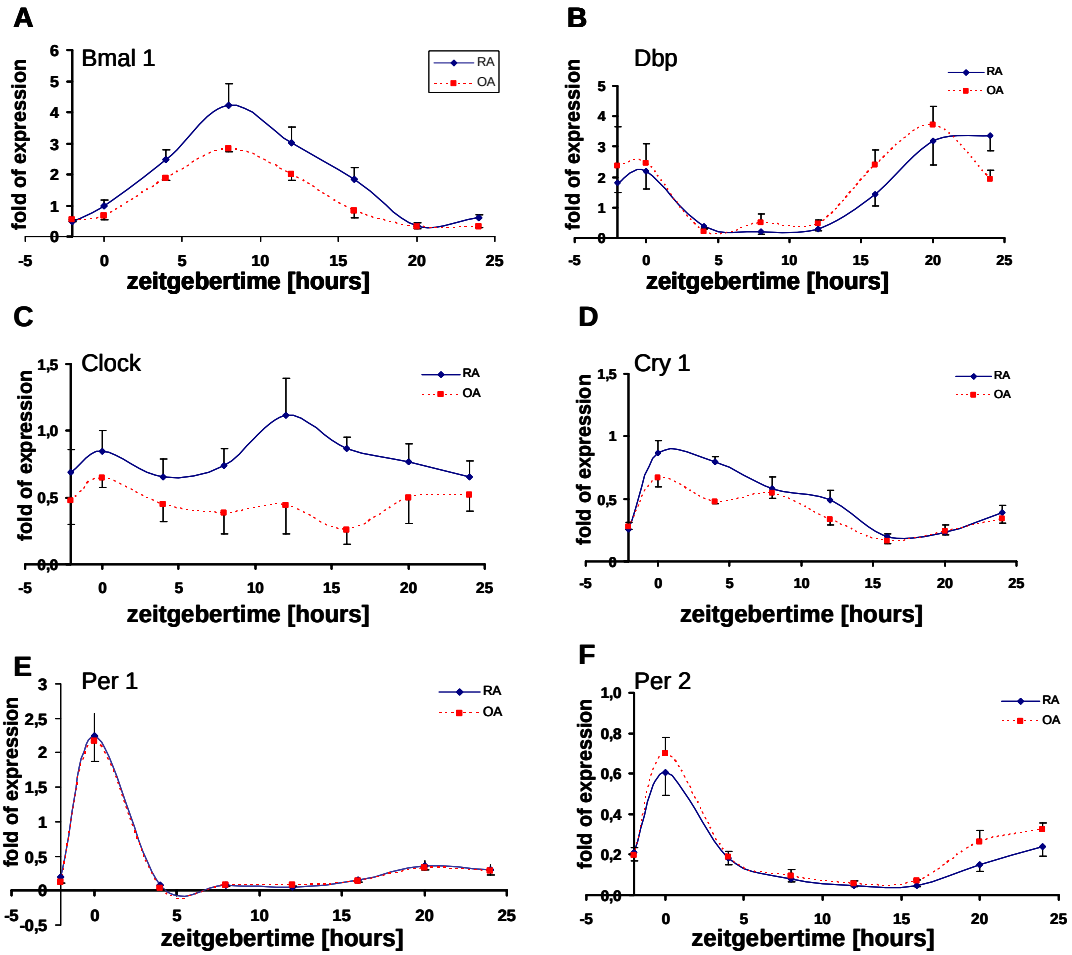

Fig 2.. mRNA expression profiles over 24h of clock genes induced by serum shock in FLS from 3 RA and 3 OA patients. Data are mean +/- SEM of three independent experiments. Bmal 1 expression (A) showed a peak after 8 hours Dbp expression (B) showed similar expressions in RA and OA FLS. Clock expression (C) showed a very low oscillation in OA FLS in contrast to the expression in RA FLS with a 2.5 fold higher expression at ZT=12. Cry 1 (D), Per1 (E) and Per2 (F) expression showed nearly the same characteristics in RA and OA FLS.

In conclusion, we showed that the most important core clock genes are expressed in synovial tissue of RA and OA patients with the most abundant expression in macrophages and SFs. The expressions were inducible in SFs not only under standard conditions but additionally also with TNF-α The strong influence of this important cytokine on the expression profiles of the clock genes implies a role of these genes in the progression of RA. Summarizing all these observations, as well as reports from the literature, it seems that inflammation has an measurable effect on the core components of the circadian clock.

References

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Research Programme REHABILITATION

LBI for Rehabilitation of Internal Diseases, Saalfelden and Gröbming

Project "REMERI"

A project (REMERI) about outcome, sustainability, and risk factor modification in stationary rehabilitation of musculoskeletal diseases combined with metabolic syndrome was planned and elaborated in 2009 in close collaboration with the LBI for Biological Rhythm Research, Bad Tatzmannsdorf. A positive vote of the ethics committee was already obtained and the study was agreed by the partner PVA to be completed in the rehabilitation centres of the PVA. However, owing to (a) the closure of the LBI Bad Tatzmannsdorf and (b) the restrictive demands of the steering board to carry out the study not with rehabilitation patients with musculoskeletal diseases (e.g. OA or after joint replacements) as planned but exclusively with patients suffering from RA the project could not be realized. Since RA patients nowadays hardly get consent for an inpatient rehabilitation stay the study would not have been possible with patients from the rehabilitation centres of the PVA in a reasonable timeframe.

Nevertheless, preliminary investigations performed during the planning phase of the project confirmed that obese patients with RA have significantly lower serum levels of the adipocytokine Adiponectin ($p<0.03$) and higher Leptin levels ($p<0.002$) than healthy people. Before and after a three-week inpatient rehabilitation stay oxLDL, hsCRP, Visfatin, and Leptin of patients suffering from Morbus Bechterew (ankylosing spondilitis, AS) without metabolic syndrome were measured. The rehabilitation stay led to decreased CuZnSOD (CuZn-superoxide-dismutase) and Visfatin levels (Figure 1), which means that positive effects of rehabilitation on inflammatory processes and radical formationare detectable.

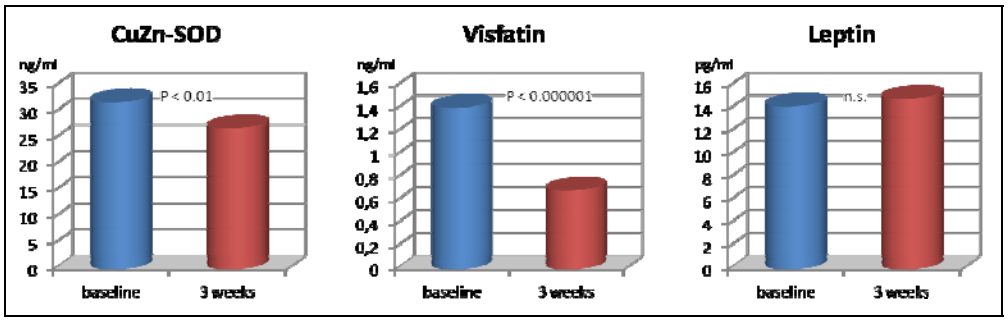

Fig 1. CuZnSOD, Visfatin, and Leptin levels of 60 patients suffering from Morbus Bechterew at baseline and after 3 weeks inpatient rehabilitation

Plasma levels of ubiquinone and oxidative stress in patients with ankylosing spondylitis and rheumatoid arthritis

In chronic inflammatory processes oxidative stress is known to be increased with accompanying decreased antioxidant capacity. The objective of the study was to obtain information about the concentration of the radical scavenger ubiquinone (coenzyme Q_{10}) in sera of patients with RA or ankylosing spondylitis (AS), possibly decreased as a consequence of oxidative imbalance.

Sixty-five patients with RA and 29 patients with AS were included in the study; 21 healthy subjects served as control group. Ubiquinone as measured by HPLC was significantly decreased in patients with RA ($p<0.03$) and AS ($p<0.01$) compared to healthy controls (Figure 2); in contrast oxidised-LDL antibodies were significantly increased ($p<0.001$ and $p<0.05$,

respectively). No significant differences between the groups were found concerning total antioxidant status and Cu/Zn superoxide dismutase SOD. However, a positive correlation was seen between ubiquinone level and total antioxidant capacity ($r = 0.546$; $p < 0.01$) in AS as well as between oxLDL-Ab and Cu/Zn SOD in RA ($r = 0.252$; $p< 0.05$).

Thus, in chronic inflammatory diseases such as RA and AS the scavenger ubiquinone is decreased leading to oxidative imbalance with raised oxLDL which may further enhance inflammation, tissue damage, and cardiovascular risk.

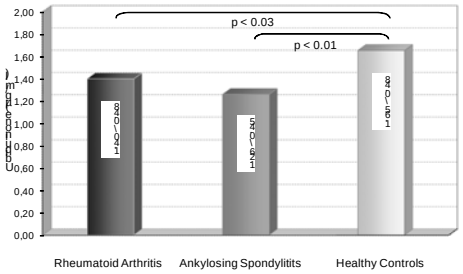

Fig. 2. Significantly decreased ubiquinone (coenzyme Q10) levels in patients with RA or AS compared to healthy controls. Mean ± standard deviation, p=significance compared to healthy controls

Outcome and sustainability of cure and inpatient rehabilitation

The outcome of the cure at the health resort Bad Hofgastein was evaluated and published in conjunction with the Paracelsus Medical University (PMU) Salzburg in the journal *Physikalische Medizin, Rehabilitationsmedizin, Kurortmedizin.* Further collaboration with the PMU, institute for physiology (head: Prof. Ritter) and the research institute Gastein (Dr. Moder) is planned adressing specific topics about rehabilitation.

The evaluation about sustainability of a "health guide of the PVA", which has been handed out to patients in 6 SKA/rehabilitation centers of the PVA, was completed in the first six months of 2011. Evaluable data of 1195 patients were transmitted from the SKAs Bad Tatzmannsdorf, Felbring, Großgmain, Hochegg, Saalfelden und St. Radegund at admission and discharge from inpatient rehabilitation as well as after 3, 6, 9 and 12 months. The measured parameters enable to draw conclusions about the coronary risk following inpatient rehabilitation. Thus, the evaluation showed significant improvements of coronary risk factors during one year, hence, a sustainable success of rehabilitation is documented (Fig. 3).

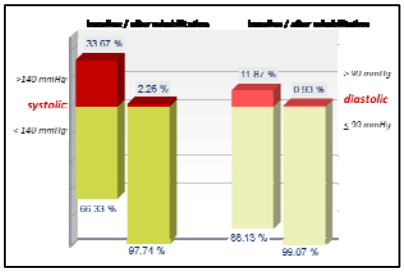

Fig. 3. Health guide – reduction of blood pressure during inpatient rehabilitation

Therapeutic application of nuclear magnetic resonance - Investigations on the effects of NMRT *in vitro*

Therapeutical nuclear magnetic resonance therapy (NMRT) uses the same physical principle of nuclear magnetic resonance like that applied in clinical diagnostics but considerably lower field strengths (millitesla range) are applied than in the imaging technique. For more than 10 years the NMRT has been used in Germany for therapy in conservative orthopedics, rheumatology, and trauma surgery, particularly for treatment of painful conditions such as osteoarthritis, sports or accidental injuries, discopathies, and osteoporosis (1-4). The LBI Saalfelden is primarily interested in investigating cellular mechanisms of NMRT such as influences on gene expression, the metabolic cell activity as well as the rapid clinical effects on pain perception which are reported by the patients.

The influence of NMRT on cellular metabolism, signal transduction pathways and cytokine expression was studied by different approaches. Initially various gene-arrays for signal transduction pathways were employed to investigate NMRT induced changes of gene-expression in two human cell lines, the osteosarcoma cell line Cal-72 and the chondrosarcoma cell line Cal-78. Cells were treated for 20h with NMRT as described (5). In Cal-78 cells these experiments indicated small changes in gene expression of components of the extracellular matrix as well as constitutive parts of MAPK and TGF-ß pathways. In Cal-72 cells most prominent changes were detected in expression of vascular endothelial growth factor (VEGF). However, these variations in gene expression could not be confirmed by qRT-PCR. Therefore we continued to study the impact of NMRT on intracellular signaling pathways by transfecting cells with different luciferase reporter gene constructs. Although altered luciferase activity of Cal72 and Cal78 cells was seen when cells were transfected with the luciferase-reporter gene constructs for MAPK/ERK, MAPK/JNK or NFkB, respectively, these differences did not reach the level of statistical significance. Nevertheless, in all three cases a trend towards an increase in relative luciferase activity could be observed in Cal-78 cells following NMRT stimulation.

To further study the influence of NMRT on the inflammatory state of the cells, the cell lines were stimulated with IL-1ß or TNF-α, exposed to NMRT for 10 h and analyzed for expression of cytokines, chemokines and metalloproteinases. Quantitative RT-PCR revealed differences for MMP10 and MMP13, IL6 and IL8 in C28I/2 cells and for MMP1, MMP3, MMP10, in Cal-78 cells (Fig 1). NMRT treatment of Cal-72 cells revealed an increase in expression of the chemokine CXCL5 (not shown).

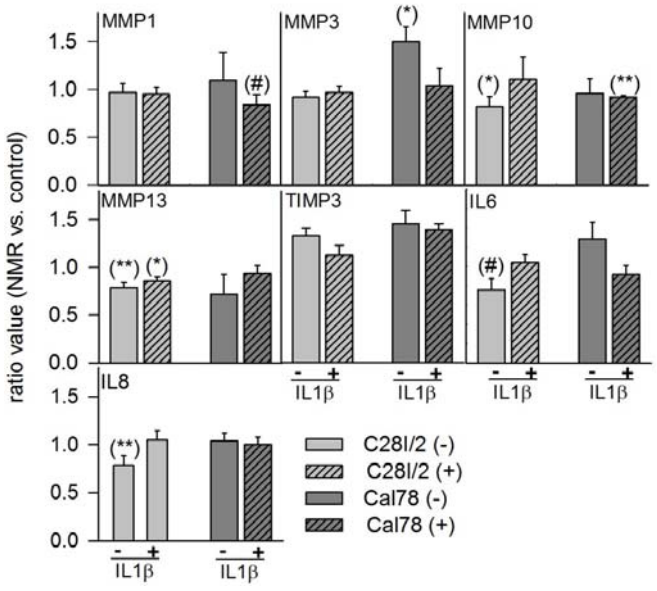

Figure 1. Influence of NMRT on expression of MMPs, IL6 and IL8 in Cal-78 and C28I/2 cells. Gene expression analysis was assessed by quantitative RT-PCR. The bar charts show the ratio values between NMR treated and control cells. Expression of MMP1, 3, 10 and 13, IL6, IL8 and TIMP3 was investigated in the presence (+) or absence (-) of IL1ß stimulation. Significant differences generated by the one sample t-test are indicated (#: p<0,1; *: p<0,05; **: p<0,01, n= 5-7). In C28I/2 cells not treated with IL-1ß NMRT induced reduction of the expression of MMP10, MMP13, IL6 and IL8, whereas in IL-1ß stimulated cells only MMP13 showed a weak decline. For Cal-78 changes in MMP3 and MMP10 could be observed when cells were stimulated with IL1ß.

Since it has been previously reported by other authors (6,7) that NMRT may influence cell growth and induce regeneration of cartilage, cell growth studies were performed with Cal78, C28I/2, Cal72, synovial cells as well as two skin fibroblast cell lines. However, these studies did not reveal any influence of NMRT on cell proliferation.

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Epidemiological studies

Research Unit for Epidemiology of Rheumatic Diseases, Baden

The activities of the Research Unit are focused on calculations of costs of illness of rheumatic diseases in Austria, and evaluation of interventions in chronic non-specific low back pain.- The research is funded by the Lower Austria Health Insurance (Niederösterreichische Gebietskrankenkasse)

The current project (since 2011) investigates the costs of soft tissue affections (periarthropathic syndromes of the shoulder). It has been approved by the local ethics review committee and will be finished in 2012.

In 2011 the study on the costs of advanced osteoarthritis of the hip and the knee was published in the journal “Wiener Medizinische Wochenschrift”. The average annual costs are shown in Table 1. The most remarkable finding in this study was the fact that patients with advanced osteoarthritis incur high costs for household assistance; the average costs for household assistance even ecxeeded those for patients with rheumatoid arthritis. The adverse impact on social participation (familiy life, leisure activities) was higher in advanced osteoarthritis than in rheumatoid arthrits and non-specific low back pain.

Cost domain	Annual costs (average)	Cost domain	Annual costs (average)
Physician´s visits	€ 89,-	Other intraarticular therapies	€ 111,-
In-patient costs	€ 149,-	Parenteral analgesic therapy	€ 39,-
NSAIDs	€ 73,-		
Chondroprotective medication	€ 27,- (oral) € 75,- (viscosupplementation)	In-patient rehabilitation	€ 243,- (patient's costs contribution € 7,-)
Analgesics (weak opioids included)	€ 35,-	Diagnostics	€ 168,-
Proton pump inhibitors	€ 96,-	Outpatient physical therapy	€ 144,- (patient's costs contribution € 34,-)
Household assistance	€ 1572,-		

Table 1: direct medical and non-medical costs of advanced osteoarthritis

The statistical calculations of the long-term project on the effect of a back-school offered by the Lower Austrian Health Insurance (NOEGKK) are performed in cooperation with the LBI Saalfelden. The publication is planned for 2012. Physical function, pain intensity, sociodemographics and disease related factors were evaluated in patients who voluntarily participated in a Back School Programme of the Lower Austrian Health Insurance by means of a specially designed questionnaire and were followed-up for two years to assess the longitudinal course of pain and function. It turned out that the number of days with low back pain per year reduces after the back school programme (Figure 1).

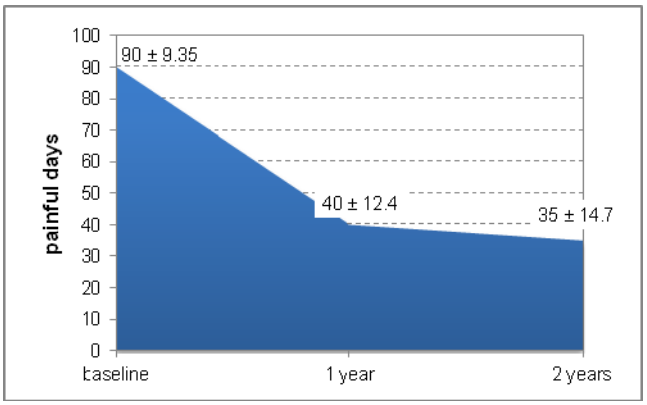

Fig 1. Influence of the back school programme of the NOEGKK on low back pain – number of painful days during the past 12 months (median ±SEM).

Oral presentations at national and international conferences

Schwann H., Kullich W.:
„Rehabilitation und Rehabilitationsforschung in der SKA und im LBI Saalfelden“
Lehrveranstaltung der Universität Innsbruck, Prof. Herold über „Kurmedizin zur Prävention und Rehabilitation rheumatischer Erkrankungen“
Saalfelden, 10. Jänner 2011

Steinecker B.:
„Untersuchungen zum Forced Use-Konzept in der stationären Rehabilitation“
Fortbildungsveranstaltung in der SKA der PVA Gröbming
Gröbming, 31. März 2011

Hackl B., Kraxner M.:
„Ergotherapie bei Anwendung der Forced Use in der stationären Rehabilitation“
Fortbildungsveranstaltung in der SKA der PVA Gröbming
Gröbming, 31. März 2011

Schumann S.:
„Sportwissenschaftliche Messungen bei Forced Use-Patienten in der Rehabilitation“
Fortbildungsveranstaltung in der SKA der PVA Gröbming
Gröbming, 31. März 2011

Müller R., Kullich W.:
„Untersuchungen des Gesundheitswegweisers in den Rehabilitationszentren der Österreichischen Pensionsversicherungsanstalt“
KOFÜ-Tagung 2011 der PVA
Saalfelden, 9. Mai 2011

Kullich W.:
„Kernspinresonanz beeinflusst Arthroseschmerz“ (Poster)
19. Wissenschaftliche Tagung der Österreichischen Schmerzgesellschaft
Zell am See, 26. – 28. Mai 2011

Kullich W.:
„Gesundheitsmodulation und Schmerzbeeinflussung durch Nahrungsinhaltsstoffe“ (Poster)
19. Wissenschaftliche Tagung der Österreichischen Schmerzgesellschaft
Zell am See, 26. – 28. Mai 2011

Kullich W.:
„Ernährung bei Schmerzen: Möglichkeiten zur Hilfe“
Diplom „Spezielle Schmerztherapie“ der Österr. Ärztekammer
Leogang, 16. Juni 2011

Kullich W.:
„Entzündungsmechanismen und Therapie: Moderne Entwicklungen“
Diplom „Spezielle Schmerztherapie“ der Österr. Ärztekammer
Leogang, 16. Juni 2011

Kullich W.:
„Therapeutischer Einsatz der Kernspinresonanz bei verschiedenen Arthroseformen“
30. Rheumatologische Fortbildungstagung
Saalfelden, 17. – 18. Juni 2011

Weigl L.:
„Intrazelluläre Ca^{2+}-Regulation als möglicher Angriffspunkt der KSRT“
30. Rheumatologische Fortbildungstagung
Saalfelden, 17. – 18. Juni 2011

Steinecker-Frohnwieser B.:
„Einfluss der Kernspinresonanz auf Arthrose-relevante Faktoren“
30. Rheumatologische Fortbildungstagung
Saalfelden, 17. – 18. Juni 2011

Kullich W.:
„30 Jahre Rheumatagung und Forschung im LBI Saalfelden“
30. Rheumatologische Fortbildungstagung
Saalfelden, 17. – 18. Juni 2011

Schwann H.:
„Aktuelles zur Arthrose und Arthrosetherapie“
30. Rheumatologische Fortbildungstagung
Saalfelden, 17. – 18. Juni 2011

Steiner G:
“Pre RA and Early RA in Vienna”
First International Workshop on Pre-RA cohorts
Amsterdam, NL, 26.1.2011

Steiner G.:
“Involvement of Nucleic Acids and Nucleic Acid binding proteins in the pathogenesis of rheumatoid arthritis”
Workshop on Modulators of RNA Fate and Function
Vienna, 1.2.2011

Steiner G.:
“New Trends and Developments in Autoimmune Diagnostics”
Orgentec Ínternational Symposium
Wiesbaden, BRD, 5.5.2011

Steiner G.:
“On the Role of Nucleic Acids and their Binding Proteins in the Pathogenesis of Autoimmune Arthritis in Rats, Mice and Men”
Visiting scientist (6.6.-11.6.) at the Center for Molecular Medicne, Karolinska Hospital
Stockholm, Sweden, 8.6.2011

Steiner G.:
„Alte Probleme und Neue Entwicklungen in der Rheumaserologie“
Rheumatologische Fortbildungstagung Saalfelden
Saalfelden, 18.6.2011

Steiner G.:
“On the Role of Nucleic Acids and their Binding Proteins in the Pathogenesis of Autoimmune Arthritis in Rats, Mice and Men”
Dresden Symposium on Autoantibodies
Dresden, BRD, 22.9.2011

Steiner G.:
“RA33 Antibodies: New data on a marker linked to CCP-negative RA with a mild prognosis
Dresden Symposium on Autoantibodies”
Dresden, BRD, 23.9.2011

Steiner G.:
“A TLR 9 antagonist diminishes arthritis severity and inhibits bone erosion in a rat model of rheumatoid arthritis”
Annual Meeting of the Austrian Society for Molecular Biology and Biotechnology
Salzburg,, 29.9.2011

OTHER ACTIVITIES

Collaborations

Major collaborations have been established with the following hospitals and research institutions inside and outside Austria:

Medical University Vienna, Department of Special Anaesthesia and Pain Therapy

Medical University Vienna, Department of Internal Medicine III/Rheumatology

University Salzburg, Department of Organismic Biology/Neurosignaling Unit in the context of the project "Primosente" supported by the FFG (Austrian Research Promotion Agency).

Medical University Innsbruck, Department of Internal Medicine/Physical Medicine

Siemens and the Austrian Institute of Technology in the context of the FFG-funded research project PORACCS

Karolinska Institute and Hospital, Stockholm, Sweden

Organisation of conferences and workshops

The Institute for Rehabilitation of Internal Diseases Saalfelden organized:

1. A professional development symposium in Gröbming about "the forced use concept in rehabilitation" in collaboration with the Austrian Pension Insurance Institution (PVA) on 31st March 2011.
2. The 30th "Rheumatological Symposium Saalfelden 2011" in collaboration with the Austrian Society for Rheumatology and the PVA in Saalfelden on June 17th/18th 2011. It was organised as a two-day anniversary symposium in celebration of 30 years existence of the institute including a practice-oriented course and a satellite symposium (topic: "therapy with nuclear magnetic resonance"). The symposium was the best-frequented so far with 145 participants.
3. Part of a course on prevention and rehabilitation of rheumatic diseases for students of the Medical University Innsbruck in Saalfelden in January 2011.

Teaching activities

Prof. Steiner and Prof. Ekmekcioglu gave lectures, held seminars and organized Journal Clubs for students of medicine and PhD students at the Medical University of Vienna.

OUTLOOK

Research Programme RHEUMA

Research on the effects of H_2S and sulphur-releasing compounds on cell types involved in the pathogenesis of rheumatic diseases such as fibrobast-like synoviocytes, monocytes, osteoclasts, osteoblasts and endothelial cells will be continued. In addition, the effects of the polyphenols curcumin and resveratol will be investigated in order to analyse their therapeutic potential. Furthermore, a pilot animal trial is planned in order to investigate the therapeutic potential of sulphur in mice with colagen-induced arthritis.

Research Program BALNEO

Although balneological therapies are among the most commonly used spa treatments worldwide, little scientific evidence exists for the effectiveness of the majority of balneological procedures, even though in some studies improvement effects of mineral baths could be shown in patients with OA. To investigate the effects of sulphur bath therapy a study has been designed in which patients with OA of the knee will be treated for three weeks with sulphur baths or sulphur-free thermal water. The main question to be explored in the context of this study is whether sulphur baths in comparison to thermal baths lead to a significant improvement in subjective pain perception and/or in the functional ability in patients with OA of the knee. The study proposal has been submitted to the ethics committee of the Center for Rheumatic Diseases, Vienna-Oberlaa.

Research Program REHABILITATION

The research programme is focused at further elucidating the mode of action and clinical efficacy of nuclear magnetic resonance therapy (NMRT). The *in vitro* investigations will be continued and a clinical study on NMRT in patients with painful shoulder disorders will be designed. Questions addressed in the context of this study are (i) Can NMRT induce a reduction of shoulder pain in a clearly defined patient population during inpatient rehabilitation? (ii) Is the NMRT induced modulation of specific biomarkers associated with pain, inflammation, and/or stress?, and (iii) If so, can there a correlation be found between laboratory and clinical parameters using validated scores? Thus, the major aim is to assess the effectiveness of NMRT as an additive treatment of shoulder disorders in inpatient rehabilitation.

PUBLICATIONS

Peer-reviewed

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8. Presch M., Hartl L., Tucek G., Minnich B., Kullich W., Bernatzky G. Einflüsse von aktiver und rezeptiver Musiktherapie auf Kognition, Verhalten, Schlaf und allgemeine Befindlichkeit von Demenzpatienten - eine Pilotstudie. Schweiz Z Ganzheitsmed 2011; 23:218-223
9. Lichtenschopf A., Kullich W., Müller R., Absenger D., Fagerer N., Falkenbach A., Gassner A., Guy-Roustayan Y., Klicpera M., Koeninger C., Laimer H., Marko C., Mayerhofer F., Rus-Machan J., Ortner S., Salomon S., Schaffelhofer A., Schwann H., Singer F., Stark B., Ulreich A., Wachter K., Walcher J., Wallner K.E., Waltl S., Wonisch M., Wotruba E.: "Erfolge einer stationären Raucherentwöhnung am Ende des Aufenthaltes und nach einem Jahr in 13 Rehabilitationszentren der PVA in Österreich". Atemwegs- und Lungenkrankheiten 2011; 37(8): 301-307.

Non peer-reviewed

1. Kullich W. Next generation of medical technology - Therapeutic effect of NMR-therapy against osteoarthritis proven. Arab Health Magazine 2011:60-62.
2. Müller R. Adipositas-Projekt SKA-RZ Alland der PVA. Soziale Sicherheit 6, 323-326 (2011)
3. Kullich W., Arnold M., Gundolf F., Schwann H., Mur E.: "The Dynamic Spinal Traction System GammaSwing Used During Inpatient Rehabilitation of Low Back Pain". J Mineralstoffwechsel 2011; 18(4): 174-175.
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