Charles University in Prague
Faculty of Medicine in Hradec Králové

7th Postgraduate Medical Students Conference

October 17th, 2011

Education Centre in the Faculty Hospital
Sokolská 581, 500 05 Hradec Králové

7. Postgraduate Medical Students Conference

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Chairman: Prof. Radek Pudil, M.D., Ph.D.

1. **THE NUMBER OF IMMUNOREGULATORY T CELLS (TREG) IS INCREASED IN PATIENTS WITH PSORIASIS AFTER GOECKERMAN THERAPY**

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   Department of Clinical Immunology and Allergology

2. **SOLUBLE TOLL-LIKE RECEPTOR 2 AND TOLL-LIKE RECEPTOR 4 IN AMNIOTIC FLUID IN WOMEN WITH PRETERM PREMATURE RUPTURE OF MEMBRANES**

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7. **CONCOMITANT FIBROMYALGIA IMPACT ON THE RHEUMATOID ARTHRITIS ACTIVITY ASSESSMENT**

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8. **CHRONIC ANTHRACYCLINE CARDIOTOXICITY: MOLECULAR AND FUNCTIONAL ALTERATIONS IN THE POST-TREATMENT FOLLOW UP**

Eduard Jirkovský
Department of Pharmacology
Tutor: Assoc. Prof. PharmDr. Martin Štěrba, Ph.D.

1.

THE NUMBER OF IMMUNOREGULATORY T CELLS (TREG) IS INCREASED IN PATIENTS WITH PSORIASIS AFTER GOECKERMAN THERAPY

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Regulatory T cells (Tregs) are a specialized subpopulation of T cells that act to suppress inadequate immune response, thereby maintaining homeostasis and self-tolerance. Psoriasis is recognized as a T-cell driven immune-mediated systemic inflammatory disease with skin manifestation. Effective therapeutical approach to treat psoriasis is Goeckerman therapy (GT).

The aim of this study was to compare the number of Tregs in the peripheral blood of 27 psoriatic patients and 19 healthy volunteers and to evaluate the influence of GT on Treg population in peripheral blood of patients with psoriasis.

There was no significant difference in the relative number of Treg cells in the peripheral blood of healthy blood donors (2.9 ± 1.0%) and patients with psoriasis before initiation of GT (3.3 ± 1.2%); P = 0.2668. In contrary, the relative number of Treg cells in peripheral blood of patients with psoriasis after GT (4.3 ± 1.6%) was significantly higher than those found in healthy blood donors (2.9 ± 1.0%); P = 0.0019. Moreover, the relative number of Treg is significantly increased in psoriatic patients after Goeckerman therapy (4.3 ± 1.6%) compared to the pre-treatment level (3.3 ± 1.2%); P = 0.0042.

In conclusion, this significant increase in Treg count after GT is probably associated with amelioration of inflammation by GT, as disease activity expressed as PASI significantly dropped from 17.5 ± 6.5 before GT to 8.4 ± 4.6 after GT (P = 0.0001).

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2.

SOLUBLE TOLL-LIKE RECEPTOR 2 AND TOLL-LIKE RECEPTOR 4 IN AMNIOTIC FLUID IN WOMEN WITH PRETERM PREMATURE RUPTURE OF MEMBRANES

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Objective:
To determine changes in the amniotic fluid, soluble Toll-like receptor 2 (sTLR2) and soluble Toll-like receptor 4 (sTLR4) concentrations in patients with preterm premature rupture of membranes (PPROM) with the presence of histological chorioamnionitis were studied.
Methods:
Fifty-four women with singleton pregnancies were enrolled. The concentrations of both sTLR2 and sTLR4 in amniotic fluid were determined using sandwich enzyme immunoassay technique.
Results:
Women with PPROM and histological chorioamnionitis had a significantly higher median of amniotic fluid sTLR2 and sTLR4 levels than those with PPROM without histological signs of chorioamnionitis (657.7 ng/mL vs. 61.6 ng/mL; $p < 0.0001$ and 271.1 ng/mL vs. 8.7 ng/mL; $p < 0.0001$, respectively)
Conclusions:
Amniotic fluid sTLR2 and sTLR4 concentrations are significantly higher in women with PPROM between 24 and 36 gestational weeks with histological chorioamnionitis than those without histological signs of inflammation.

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3.

OSTEOCHONDROGENIC POTENTIAL OF MESENCHYMAL STEM CELL IN PRIMARY OSTEOARTHRITIS

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Primary osteoarthritis is the most common degenerative arthritic condition with unknown aeitology leading to significant denudation of the articular bone. With increase in life expectancy the greatest burden of disease is in older people, where total hip replacement surgery is the only available treatment option. Despite recent advances in surgical and non-surgical intervention, the treatment of cartilage lesion remains an intractable problem.

MSCs are a promising cell source for the biological repair of articular cartilage. The self replicatory and differentiation potentials of MSCs open up therapeutic opportunities for treatment of lesions in musculoskeletal tissue. Unlike most other human adult stem cells, MSCs can be obtained in quantities appropriate for clinical applications, making them good candidates for use in tissue repair. MSCs have clinical advantage because it is readily available from bone marrow aspirated under local anesthesia with minimal morbidity.

The aim of this study was to identify the characteristic of MSCs in patients with primary OA of hip and its potential use in the treatment of OA. Bone marrow obtained from head of femur of 20 patients with primary OA of hip joint undergoing THR was compared with MSCs obtained from 6 healthy donors in terms of their availability, morphological features, proliferation and differentiation capacity. MSCs of appropriate quantity could be obtained from the head of femur in patients with sever OA of hip undergoing THR.

Cells obtained had same morphological characteristic as MSCs and could differentiate into chondrogenic, adipogenic and osteogenic pathways. MSCs from OA patients had very low proliferative capacity and found to have lost their multipotential ability. MSCs ability to undergo chondrogenic and osteogenic differentiation was found to be reduced, and adipogenic differentiation was increased in OA when compared with healthy donors. The decrease in chondrogenic linage could explain the inability of the articular cartilage to repair itself or even leading to its degeneration. Increase in adipogenic linage could explain the increased marrow fat in these patients. Mild reduction in the osteogenic linage was observed but activity of these cells could explain the increased bone density and bone changes that are seen in OA patients.

The basis for autologous MSCs transplantation is that, cells should have a high proliferative capacity, multipotent and the ability to deposit the required matrix protein. Thus it does not seem feasible to use autologus transplantation of MSCs in OA patient as a clinical mode of treatment. Also from the results obtained we could speculate that changes in MSCs may be leading to the process of OA.

Key words: OA- Osteoarthritis, MSCs- Mesenchymal stem cells, THR- Total hip replacement

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4.

INSIGHTS FROM TRANSCRIPTIONAL ANALYSIS OF LIVER REGENERATION TERMINATION

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Transcriptional control of late phase liver regeneration in a rat 2/3 partial hepatectomy model was studied, motivated by the lack of its understanding and high clinical relevance. Microarray gene expression profiling in adult male Wistar rats during experiment with rather exceptionally wide range of recovery times, spanning up to 14 days after the surgery, was followed by real-time RT-PCR assessment of 11 genes relevant to the process. These genes recruited from pathways selected by gene enrichment analysis of microarray data. Hierarchical clustering and principal component analysis resulted in accord to distinction of three temporal phases of liver regenerative response, of which the late stage, mapped beyond the 5^{th} day, was focus of further analyses. Besides the computational pathway analysis, manual review of the 359 genes found to be specific for the late phase, revealed several gene functional groups highly probably involved in the advanced regeneration. These were PPAR signalling pathway, lipid metabolism-related pathways and complement, coagulation and thrombolytic cascades. Our results also support indispensable role of extracellular matrix remodelling. Xenobiotic biotransformation enzyme abundance also had distinct temporal pattern in respective phases of regeneration. Our findings expanded those of recent pivotal work of Yuan and co-workers (J Proteome Res. 2011;10:1179-1190) and were achieved using a novel combination of □ioinformatics genome-scale data processing approaches and substantiated by widely trusted real-time RT-PCR assessment. Taking posttranscriptional regulation mechanisms into account, our results form reasonable basis for subsequent research studies in a vivid field of liver regeneration.

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5.

TREATMENT OF NEUROGENIC DETRUSOR OVERACTIVITY USING BOTULINUM A TOXIN: COMPARISON OF SUBMUCOSAL AND INTRAMUSCULAR ROUTE OF APPLICATION

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Summary: Treatment of neurogenic detrusor overactivity using botulinum A toxin is the first option for patients with refractory disease. This study compares the changes of urodynamic parameters in different routes of applying of botulinum A toxin.

Patients and Methods: Total of 15 patients with neurogenic detrusor overactivity after spinal cord injury in age between 25 and 46 years were randomized into two groups to receive 300 U of Botox® by cystoscopic approach. In group A the drug was administered submucosaly in nine patients and in group B into detrusor in six patients. Evaluation of urodynamic parameters and quality of life questionnaires were done before and 12 weeks after treatment.

Results: Maximal cystometric capacity increased from 236.6±76.1 ml to 456±73.8 ml in the group with submucosal application ($p<0.05$) and from 242.7±96.8 ml to 432.8±79.1 ml in the group with application into detrusor ($p<0.05$). Maximal detrusor pressure during involuntary contraction decreased in both groups ($p<0.05$). After treatment the number of episodes of incontinence decreased from 13.4±5.3 to 3.1±1.7 in group A ($p<0.05$) and from 13.2±4.6 to 3±1.9 in group B ($p<0.05$). Continence rate was 88% for group A and 83 % for group B. Quality of life scores significantly increased in both group ($p<0.05$). We did not observed differences between these form of application ($p>0.05$).

Conclusion: Application of botulinum A toxin in the period 3 months after treatment improved monitored urodynamic parameters and reduced the number of episodes of incontinence in both group. From these initial preliminary results both methods of application seem to be comparable.

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6.

DOPAMINE 2 RECEPTOR EXPRESSION IN VARIOUS PATHOLOGICAL TYPES OF CLINICALLY NON-FUNCTIONING PITUITARY ADENOMAS

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Clinically non-functioning pituitary adenomas account for about one-third of pituitary tumors. The majority of them are pathologically classified as gonadotropinomas or null-cell adenomas without hormonal expression. The rest represent silent corticotroph adenomas and plurihormonal tumors. Conservative therapy with dopamine agonists is effective in some cases only depending on the expression of dopamine 2 receptors (D2R). The aim of this study was to quantitatively estimate D2R expression in clinically nonfunctioning pituitary adenomas and correlate the results with adenoma type according to pathological classification. Out of the 87 adenomas investigated, 63 expressed gonadotropins, 7 were silent corticotroph adenomas, 7 were plurihormonal tumors, and only 6 did not express any pituitary hormone on immunohistochemical investigation. With the use of the reverse transcriptase PCR technique, D2R mRNA was expressed in all adenomas with very heterogeneous quantity. The expression was very low in corticotroph adenomas (relative median quantity after normalization to housekeeping gene 0.01) and lower in plurihormonal tumors (median 0.4) than in gonadotroph (median 1.3) and null-cell adenomas (median 1.9). The difference between corticotroph adenomas and plurihormonal tumors in comparison with other pathological types was statistically significant. The expression of D2R did not depend on the presence or absence of gonadotropins. We conclude that D2R expression is very low in corticotroph adenomas and significantly lower in plurihormonal tumors. The positivity of gonadotropins does not predict the D2R quantity.

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7.

CONCOMITANT FIBROMYALGIA IMPACT ON THE RHEUMATOID ARTHRITIS ACTIVITY ASSESSMENT

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Monitoring disease activity in patients with rheumatoid arthritis (RA) is a cornerstone for successful treatment. Composite indices DAS-28 (Disease Activity Score), CDAI (Clinical Disease Activity Index) and SDAI (Simplified Disease Activity Index) are available instruments for such an assessment, used in clinical practice, but they could be influenced in the presence of associated chronic pain syndrome such as fibromyalgia (FM). The aim of our study was to explore the FM impact on disease activity assessment in the patients with RA.

We examined 120 patients (29 men, 91 women) with RA on the presence of concomitant FM. Tender joint count (TJC), swollen joint count (SJC), tender point count (TPC), patient general health by visual analog scale (VAS-GH) were assessed. Laboratory parameters (ESR, CRP, rheumatoid factor - RF) were recorded.

FM diagnosis based on ACR criteria was established in 25 (20,8%) patients (4 men, 21 women). There were no significant difference in sex ratio, age, RA duration, RF positivity, ESR and CRP between RA and RA/FM patients. RA/FM patients had significantly higher score of DAS-28, SDAI and CDAI in comparison to RA patients. TJC and VAS-GH, which are subjectively influenced measurements, contributed mostly to the disease activity differences.

The composite indices, widely used in RA assessment, can be insufficient to evaluate real inflammatory activity in cases of RA associated with FM. Each rheumatologist should be aware of these limitations during planning immunosupressive treatment strategy.

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8.

CHRONIC ANTHRACYCLINE CARDIOTOXICITY: Molecular and functional alterations in the post-treatment follow up

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So far, only little experimental data are available on changes ocurring in the myocardium during post-treatment follow up of chronic anthracycline (ANT) cardiotoxicity. Therefore, this was the aim of this study. Chronic ANT cardiotoxicity was induced in rabbits with daunorubicin (DAU; n=27, 3 mg/kg, weekly for 10 weeks), while controls received saline (n=19). A half of animals was sacrificed after the last dose, while the rest was followed-up for next 10 weeks (FU). DAU treatment caused significant decrease of the left ventricle (LV) fractional shortening with further significant progression in the FU ($p<0.001$) and development of significant LV dilation ($p<0.01$). Cardiomyocyte damage was documented by elevation of plasma troponin T during DAU-treatment ($p<0.001$) and the values remained increased for several weeks in the FU which indicated ongoing myocardial damage. DAU-induced an increase in LV lipoperoxidation, which significantly progressed further in the FU ($p<0.001$). However, expression and transcriptional activity of master regulator of antioxidant response (Nrf2) stayed unchanged. This corresponded with no changes in majority of its gene targets. Interestingly, significant decrease in gene expression of MnSOD ($p<0.001$) and NQO1 ($p<0.01$) and marked overexpression of HO1 ($p<0.001$) were found which pointed to involvement of different regulation pathway. Significant decrease in citrate synthase ($p<0.05$) and complex I ($p<0.001$) activities documented continuous mitochondrial damage even in the FU. However, no response of the mitochondrial biogenesis pathway directed by PGC-1α was found, instead its marked suppresion mainly in the FU was apparent. These findings may help to understand the mechanisms responsible for progression of ANT cardiotoxicity in the FU. Supported by GACR 305/09/0416 and SVV 2011-269901.

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