AB0055

HYDROXYCHLOROQUINE INHIBITS SOLUBLE CD154 PRODUCTION THROUGH CA2+ AND PKC SIGNALLING PATHWAY

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Background: Over-expression of membranous CD154 and soluble CD154 (sCD154) in T lymphocytes is important in pathogenesis of autoimmune diseases. PKC pathway induces sCD154 production through promoting shedding of membranous CD154.

Objectives: Hydroxychloroquine (HCQ) has been used in the treatment of autoimmune diseases for decades. We sought to identify the effects of HCQ on sCD154 and a possibly regulatory mechanism.

Methods: CD4⁺ T cells were isolated from the blood of healthy donor. After stimulated with ionomycin +PMA and various concentrations of HCQ, concentration of sCD154 in the medium, expression of membranous CD154, Ca²⁺ pathway and PKC signalling pathway were assessed.

Results: HCQ attenuated intracellular sustained calcium storage release and membranous CD154 synthesis in activated T cells. Besides, HCQ inhibited PKC activation and subsequently shedding of membranous CD154.

Conclusions: HCQ inhibited production of sCD154 in activated T cells through suppressing Ca²⁺ and PKC signalling pathway. These findings provide one of the mechanistic insights into HCQ treatment.

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AB0056

MEASUREMENT OF THE PRO-COAGULANT ACTIVITY OF MICROPARTICLES IN PATIENTS WITH INFLAMMATORY RHEUMATIC DISEASES: PROSPECTIVE STUDY

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Background: Microparticles (MPs) are small membrane-bound vesicles that arise from activated and dying cells. Although the majority of MPs in the blood originate from platelets, all cells appear to be able to release MPs. Many studies have raised the implication of these MP in various processes: inflammation, thrombosis, angiogenesis. Previous studies reported inconsistent results in inflammatory rheumatic diseases. Studies have shown the correlation between the circulating MPs, platelet MPs and lymphocyte MPs in patients with spondyloarthritis (SpA) compared to control patients. For rheumatoid arthritis (RA), platelet MPs levels were correlated with DAS28.

Objectives: The aim of this study was to search a possible correlation between the disease activity and the pro-coagulant property of microparticles, potential indirect marker of inflammation.

Methods: The test used (STA Procoag PPL) is a standardised automated test. Results are expressed as coagulation times (in seconds). It is a functional test that provides information on the procoagulant potential of microparticles. The microparticles supply the phospholipids expressed on their membrane surface and the test provides calcium and factor Xa necessary to initiate coagulation: the shorter the coagulation time the greater the procoagulant activity of the phospholipids being studied, suggesting a higher number of MP.

This is a prospective, single-centre study, including 39 patients with spondyloarthritis (ASAS criteria), 37 with rheumatoid arthritis (ACR criteria) and 26 control patients (healthy subjects, osteoarthritis). All patients underwent STA Procoag PPL test, and we collected medical data: disease activity (BASDAI, BASFI,

DAS28vs and DAS28crp and HAQ), biological inflammation (VS, CRP), duration of disease, and current treatment.

Results: The *in vitro* clotting time of serum of patients with spondyloarthritis and rheumatoid arthritis compared with controls was not significantly different (p=0.23 and p=0.44, respectively). Regarding the activity scores of inflammatory rheumatic disease: BASDAI and BASFI, DAS28esr, DAS28crp and HAQ for patients with RA, no correlation between these data and coagulation time was found; the same goes for biological inflammation (ESR, CRP), duration and type of treatment (Nonsteroidal anti-inflammatory drugs, DMARDs, biologics).

Abstract AB0056 – Table 1

STA PPL	Controls (n=26)	SpA (n=39)	p	RA (n=37)	p
Mean±SD	62,70±11,58	66,48±12,77	p=0,23	65,25±13,54	p=0,44
BASDAI	r=0,0602	p=0,72	DAS28 esr	r=0,0230	p=0,17
BASFI	r=-0,125	p=0,45	DAS28 crp	r=0,0151	p=0,37

Conclusions: In this study, there is neither difference in values of procoagulant activity of MPs between inflammatory rheumatic diseases and control subjects, nor correlation with their activity scores or biological inflammation.

Disclosure of Interest: None declared

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AB0057

DECREASED IRISIN LEVEL AS RISK FACTOR OF PATHOLOGICAL FRACTURES IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Recent studies have been suggested that adipokines and myokines may be implicated in bone metabolism and pathogenesis of osteoporosis (OP).¹ Previous studies have revealed inverse correlation between irisin levels and vertebral fragility fractures, but no significant correlation was found between irisin and bone mineral density (BMD) as well as with lean weight. There is no previously published data about irisin levels in rheumatoid arthritis (RA) patients.²⁻⁴

Objectives: To investigate serum irisin levels in patients with RA.

Methods: We studied 170 people: 110 RA patients (mean age 53.58±12.32; hereinafter M±SD) and 60 healthy controls. All patients with RA were examined using dual-energy X-ray absorptiometry using LUNAR DPX-Pro densitometer. Osteoporotic fractures were confirmed by X-ray examination and/or by anamnesis data. All patients were passed through extensive clinical and laboratory examination, including N-terminal propeptide of procollagen type I, C-telopeptide of type I collagen, 25(OH)-vitamin D concentration. Serum irisin levels were measured by ELISA (BioVender test system, Cat №RAG018R).

Results: The mean concentration of irisin in RA group was 14.48±7.07 mg/ml, which was significantly lower than of healthy donors – 20.49±4.82 mg/ml (p<0,001). We subsequently divided all of RA patients into two groups: the first one (n=44) included patients with reduced serum irisin levels (below 10.85 mg/mL), and the second one (n=66) with normal irisin levels (above 10.85 mg/ml). The first group had significantly higher activity (DAS28), increased frequency of extra-articular manifestations, disease duration more than 5 years, class III of functional joints disability and lower levels of 25(OH)-vitamin D. We also observed higher incidence of pathological bone fractures in this group (p=0,047). There wasn't any significant correlation between serum irisin level and BMD at any localization, lean, or fat weight. We did not reveal any difference of bone turnover markers (serum C-terminal telopeptide of type I collagen, serum N-terminal propeptide of type I procollagen (P1NP)) between these groups.

Conclusions: We have therefore revealed relationships between decreased serum irisin levels, 25(OH)-vitamin D concentration and higher incidence of pathological bone fractures in RA patients. We found no connexion between serum irisin levels and BMD at any site and body composition. We could consequently suppose, that irisin levels may reflect bone quality or increased fall risk.

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AB0058 IL2 DECREASE AND INCREASE OF IL10 AND INF1A ARE ASSOCIATED TO CLINICAL ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOUS PATIENTS

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by deregulation of cytokine production. INF1A is a proinflammatory cytokine considered as a key molecule in the SLE etiopathogenesis, being responsible indirectly of IL10 upregulation. BLYS is involved in autoantibodies production and clinical activity in SLE, and its expression is regulated by other cytokines as IL10 and INF1A. IL2 is an anti-inflammatory cytokine in SLE, but its loss leads to the production of Th2 proinflammatory cytokines as IL4, IL5 and IL13.

Objectives: To analyse the association among inflammatory cytokine levels and clinical activity, as well as to identify a cytokine profile related to disease activity in SLE.

Methods: A cross-sectional, observational study of 142 patients diagnosed of SLE^{SLECC 2012} criteria), and 35 healthy controls, was performed. A complete blood-test and an interview were carried out to collect their clinical data. We analysed inflammatory cytokines serum levels by colorimetric methods. Biostatistical analysis with R was performed.

Results: Mean cytokine levels for the SLE patients and healthy controls are shown in the table 1.

Abstract AB0058 – Table 1

	SLE patients (pg/ mL)	Healthy controls (pg/ mL)
	Mean (SD)	Mean (SD)
IL2	4.34 (12.2)	4.96 ^{4,5}
IL4	58.65 (64.6)	89.05 ^{70,18}
IL5	18 (5.71)	7.33 ^{8,59}
IL10	12.29 (32.82)	1.92 ^{3,67}
IL13	44.97 (273.78)	42.85 ^{93,05}
IL21	3.18 (5.61)	2.82 ^{3,35}
INF1A	15.69 (24.59)	4.84 ^{1,91}
BLYS	2293.82 (6948.46)	1181.15 ^{260,04}

The mean clinical activity measured by SLEDAI was 5.91±5.06, and we observed a statistically significant association between high levels of IL10 and high clinical activity (p=0.001).

Statistical analysis indicates that complement consumption is associated with increased levels of IL10 (p=0.029), INF1A (p<0.001), IL4 (p=0.004), IL5 (p=0.009) and decreased levels of IL2 (p=0.045); or anti-DNA positivity is associated with increased levels of INF1A (p=0.002) and decreased levels of IL2 (p=0.045), IL4 (p=0.034) and IL5 (p=0.007).

Moreover, BLYS seems to have increased in patients with positive antiphospholipid antibodies and anti-DNA. IL10 is associated with ENAs positivity (p=0.022). In patients with other autoimmune disease associated with SLE, an increase of INF1A (p=0.008) and IL5 (p=0.044) is observed.

SLE patients were categorised by normal, low or high level of the eight cytokines. Despite the fact that no specific cytokine profile associated with clinical activity was observed, those patients with high SLEDAI score had increased levels of IL10 and INF1A and decreased levels of IL2 and IL21.

Conclusions: Our SLE patients displayed mainly IL10, INF1A and BLYS increased, and IL2 decreased. Although IL10 seems to be the cytokine which best fits to clinical activity in SLE, altered levels of INF1A, IL2, IL4, and IL5 are associated with complement consumption or anti-DNA positivity.

Disclosure of Interest: None declared

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AB0059 FETUIN-A: CLINICAL AND LABORATORY ASSOCIATIONS IN WOMEN WITH RHEUMATOID ARTHRITIS

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Background: Fetuin-A is an acute-phase protein with contradictory effects. It is well known that fetuin-A low levels are associated with calcification and higher risk of cardiovascular diseases and its level is downregulated by pro-inflammatory cytokines.¹ Nevertheless, it was shown, that fetuin-A induces synthesis of pro-inflammatory cytokines in adipocytes and macrophages.²

Objectives: To investigate the level of fetuin-A in women with rheumatoid arthritis (RA).

Methods: At baseline we measured fetuin-A level, femoral neck, total hip and L₁₋₄ BMD by DXA in 110 women with RA (mean age 54.5±12.6; hereinafter M±Std. dev.) and 30 healthy controls. Serum CRP and ESR were measured to assess inflammation. DAS28 was calculated to determine RA activity. The diagnosis of osteoporosis was set according to the recommendations of world health organisation – T-scores≤-2.5 for patients without glucocorticoid therapy in anamnesis, T-scores≤-1.5 for patients treated with glucocorticoid for 3 months in anamnesis or with an osteoporotic fracture in anamnesis. Fetuin-A in serum was determined by enzyme-linked immunosorbent assay.

Results: Mean concentration of fetuin-A in group with RA was 765,69 ±120,64 ug/ml, which was lower than of healthy controls – 812,95 ug/ml (p=0,0438). Secondary osteoporosis was revealed in 52 patients (47%) with RA with mean level of fetuin-A at 733,7±18,83 ug/ml vs. 794,36±12,83 ug/ml (p=0,0078) of 58 (53%) non-osteoporotic patients. Moderate negative correlations were observed between fetuin-A and DAS28 (r=-0,4334; p<0,001), fetuin-A and CRP (r=-0,3148; p<0,001), fetuin-A and ESR (p=-0,344; p<0,001). Mean concentrations of fetuin-A were significantly different between the subgroups with moderate (3,2≤DAS28<5,1) and high disease activity (5,1≤DAS28) of RA patients and healthy controls: 742,41±12,07 ug/ml vs. 812,95 ug/ml (p=0,0021) and 663,9 ±39,14 ug/ml vs. 812,95 ug/ml (p<0,001).

Conclusions: Our study confirms that lower levels of fetuin-A are associated with higher activity of RA and with the loss of bone mineral density.

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AB0060 MECHANISMS OF ACTION OF CHONDROITIN SULFATE AND GLUCOSAMINE IN MUSCLE TISSUE: IN VITRO AND IN VIVO RESULTS. A NEW POTENTIAL TREATMENT FOR MUSCLE INJURIES?

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Background: Musculoskeletal injuries are the most common cause for severe, chronic pain and physical disability affecting hundreds of millions of people around the world and represent a major concern also in sports medicine. A preclinical study evaluated the impact of chondroitin sulfate (CS) and glucosamine (GLU) combination (both compounds used in the treatment of osteoarthritis) on muscle healing and force recovery. Although the mechanisms of action of the combination CS +GLU have been largely studied in articular tissue, its potential therapeutic effects for muscle healing remain still unknown.

Objectives: The aim of the present study is to elucidate the mechanisms of action responsible for this interesting benefit.

Methods: Human skeletal muscle biopsies were digested with Protease type XIV and the resulting tissue suspension were collected by centrifugation. The digested muscle pellet was then triturated to liberate the human satellite cells. Differential centrifugations were used to enrich the cell fraction. Cell suspension was then transferred onto cell-culture dishes in Growth media (DMEM/M-199 medium (3:1) with 10% FBS, 10 µg/ml insulin, 2 mM glutamine, 25 ng/ml fibroblast growth factor, and 10 ng/ml epidermal growth factor) and cells were expanded in a growing monolayer. The effect of CS+GLU treatment in primary human skeletal muscle