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TEMAS LIVRES – ÁREA BÁSICA

1º LUGAR

CO.02

Identificação de peptídeos imunogênicos do colágeno V na esclerose sistêmica precoce: um potencial alvo imunoterapêutico

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Introdução

O colágeno tipo V (Col V) tem sido considerado um antígeno sequestrado dentro da fibra colágena, associado à autoimunidade em vários processos patológicos. O nosso grupo estabeleceu um novo modelo de esclerose sistêmica (ES) induzido pelo Col V. Ainda, mostramos que a indução de tolerância nasal com Col V diminuiu o processo inflamatório e fibrótico no pulmão e pele, aliado à diminuição da expressão de TGF- β no modelo de ES. Recentemente foi demonstrada a presença de anticorpos anti-Col V em pacientes com ES precoce. Entretanto, até o momento não foram determinadas as regiões do Col V com potencial autoimune na ES que podem ser possíveis alvos terapêuticos nesta doença.

Objetivos

Avaliar a autoimunidade para as cadeias $\alpha 1(V)$ e $\alpha 2(V)$ do Col V e para os peptídeos imunogênicos desta proteína em pacientes com ES precoce.

Método

Na primeira etapa do estudo o soro de 19 pacientes com ES precoce (Critérios EULAR) e de indivíduos saudáveis ($n=6$) foram utilizados para determinar a presença de anticorpos anti-Col V por citometria de fluxo. A IgG, proveniente da mistura de 3 amostras soro positivas para anti-Col V, foi isolada por cromatografia de afinidade e depois biotinilada (ColV-IgG+). Posteriormente, amostras de ColV-IgG+ foram adsorvidas, separadamente, com a cadeia $\alpha 1(V)$ [ColV-IgG+/ads- $\alpha 1(V)$] e com a cadeia $\alpha 2(V)$ [ColV-IgG+/ads- $\alpha 2(V)$]. Na sequência, procedeu-se à avaliação da reatividade para $\alpha 1(V)$ e $\alpha 2(V)$ do Col V por imunofluorescência, comparando biópsias de pulmão ($n=4$) de pacientes com doença pulmonar intersticial em decorrência da ES (ES-DPI) e de controles saudáveis ($n=6$). Numa segunda etapa, os soros positivos para anti-Col V foram testados por citometria de fluxo, com oito peptídeos sintéticos, relacionados com a autoimunidade do Col V, já descritos na literatura em outras doenças.

Resultados

Sete pacientes (36,84%) apresentaram anticorpos anti-Col V. As biópsias de tecido pulmonar de ES-DPI imunomarcadas com ColV-IgG+ mostraram intensa imunofluorescência ao longo da membrana basal vascular, no músculo liso do brônquio e na camada adventícia, contrastando com a tênue expressão no tecido pulmonar controle (respectivamente $32,86 \pm 8,02$ vs. $0,49 \pm 0,48$, $p < 0,05$). A imunomarcagem do tecido pulmonar ES-DPI com a amostra de ColV-IgG+/ads- $\alpha 1(V)$ apresentou significativa redução de fluorescência comparada com ColV-IgG+ ($10,05 \pm 4,59$ vs. $32,86 \pm 8,02$, $p < 0,05$) e ColV-IgG+/ads- $\alpha 2(V)$ ($10,05 \pm 4,59$ vs. $30,21 \pm 4,29$, $p < 0,05$), confirmando a reatividade para a cadeia $\alpha 1(V)$ tecidual. Na segunda etapa do estudo, foi demonstrado que as amostras de soro dos pacientes com ES precoce, positivas para anti-Col V, apresentaram imunorreatividade para os peptídeos Col5A1(599) e Col5A1(1.049) da cadeia $\alpha 1(V)$ do Col V.

Conclusão

A autoimunidade para o Col V em ES precoce é direcionada para a cadeia $\alpha 1(V)$ do Col V, nos peptídeos Col5A1(599) e Col5A1(1.049) desta cadeia. Esses resultados abrem a perspectiva do uso desses peptídeos para a imunoterapia da ES.

2º LUGAR

CO.09

Altered distribution of monocytes subsets in peripheral blood from Takayasu arteritis patients

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Introduction

Takayasu arteritis (TA) is a chronic granulomatous vasculitis that affects large arteries, and is characterized by an infiltration of macrophages, lymphocytes, and NK cells in all layers of the vessel wall. Monocytes, the precursors of macrophages/dendritic cells, can be distinguished into three subsets based on phenotypic and functional characteristics. No previous studies have evaluated monocyte subsets' distribution in TA and relations with disease activity.

Objectives

This study aims to compare monocyte subsets' distribution in peripheral blood between TA patients and controls, as well as between TA patients with active disease and those in remission.

Materials and methods

This cross-sectional study included 32 TA patients who fulfilled the 1990 American College of Rheumatology (ACR) classification criteria and 30 controls. Disease activity was evaluated by the modified National Institutes of Health (NIH) criteria and physician's global assessment (PGA). Monocytes were assessed by flow cytometry and cells were divided into three subtypes, based on CD14 and CD16 expression: classical (CD14⁺CD16⁻), intermediate (CD14⁺CD16^{dim}) and non-classical monocytes (CD14^{dim}CD16^{high}). Results were displayed as number of cells x10⁶/L or as percentage.

Results

TA patients presented a higher number (cells x10⁶/L) of intermediate monocytes than controls [23.69 (15.99-44.17) vs. 17.20 (9.16-25.33); $p = 0.030$]. In highly active disease, a significant increase in the median number (cells x10⁶/L) of intermediate [56.64 (24.49-155.97) vs. 17.20 (9.16-25.33); $p = 0.008$] and classical monocytes [748.47 (511.97-1628.19) vs. 420.66 (278.04-506.39); $p = 0.005$] compared to controls was found. However, no differences between active patients and those in remission were found. There was a trend towards a decrease in the relative proportion of non-classical monocytes with glucocorticoid treatment (3.97% vs. 7.08%, $p = 0.056$). The percentage of non-classical monocytes had a strong negative correlation with daily prednisone dose ($\rho = -0.796$, $p = 0.002$) and a positive correlation with C-reactive protein (CRP) levels ($\rho = 0.434$, $p = 0.030$). Hypertension, diabetes, ischemic events, and the use of statins or other immunosuppressants did not impact the distribution of monocytes' subsets in TA patients.

Conclusions

There is an altered distribution of monocytes' subsets in the peripheral blood of TA patients compared to controls with an increased number of intermediate monocytes and classical monocytes, especially in those with active disease, despite no differences were found between active disease and the remission state. Prednisone daily dose and serum CRP levels were significantly correlated with non-classical monocytes.

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MENÇÃO HONROSA

CO.03

Nanocápsulas de núcleo lipídico carregadas com diclofenaco reduzem a sinovite e preservam cartilagem articular em modelo experimental de artrite

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Introdução

O diclofenaco (DIC) tem sido usado no tratamento de controle da dor e inflamação em diversas condições inflamatórias agudas e crônicas. O DIC tem diversos efeitos colaterais, sobretudo em pacientes idosos e com comorbidades. Sistemas de liberação direcionada de drogas, tais como formulações nanoencapsuladas, são capazes de aumentar a eficiência de drogas por concentrá-las no sítio de inflamação. Neste trabalho propõe-se um novo sistema nanoparticulado constituído de uma dispersão de monosorbitano em triglicerídeos de cadeia média, com uma parede polimérica de poly(ϵ -caproalctona) estabilizados em micelas de polysorbato 80. Esse novo sistema foi denominado de Nanocápsulas de Núcleo Lipídico Carregadas com Diclofenaco (LNC-DIC).

Objetivo

Avaliar o efeito de LNC-DIC em modelo experimental de artrite e sobre células sinoviais mononucleares ativadas de pacientes com artrite reumatoide (AR).

Metodologia

Foi induzida artrite por adjuvante de Freund em ratos Lewis, e o efeito dos diferentes tratamentos sobre o edema, citocinas (TNF- α e IL1), foram analisados nos grupos: a) artrite sem tratamento; b) tratamento com DIC; c) nanocápsulas de núcleo lipídico placebo (LNC); d) LNC carregadas com DIC (LNC-DIC). Adicionalmente, células mononucleares ativadas coletadas do fluido sinovial de pacientes (CMSA) com artrite reumatoide (AR) em cultura foram submetidas a tratamentos com diferentes doses de DIC ou LNC-DIC. A avaliação do efeito dos tratamentos sobre componentes das articulações metatarsofalangeanas (membrana sinovial, cartilagem, condrócitos, espaço sinovial) foi realizada por morfometria quantitativa. Por citometria de fluxo (CBA) do sobrenadante de cultura para foram quantificadas as TNF- α e IL17A.

Resultados

As formulações LNC e LNC-DIC foram preparadas com metodologia *self-assembling* e apresentaram pH $5,43 \pm 0,24$ e $5,39 \pm 0,16$ para LNC e LNC-DIC, respectivamente, com perfil nanométrico e monomodal com diâmetro médio $D[4,3] 204 \pm 46$ nm (Span < 1,7) para as duas formulações. O LNC-DIC mostrou efeito significativamente superior ao DIC na redução de edema da pata (ANOVA $p < 0,0001$). O efeito antiedema de DIC-LNC é alcançado precocemente no 21º dia de experimento ($P < 0,0001$), quando comparado ao DIC. O efeito redutor de edema de DIC só foi significativo no 28º dia de experimento, quando comparado ao controle. Os resultados mostraram que LNC-DIC apresentou maior efeito mais precoce e com maior magnitude que DIC. LNC-DIC foi capaz de reduzir TNF- α e IL1 do soro dos animais ($P = 0,004$ e $0,018$, respectivamente). O LNC-DIC mostrou-se mais eficiente em reduzir citocinas de CMSA quando comparado ao DIC – regressão não-linear: TNF- α ($r^2 = 0,89$ e $r^2 = 0,31$, $P = 0,008$), IL17A ($r^2 = 0,84$ e $r^2 = 0,16$, $P = 0,019$). A análise dos componentes articulares por morfometria quantitativa mostrou que LNC-DIC reduziu o volume da membrana sinovial ($p = 0,0053$), preservou o volume do espaço sinovial ($p = 0,007$) e da cartilagem articular ($p = 0,0148$) e aumentou o volume total de condrócitos ($p = 0,004$) quando comparados do DIC.

Conclusões

Os resultados indicam que a formulação LNC-DIC tem efeito comparativamente melhor que o DIC, especialmente em reduzir a sinovite, preservar o espaço sinovial, cartilagem e condrócitos, indicando grande potencial desta formulação nanoparticulada no tratamento de doenças inflamatórias articulares.

TEMAS LIVRES – ÁREA CLÍNICA

1º LUGAR

CO.01

Chronic use of hydroxychloroquine did not protect against COVID-19 in a large cohort of patients with rheumatic diseases in Brazil

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Background

There is a lack of information on the role of chronic use of hydroxychloroquine (HCQ) during the SARS-CoV-2 outbreak. Our aim is to compare the occurrence of COVID-19 between rheumatic disease (RD) patients on HCQ with individuals from the same household not taking the drug during the first 12 weeks of community viral transmission in Brazil.

Methods

This baseline cross-sectional analysis is part of a 24-week observational multi-center study. All information regarding COVID-19

symptoms, epidemiological, clinical and demographic data were recorded on a specific web-based platform using telephone calls from physicians and medical students. Clinically confirmed COVID-19 was defined according to the Brazilian Ministry of Health (BMH) criteria. A significant p value was set as <0.05.

Results

From March 29th to May 17st, 2020, a total of 10,443 participants were enrolled, including 5,166 (53.9%) RD patients, of whom 82.5% with systemic erythematosus lupus, 7.8% rheumatoid arthritis, 3.7% Sjögren's syndrome and 0.8% systemic sclerosis (SS). In total, 1,822 (19.1%) participants reported flu symptoms within the 30 days prior to enrollment, of which 3.1% fulfilled the BMH criteria, but with no significant difference between RD patients (4.03%) and controls (3.25%). After adjustments for multiple confounders, the main risk factor significantly associated with clinically confirmed COVID-19 was lung disease (OR=1.63; 95%CI 1.03-2.58); and for RD patients were diagnosis of SS (OR=2.8; 95%CI 1.19-6.63) and glucocorticoids above 10 mg/day (OR=2.05; 95%CI 1.31-3.19). In addition, a recent influenza vaccination had a protective effect (OR=0.674; 95%CI 0.46-0.98).

Conclusion

Patients with RD on HCQ presented a similar occurrence of clinically confirmed COVID-19 to household cohabitants, suggesting a lack of any protective role against SARS-CoV-2 infection.

Funding

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Conflict of interest

The authors declare no conflict of interest.

Trial registration

CONEP (CAAE 30246120.3.1001.5505) and ReBEC (RBR – 9KTWX6).

Keywords

COVID-19; SARS-CoV-2; coronavirus; hydroxychloroquine; antimalarials; rheumatic diseases.

2º LUGAR

CO.07

COVID-19 in SLE patients that developed acute respiratory syndrome: does SLE contributes to a higher mortality in hospitalized patients?

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Background/purpose

The pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has progressed rapidly around the world reaching lethality up to 20% in different regions due to acute respiratory distress syndrome (ARDS)¹. COVID-19 raised a particular concern for people

with rheumatic diseases^{2,3}, especially systemic lupus erythematosus (SLE) that is known to increase susceptibility to infections^{3,5}. Currently, little is known about the impact of COVID-19 in patients with SLE^{3,4}, and even less regarding the evolution of SLE patient who developed ARDS. The primary aim of this study was to analyze the mortality rates in SLE patients that developed ARDS due to COVID-19 and compare with the mortality rates of healthy and comorbid patients.

Methods

In Brazil, ARDS is a compulsory reporting disease, and the standardized electronic database (SIVEP gripe)⁶ is supposed to capture all cases of ARDS from the health system. This database capture demographic, clinical and laboratorial features of the hospitalized patients. In this epidemiologic study, all 2020 COVID19-ARDS notifications were analyzed. Patients were divided in 3 groups: SLE patients, comorbid patients in general and healthy patients. Features analyzed were: demographic features such as age and gender, clinical features such as risk factors (smoking, alcoholism and obesity), comorbidities (hypertension, diabetes, heart disease, hepatic disease, pulmonary disease, renal disease, neurological disease, oncological disease, transplant patients and dyslipidemia), ARDS and COVID-19 symptoms (fever, cough, sore throat, dyspnea, respiratory discomfort, desaturation, diarrhea, vomiting, abdominal pain, fatigue, anosmia and ageusia) and patients outcomes (intensive care need, ventilatory support, disease cures and deaths). A comparison of data between groups was performed and the mortality rate and relative risks for each group were calculated.

Results

In total, 312,288 patients were analyzed (SLE = 382, comorbid = 185,424 and healthy = 126,482). The mortality rate was 40.3% for SLE, 39.7% for comorbid and 23.0% healthy patients (Table 1). Comparing the mortality rate of the SLE group with healthy group the unadjusted RR was 1.75 (95% IC: 1.55-1.97, $p < 0.0001$). Comparing the SLE group with comorbid group the unadjusted RR was 1.01 (95% IC: 0.89 to 1.14, $p = 0.82$). Comparing the SLE group without any other comorbidity with healthy group the unadjusted RR was 1.43 (95% IC: 1.14 to 1.79, $p = 0.001$). Comparing the SLE group that had other comorbidities with comorbid group the unadjusted RR was 1.14 (95% IC: 0.98 to 1.31, $p = 0.07$).

Conclusion

In this unadjusted analysis, the mortality rate in SLE patients with COVID-19 that were hospitalized due to ARDS seems to be higher than the mortality of healthy patients. When excluding patients that had SLE combined with other comorbidities from the analysis the risk seems to remain a slightly higher for SLE patients. However, no increase in mortality rate was observed in SLE group compared to other comorbidities.

TABLE 1 Demographic and clinical features of lupus patients hospitalized with SARS-Cov-2 in 2020.

	SLE n = 382	Comorbid patients n = 185,424	Healthy patients n = 126,482
Age (mean, SD)	45.1 (16.5)	63.7 (16.8)	50.9 (19.7)
Age > 65 (mean, SD)	73.0 (6.2)	76.8 (8.4)	76.9 (8.0)
Gender			
Female (n,%)	327 (85.6)	84,712 (45.6)	51,802 (40.9)
Male (n,%)	55 (14.4)	100,667 (54.2)	74,637 (59.0)

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TABLE 1 Demographic and clinical features of lupus patients hospitalized with SARS-Cov-2 in 2020.

	SLE n = 382	Comorbid patients n = 185,424	Healthy patients n = 126,482
Risk factors			
None (n,%)	351 (91.8)	163,914 (88.4)	125,333 (99.0)
Smoking (n,%)	3 (0.7)	2401 (1.2)	823 (0.6)
Alcoholism (n,%)	1 (0.2)	903 (0.4)	471 (0.3)
Obesity (n,%)	27 (7.0)	18,793 (10.1)	10 (0.0)
Comorbidities			
None (n,%)	152 (39.7)	0 (0.0)	0 (0.0)
Any comorbidity (n,%)	230 (60.3)	185,424 (100)	0 (0.0)
Hypertension (n,%)	60 (15.7)	37,799 (20.3)	0 (0.0)
Diabetes (n,%)	74 (19.4)	76,689 (41.3)	0 (0.0)
Heart disease (n,%)	110 (28.8)	99,909 (53.8)	0 (0.0)
Hepatic disease (n,%)	10 (2.6)	2,900 (1.5)	0 (0.0)
Pulmonary disease (n,%)	24 (6.2)	19,577 (10.5)	0 (0.0)
Renal disease (n,%)	66 (17.2)	12,854 (6.9)	0 (0.0)
Neurological disease (n,%)	16 (4.1)	15,656 (8.4)	0 (0.0)
Oncological disease (n,%)	8 (2.0)	7,605 (4.1)	0 (0.0)
Transplant patients (n,%)	3 (0.7)	548 (0.3)	0 (0.0)
Dyslipidemia (n,%)	1 (0.2)	2,657 (1.4)	0 (0.0)
COVID-19			
Positive PCR (n,%)	319 (83.5)	153,215 (82.6)	98,490 (77.8)
Symptoms			
Fever (n,%)	245 (64.1)	121,204 (65.3)	86,459 (68.3)
Cough (n,%)	259 (67.8)	134,627 (72.6)	90,732 (71.7)
Sore throat (n,%)	68 (17.8)	31,767 (17.1)	30,176 (23.8)
Dyspnea (n,%)	287 (75.1)	135,483 (73.0)	76,797 (60.7)
Respiratory discomfort (n,%)	232 (60.7)	108,553 (58.5)	61,294 (48.4)
Desaturation (n,%)	229 (59.9)	113,977 (61.4)	54,384 (43.0)
Diarrhea (n,%)	69 (18.0)	25,247 (13.6)	16,814 (13.2)
Vomiting (n,%)	50 (13.0)	14,929 (8.0)	9,279 (7.3)
Abdominal pain (n,%)	8 (2.0)	2,919 (1.5)	1,771 (1.4)
Fatigue (n,%)	31 (8.1)	17,797 (9.6)	8,802 (6.9)
Anosmia (n,%)	26 (6.8)	8,880 (4.7)	8,215 (6.4)
Ageusia (n,%)	10 (2.6)	6,364 (3.4)	6,049 (4.7)
ICU (n,%)	159 (41.6)	65,531 (35.3)	25,441 (20.1)
Ventilatory support (n,%)	251 (65.7)	118,652 (63.9)	53,603 (42.3)
Invasive ventilation (n,%)	105 (27.4)	39,379 (21.2)	13,617 (10.7)
Non-invasive ventilation (n,%)	146 (38.2)	79,273 (42.7)	39,986 (31.6)
Outcome			
Cures (n,%)	164 (42.9)	79,304 (42.7)	71,621 (56.6)
Unknown outcome (n,%)	64 (16.7)	32,288 (17.4)	25,642 (20.2)
Deaths (n,%)	154 (40.3)	73,752 (39.7)	29,183 (23.0)

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3º LUGAR

CO.05

Transcranial direct current stimulation is safe and efficient in patients with systemic autoimmune myopathies

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Background

There is currently little information regarding rehabilitation in systemic autoimmune myopathies (SAMs). Transcranial direct

current stimulation (tDCS) has shown promising results for the motor performance of healthy individuals, and patients with, for example, post-stroke hemiparetic limbs. Therefore, the present study aimed to assess the safety and efficiency of tDCS in patients with SAMs.

Material and methods

This study is a prospective, randomized, sham-controlled, double-blind clinical trial with ethical approval. Eighteen adult patients with dermatomyositis, polymyositis, antisynthetase syndrome or immune-mediated necrotizing myopathies in remission or with minimal disease activity were enrolled from 2018 to 2019. Patients were allocated randomly in two groups to receive sham or active tDCS, with 2mA amplitude for 20 minutes for three consecutive days. The 5x7cm sponge-electrodes were positioned with the anode over the left (C1) or right (C2), contralateral to the dominant limb, whereas the cathode was positioned over the FP2 or FP1, respectively (10-20 EEG electrodes placement). The groups were evaluated in four moments: pre-stimulation, and 30 minutes, 3 weeks, and 8 weeks post-tDCS. They were evaluated in the different moments with International Myositis Assessment and Clinical Studies Group set scores, Short-Form health survey (SF-36), state-trait anxiety inventory (STAI), Beck depression inventory (BDI), timed up-and-go test (TUG), time-stands test (TST), and isokinetic extension and flexion testing of the bilateral knee and elbow. A specific security questionnaire for tDCS was used after the active or sham stimulation for all patients.

Results

The demographic data, kind of myositis, disease duration, and disease status (all with low disease activity) were comparable between the active and sham tDCS groups. After interventions, there was improvement in the values of the patient's VAS ($P=0.042$) and serum levels of creatine phosphokinase ($P=0.005$), independent of the group. Moreover, in the active tDCS group week 8, the physical aspects of SF-36 ($P<0.001$), the mean and better TST at each evaluation ($P<0.001$), absolute peak torque ($P<0.001$), and peak torque adjusted for body weight values ($P<0.001$) of stimulated inferior limb extension also improved. No differences were observed in the STAI, BDI, or TUG in both groups. The patients' adherence to the protocol was 100% and no adverse event was reported, including disease relapse.

Conclusions

This unprecedented study evidences the safety of tDCS, as well as the potential efficiency in improving rehabilitation of tDCS in SAMs. More studies with a large samples and longer tDCS sessions are necessary to corroborate the present study. ClinicalTrials.gov Identifier: NCT03749538.

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