4.001 Monoclonal antibodies applied to recovery of INS-R of the salivary glands of spontaneously diabetic mice

Morfologia e Patologia Básica - FMJ
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Introdução:
In relation to classic treatment for diabetes, the insulin, per se, is ineffective to cellular recovery of organs and tissues. In this aspect, the immunotherapy can be a way out, however according to literature, this therapy also is ineffective, as an agent to control and recover the damages caused by diabetes. On the other hand, the immune treatment with antibodies anti-CD4 and anti-CD8 can promote effective results in reduction of inflammatory process in different organs and also on the control of hyperglycemic condition.

Objetivos:
Thus, the aim of this study was to evaluate the long-term therapy with anti-CD4 and anti-CD8 monoclonal antibodies in the recovery of insulin receptors of salivary glands of the spontaneously diabetic mice.

Métodos:
Fifteen mice, among BalbC and NOD (diabetic mice), were divided into three groups with 5 animals each: group I (Balb/C control mice), group II (untreated NOD mice), group III (NOD mice treated with anti-CD4 and CD8 antibodies). The anti-CD4 and CD8 antibodies (IMUNY, Rheabiotec Ltda, Brazil) were administered by intravenously injection (25 ug/ single injections on days: 0, 7, 14, and 21). After treatment, salivary glands samples were analyzed by immunofluorescence microscopy (ethical approval process: 342/11). Also, the analysis of variance (ANOVA) was used to evaluate the glucose levels.

Resultados:
Glucose levels (mg/dl) were high in untreated animals (group II) (685.23±21.26 p&le;0.05), whereas in treated animals (group III), was noted a significant decrease in this level (465.77 ± 39.66 p&le;0.05). Intense signaling (+++) of insulin receptors was observed in animals of group I. On the other hand, in group II was noted a reduction of these receptors (+). In treated animals (group III) were observed a recovery of the insulin receptors (+++).

Conclusão:
These results show that therapy with monoclonal antibodies, can promoted the recovery of insulin
receptor in the salivary glands, and also can assist in the reduction of glucose levels in NOD animals. The association of these monoclonal therapies promoted a beneficial effect on the recovery of the salivary tissues damaged by diabetic condition.

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**Comitê de Ética:**
342/11
4.002 4`,5,7-trihydroxyisoflavoneto recovery the salivary glands in experimental hyperglycemic condition

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Department of Physiology - UNIP
Department of Functional and Structural Biology - UNICAMP
Department of Environmental & Molecular Toxicology - OSU

Introducao:
The diabetes affects the structure and function of various organs and tissues, including the salivary glands. Different studies show that the antioxidants and estrogens, can be agents with capacity of promoting the cell recovery. Among these, the isoflavones are phenolic chemical compounds of phyto-estrogen class and, in this way, could be an alternative treatment for this hyperglycemic condition.

Objetivos:
The present study aims to evaluate the treatment with 4`,5,7-trihydroxyisoflavone and the effects on recovery of cells producing saliva in spontaneously diabetic mice.

Metodos:
Ten female mice with 15 weeks-old were organized into two sets of 5 animals each: group I (Balb/C control mice), group II (NOD mice treated with 4`,5,7-trihydroxy isoflavone). The mice were obtained of UNICAMP (CEMIB) with 25g of average weight. The genistein was mixed to the diet at the dosage of 0.04 g/kg of body weight (Genistein, Cayman Chemical, Ann Arbor, MI, USA) for 5 days. During this time, the animals had access to food and water ad libitum. After treatment, the samples of submandibular and parotid glands were collected and submitted to morphometric analysis (ethical process approval: 123/13). ANOVA and nonparametric tests were performed.

Resultados:
High levels of glucose (mg/dl) were observed in treated animals (group II) (563.1±60.52 p≤0.05) when comparing to the control group (group I) (102.9±7.40 p≤0.05). Salivary tissue in recovery process was observed in animals of group II. This tissue restructuring was characterized by cellular volume recovery: nuclear volume of parotid glands (µm3): 63.84±7.52 p≤0.05 and submandibular glands (µm3): 52.87±4.97 p≤0.05; cytoplasmic volume of parotid glands (µm3): 163.58 ± 8.93 p≤0.05 and submandibular glands(µm3): 116.30±12.52 p≤0.05.

Conclusao:
Thus, it was concluded that the dosages of genistein and the treatment period used in this study,
were not sufficient to show improvements in the general metabolism of diabetic animals. However, in relation to salivary tissue, comparing the healthy group with the treated group, the acinar cells presented recovery in nuclear and cytoplasmic volume. This phytoestrogen, probably, regulated the local action of insulin, cellular growth factors, and the inflammatory activity, promoting this morphofunctional recuperation. These results demonstrate the positive properties of genistein in the recovery process of salivary glands in hyperglycemic conditions.

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**Comitê de Ética:**

123/13
4.003 INCRETIN MIMETIC IMPROVED THE EXPRESSION OF INS-R IN THE SALIVARY TISSUES UNDER HYPERGLYCEMIC CONDITION

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Introducao:
Incretin mimetics inhibits the DPP-4 enzyme (dipeptidyl peptidase 4) and in this form can stimulate the action of GLP-1 (glucagon-like peptide-1). The GLP-1 acts in relation to synthesis and production of insulin and participates in this way of glucose level control. In this respect, the use of incretin mimetics, has proven effective in glycemic control and also in the recovery of pancreatic beta cells, mainly in type 2 diabetes. However, doubts still exist about the efficacy of this therapy in cases of type 1 diabetes.

Objetivos:
The aim of this study was to evaluate the therapeutic effects of incretin mimetic (MKO-431) on signaling and restoration of insulin receptors (INS-R) in salivary glands of spontaneously diabetic mice (NOD).

Metodos:
Twenty-one females mice were divided into three groups of seven animals each: Group I (control mice Balb/C), group II (untreated NOD mice) and group III (NOD mice treated with MKO-431). Diabetic animals (group III) received diet mixed with MKO-431 (43mg/kg) (Merck & Co, Inc, Whitehouse Station, NJ, USA) for a period of 4 weeks. After the treatment period, samples of salivary glands were obtained to immunofluorescence analysis of the insulin receptors (INS-R). The signaling of these receptors was assessed by semi-quantitatively procedure with the scores: intense (+++), moderate (++), weak (+); according to expression, concentration and distribution of these receptors in glandular tissue (ethical process approval: 180/10). ANOVA and Nonparametric tests analyses were performed.

Resultados:
In Group I was observed intense signalling of INS-R in the parotid glands (+ + +), especially closed to the salivary ducts. Similarly, was observed in relation to submandibular glands. In group II, weak (+) signalling was observed in salivary glands. In treated NOD animals (group III), moderated signalling of INS-R was noted in both salivary glands.

Conclusão:
The MKO-431 contributed to the recovery of the expression of insulin receptors in the salivary
glands. These results can be associated, possibly, to inhibiting of DPP-4, thus stimulating the activity of GLP-1, which favoring the insulin sensitivity and the hormone-receptor interaction. Thus, this treatment promoted the recovery of salivary tissues, especially in relation to insulin receptors in the autoimmune diabetes condition.

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Comité de Ética:
180/10
Introdução:

GASTROESOPHAGEAL REFUX IN ELDERLY PATIENTS FJHN Braga (1), Ayres, EM (1,2) (1) HSao Lucas Hospital, Ribeirao Preto, SP (2) Barao de Maua School of Medicine, Ribeirão Preto, SP

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INTRODUCTION

Age is known for reducing function of organs and systems. Such reduction is considered normal and literature data have shown that the oesophagus is also affected by age in normal elderly patients [1]. Nevertheless, there are no data concerning the oesophageal function of elderly patients in the presence of signs and symptoms related to the oesophagus. Scintigraphy is known for being a very sensitive method in the evaluation of gastrointestinal motility and has been in the nuclear medicine routine for several decades.

Objetivos:

The aim of this study was to evaluate the role of the Gastroesophageal Reflux (GOR) Test in detecting abnormality in elderly patients presenting signs and symptoms related to the oesophagus, routinely referred to the Nuclear Medicine Service, and compare results to those obtained from younger patients presenting similar signs and symptoms.

Métodos:

MATERIALS AND METHODS

We studied two groups (G) of patients after agreement and consent: G1, n = 12, aged 61 - 84 y, x = 66.7, referred to the Service for the GOR test for asthma (4), epigastrial discomfort (6), chest pain (1), cough after meals (1) and G 2, n = 16, aged 18 - 30 years, x = 23.8, referred for epigastrial discomfort (5), epigastrial burning (4), asthma (3), chest pain (2) and other reasons (2). We used 370 MBq of 99mTc-Stanous colloid in 10 ml of water solution. Patients were instructed to swallow the tracer only once and received pure water after the oral administration of the tracer to wash the oesophagus of any possible remaining activity. Images (anterior view of the chest, LEAP collimator, camera peaked in 140 keV) were acquired dynamically (one frame/15 seconds) for 10 minutes, the patients lying in dorsal decubitus. Patients were then instructed to stand up by their own means and walk for 1 minute. Acquisition re-started thereafter (external marks being used to place patient in the same position as in the beginning of the test) and continued for 10 more minutes. This procedure has recently been reported as being useful to increase the sensitivity of the test [2]. Time / activity curves were generated in a computer after ROIs were drawn on the oesophagus projection.
Resultados:
Ten of twelve patients in G1 had episodes of GOR (83.3%), as compared to 6/16 in G2 (37.5%). More than 1 episode of GOR was noted in 6 patients in G1 (9 episodes in one sole patient), but that was not noted in any G2 patients. All patients in G2 who had GOR presented fast and complete emptying of the refluxate (10-20 sec). Oppositely, 7 patients in G1 presented prolonged emptying time (washout time 2-7 minutes, $x = 3.4$ min). One patient in G1 could not clear refluxate activity out of the oesophagus at all, and that was only achieved after the ingestion of water. Different and unexpected patterns of refluxate retention were observed in G1: · irregular and diffuse retention along the oesophagus, · 3 foci of retention (one in each third of the oesophagus), · heterogeneous and segmented retention and · episode of GOR reaching the proximal third of the oesophagus, with retention limited to the middle third.

Conclusão:
Differently from the data obtained from normal elderly [1], our results indicate that the physiological reduction of function related to age is accompanied by an increase in the number of episodes of GOR when disease is present. Ferrioli has shown that the number of GORs in normal elderly patients is not significantly different from the number of episodes detected in younger patients [1], but the difference we noted in the present study was significant. In addition, the detection of infrequent patterns of retention after GOR episodes supports the hypothesis that the response of the oesophagus to disease is different in the elderly, as compared to younger patients, possibly as a consequence of the normal reduction in function related to age, which precedes disease. We conclude that GOR episodes are more frequent in elderly patients, as compared to younger ones with similar clinical symptoms. Retention also occurs more often in the elderly and different patterns of retention may be recognised. The scintigraphic GOR test is a very good tool to evaluate such patients.

REFERENCES

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REFERENCES  

Comitê de Ética:

não se aplica
4.005 EVALUATION OF ALTERATIONS IN THE CONTRACTILE ACTIVITY OF SMOOTH MUSCLE OF THE BLADDER AND ILEUM INDUCED BY POLYMICROBIAL SEPSIS MODEL

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Introdução:
Sepsis is a Systemic Inflammatory Response Syndrome (SIRS) caused by an infection, having a high mortality rate, up to 70% in severe sepsis. The systemic inflammatory overreaction associated with hypoperfusion leads to tissue damage and organ dysfunction, alterations in the smooth muscle contractility in organs like small and large intestines and urinary bladder may take part in the dysfunction of these organs.

Objetivos:
This study aims to evaluate the alterations caused by sepsis on contractile response of the smooth muscles of the bladder and ileum of rats in vitro.

Métodos:
In all experiments were used Wistar rats (200–250g) and all procedures performed in accordance with the local Animal Ethics Committee under registration no. 11586298-6/15. Sepsis was induced by cecal ligation and puncture (CLP) model. Briefly, midline laparotomy was performed in anesthetized rats (Ketamine and Xylazine, 90 and 15 mg/kg respectively) to allow exposure of the cecum, which was tightly ligated at ileocecal valve; perforated 10 times with a 21-gauge needle; placed back and abdomen was closed. Sham group underwent similar procedure, but the cecum was neither ligated nor punctured. After 24 h of sepsis induction, the animals were sacrificed by CO2 asphyxiation and ileum and urinary bladder dissected, cut into strips of 10 mm and connected to a isometric force transducer (Grass, FT03 Model) to measure tension in the vessels. The tissues were placed in 5ml organ chamber containing Krebs solution, gassed with 95% O2 and 5% CO2, at 37 °C and pH 7.4. Strips were mounted under 1g passive tension and allowed to equilibrate for 60 min. After, non-cumulative concentration-response curves were produced using KCl (10 - 100 mM) and Carbachol (Cch) (0.01 – 10 uM) the contracting (g) was measured as the difference between baseline tension and the plateau. Data were expressed as mean ± SEM and statistical significance (p<0.05) was assessed by unpaired t-test.

Resultados:
Our data showed that, in ileum, sepsis significant increases the maximum contraction force, induced by Cch, in both Phasic (2.22±0.22g, n=8) and tonic (1.64±0.12g, n=7) components of contraction,
compared to sham-operated rats (1.43±0.20g, n=7), (0.68±0.16g, n=6) respectively. As for EC50, the only altered was from phasic phase (0.10±0.01 uM), compared to (0.29±0.01, n=6) sham-operated rats. It was not observe none alteration in the bladder in response to Cch. For KCl responses, was observed that sepsis decreases the maximum contractile force in ileum (7.00±0.77g) but increases in the bladder (8.00±0.77g) compared to sham-operated rats (11.8±0.93g, n=8), (4.10±0.60g, n=8) respectively.

Conclusão:
In conclusion, our data indicate that sepsis affects the smooth muscle response in ileum and bladder in different ways, in ileum the effect on pharmacological coupling appears to be greater than on electromechanical coupling, but seems to be the opposite bladder. Further experiments will undertake to assess the molecular and cellular mechanism involved in alteration in contractile response caused by sepsis in these organs.

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Comitê de Ética:
11586298-6/15
Introdução:

O Diabetes Melitus (DM) está associado a alterações na função do trato gastrointestinal (TGI) incluindo distúrbios na motilidade. Poucos trabalhos avaliaram alterações da inervação intrínseca ou extrínseca do TGI no DM.

Objetivos:

Nesse estudo, buscou-se avaliar modificações na morfologia do músculo liso ileal, na inervação colinérgica e na expressão de canais para sódio dependentes de voltagem em neurônios do plexo mioentérico de ratos.

Metodos:

Os procedimentos envolvendo experimentação animal foram aprovados pelo CEUA-UFTM (protocolo 233). Foram utilizados ratos Wistar machos e fêmeas de 70-80 dias e aproximadamente 200 g. O grupo diabetes foi tratado com injeção intraperitoneal de estreptozotocina (60 mg/kg) enquanto o controle (Con) com tampão citrato de sódio, pH 4,5. A coleta de segmentos do íleo distal foi feita em solução fisiológica contendo nifedipina e atropina, após oito semanas de comprovado estado hiperglicêmico (glicemia em jejum maior que 220 mg/dL). Cortes transversais do íleo foram corados por hematoxilina-eosina (HE), fotografados (Leica DM500) e analisados (Microsoft Image Composite Editor e ImageJ). Reações de imunofluorescência (IF) de dupla marcação foram realizadas utilizando anticorpos anti colina-acetiltranferase e anti-canais para sódio dependentes de voltagem (anti-Nav1.1,1.2,1.3,1.6, 1.7 e anti-PAN Nav) na preparação músculo longitudinal-plexo mioentérico (LMMP). A marcação foi documentada com microscopia confocal (LSM510 ou 710, Zeiss). A inervação colinérgica foi adicionalmente avaliada por western-blot e a expressão de canais para sódio por RT-PCR. Para análise estatística foi utilizado o teste t-student ou Mann-Whitney Rank para os dados não paramétricos sendo considerado p < 0,05 como nível de significância.

Resultados:

Não foram observadas diferenças significativas na área circunferencial total (Con 1255 ± 68 x 103; DM 1387,8 ± 57,9 x 103 µm², n=5) e espessura do músculo liso intestinal (Con 100 ± 1,8 &mm;m; DM 114 ± 5,4 &mm;m, n=9). Não foi detectada alteração na área relativa ocupada por fibras
colinérgicas do plexo terciário quando comparado os dois grupos. A expressão de ChAT apresentou uma tendência ao aumento no tecido diabético, embora não estatisticamente significante. As isoformas Nav1.6 e 1.7 foram detectadas em reações de IF nos dois grupos sendo o Nav1.6 associado a neurônios colinérgicos. Resultados dos experimentos de RT-PCR indicaram a expressão das isoformas Nav1.3, Nav1.6 e Nav1.7 no plexo mioentérico ileal de ambos grupos e sugerem uma redução na expressão do Nav1.6 no grupo diabético. A área de silhueta somática de células com marcação positiva para o Nav1.6 também não apresentou diferença significativa entre controle e o diabético (297,4 ± 10 e 372,5 ± 8,4 μm², respectivamente), sugerindo que esses neurônios não sofrem o processo de degeneração hidrópica durante o curso da doença.

**Conclusão:**
Os dados não indicam alterações morfológicas no músculo liso ou em neurônios do íleo de animais diabéticos, enquanto sugerem que mudanças na expressão gênica neuronal podem estar envolvidas em alterações motoras do TGI associadas ao diabetes.

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**Comitê de Ética:**
CEUA-UFTM 233