

Session:

BASIC SCIENCE I

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TITLE: IS SB 334867, AN OREXIN 1 RECEPTOR ANTAGONIST AFFECTING AN ANTIEPILEPTIC DRUG, ETHOSUXIMIDE, IN CHEMICALLY INDUCED SEIZURE TEST IN MICE?

AUTHOR: Piotr Gorlach

CO-AUTHORS: Paweł Gryta, Michał Hader, Wojciech Fila

SUPERVISOR: Prof. Stanisław Jerzy Czuczwar MD, PhD, Barbara Miziak MD, PhD

AFFILIATION: Student's Science Club of Pathophysiology, Medical University of Lublin

Introduction: Epilepsy is one of the most known neurological disease in the world. It comes from abnormal, excessive and synchronized neuronal discharges in the brain. The number of people suffering from this disease reaches over 60 million. In addition, more than 30% of people affected by epilepsy is drug resistant. Pharmacological methods are the most known medications against epilepsy, but considering the increasing resistance to antiepileptic drugs in patients with epilepsy, there is a necessity to discover new possibilities and substances, which can be useful in the treatment of epilepsy. The mechanism by which ethosuximide (ETX) affects neuronal excitability includes a blockade of T-type calcium channels in thalamic neuronal cells. This results in stopping the generation of absence seizures. Pentylenetetrazole (PTZ) is used to study seizure phenomena and to identify pharmaceuticals that may control seizure susceptibility. It binds to the GABA-A receptor, as well as increases calcium influx and sodium influx, both of which depolarize the neuron.

Aim of study: The target of this study was to rate the influence of SB 334867, an orexin 1 receptor antagonist, on the anticonvulsant effect of ETX in PTZ-induced seizure test in mice.

Material and methods: Swiss male mice were used. The animals received SB 334867 in a dose of 2.5 mg/kg in combination with ETX in ranged doses from 50 to 84 mg/kg, both treatments being injected intraperitoneally. After drug application, the convulsive activity was induced with PTZ subcutaneous injection.

Results: SB 334867 in dose of 2.5 mg/kg combined with ethosuximide (50-84mg/kg) has not changed its anticonvulsant action.

Conclusions: SB 334867, an orexin 1 receptor antagonist, did not modify the anticonvulsant potential of ethosuximide.

TITLE: EFFICIENCY OF WEB APPLICATION AND SPACED REPETITION ALGORITHMS AS AN AID IN PREPARING TO PRACTICAL EXAMINATION OF HISTOLOGY.

AUTHOR: Dominik Karch

CO-AUTHORS: Krzysztof Kopyt

SUPERVISOR: Michał Nowakowski MD, PhD

AFFILIATION: Department of Medical Education, Jagiellonian University Medical College

Introduction: Educational methods evolve with development of technology. Students in medical disciplines are looking for new learning strategies. Computer applications are becoming more popular as they use a variety of strategies to improve efficiency of study. One of them are spaced repetition algorithms like SuperMemo.

Aim of study: We conducted this study to evaluate impact of using web application on the results of histology practical exam. We also want to check if the SuperMemo-based algorithm is a useful tool in medical education.

Material and methods: We prepared web application which shows the photography of histological slide to recognize. Students had to decide if they have known the answer or not (by clicking proper button) and the program was measuring time of each answer. Then the algorithm allocated new slide to display. Users were randomly divided into two groups: - study – where the slides were displayed in the order of SuperMemo2-based algorithm (difficult slides were shown more frequently), - control – where the slides were displayed randomly. Quality of the students answers was evaluated according to the 6-steps scale, where 0 means incorrect answer, and from 1 to 5 – correct answer depending on time. We also took into consideration results of official histology practical exam. For the calculations we used standard statistical methods. The level of statistical significance was set at $p < 0.05$.

Results: The study involved 79.4% of students approaching the exam. The study group ($n=98$) and control ($n=106$) were similar in terms of the average number of responses in application (901 vs 858; $p=0.73$). The average usage time of the application for a single student was about 1 hour and 45 minutes. We have shown a statistically significant difference which indicate obtaining higher examination results by students who used our application. The average exam score on a scale of 0-15 points among students who used the application was 11.8, while among students who did not: 10.98 (Mann-Whitney U test: $U=3688$; $Z=2.4213$; $p=0.016$). The study showed also no superiority of spaced repetition algorithm over the random allocation of slides, based on the examination results (11.7 vs 11.9; $p=0.73$).

Conclusions: The study involved 79.4% of students approaching the exam. The study group ($n=98$) and control ($n=106$) were similar in terms of the average number of responses in application (901 vs 858; $p=0.73$). The average usage time of the application for a single student was about 1 hour and 45 minutes. We have shown a statistically significant difference which indicate obtaining higher examination results by students who used our application. The average exam score on a scale of 0-15 points among students who used the application was 11.8, while among students who did not: 10.98 (Mann-Whitney U test: $U=3688$; $Z=2.4213$; $p=0.016$). The study showed also no superiority of spaced repetition algorithm over the random allocation of slides, based on the examination results (11.7 vs 11.9; $p=0.73$).

TITLE: INFLUENCE OF PNEUMOCOCCAL POLYSACCHARIDES VACCINE STIMULATION ON LYMPHOCYTE SUBPOPULATIONS AMONG PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL).

AUTHOR: Maciej J. Rutkowski

CO-AUTHORS: Anna Roszkowska, Łukasz Świerszcz

SUPERVISOR: Assoc. prof. Ewelina Grywalska MD, PhD

AFFILIATION: Chair and Department of Clinical Immunology, Medical University of Lublin

Introduction: Chronic Lymphocytic Leukemia (CLL) is an example of secondary immunodeficiency. Leukemic lymphocytes in CLL are characterized by CD19, CD20, CD5, CD 23 and low expression of immunoglobulines on surface. Activation of B lymphocytes in CLL is achieved by antigen stimulation on specific receptor but usually it does not cause proliferation of cells. Ability to induce apoptosis is reduced in the leukemic cells and CD95 expression is lowered.

Aim of study: The objective of the research is the analysis of influence of PPV23 stimulation on amount of basic subpopulations and activated lymphocytes and on expression of CD95 marker.

Material and methods: A study group of 15 subjects with CLL, with an average age of 64.9 ± 9.1 years, patients of Immunology Dept. at Clinical Hospital No. 4 in Lublin was recruited. 10 of them was in stage 0 and 5 of them in stage 1 in Rai classification. None of them was taking immunosuppressive, immunomodulative or steroid treatment within the last 2 months and complained of ailments characteristic of the current infection. Blood sample (30 ml) taken from basilica vein into EDTA tubes was research material. Assessment of immunophenotype and isolation was perform immediately. Three-colour immunofluorescence analyses were performed using a FACS Calibur flow cytometer (Becton Dickinson) equipped with 488 nm argon laser. Statistical analysis was performed using Statistica 10.0 (Stat Soft Inc.) software. The Local Ethical Committee at the Medical University of Lublin approved the research and patients gave their prior written consent

Results: There was significant higher amount of CD3+CD69+ lymphocytes in PPV23 stimulated cell culture ($p=0.004$ after 24h, $p=0.012/48h$, $p=0.022/72h$). The same relation occur in CD3+CD25+ subpopulation ($p=0.021/24h$, $p=0.01/72h$), CD19+CD69+ ($p=0.003/24h$, $0.025/48h$, $0.012/72h$) and CD19+CD25+ ($p=0.003/24h$). Expression of CD95 was significantly higher in CD3+ lymphocytes after PPV23 stimulation ($p=0.003/24h$, $p=0.016/48h$) and in CD19+ lymphocytes after PPV23 stimulation ($p=0.003/24h$, $p=0.015/48h$, $0.006/72h$).

Conclusions: PPV23 stimulation lead to increase of immunological activation markers especially on T lymphocytes which may indicate their function preserved despite leukemia. PPV23 stimulation caused increase of CD3+CD95+ and CD19+CD95+ compared to non-stimulated cell cultures which may indicate induction of leukemic cells apoptosis by PPV 23 stimulation.

TITLE: MEASUREMENT OF BODY FAT DEVELOPMENT BY USING BODYMETRIX ULTRASOUND METHOD.

AUTHOR: Paweł Szybisty

CO-AUTHORS:

SUPERVISOR: Mariusz Teter MD, PhD, assoc. prof Teresa Małecka Massalska MD, PhD

AFFILIATION: Chair and Department of Human Physiology, Medical University of Lublin

Introduction: The issue of measuring body composition expands rapidly not only for purely medical reason but which is not surprising for widely evolving “health and beauty” lifestyle. This entails necessity of using relatively inexpensive and simple method, that can show us regional development of muscle and fat tissue. The BodyMetrix method use ultrasound waves to penetrate the tissue and detect the reflections that occur at different tissues boundaries. Obvious advantage is constancy of measurements regardless of alcohol or hydration level.

Aim of study: The aim of the study was to confirm that regional and overall body fat estimation by using ultrasound method is accurate and repeatable method in young population of students.

Material and methods: To this study we included 26 young students from regional high-school next divided into two sub-groups by gender. The first group include 14 men with average age of $19,35 \pm 2,2$. The second group consist of 12 women with average age of $17,41 \pm 0,51$. The device used to perform the study was BodyMetrix ultrasound system and BodyViewProFit software. The following parameter were obtained: Body fat, fat thickness measured in: thigh, subscapular, chest, waist, triceps, hips, axillary and BMI.

Results: The two groups did not differ statistically BMI $21,35 \pm 2,53$ kg/m². Due to physiologically more developed fat tissue in women all parameters were more or less great in female sub-group. Difference in overall body fat were ($20,95 \pm 3,47\%$ in woman group vs $9,68 \pm 1,72\%$ in man $p < 0,00001$). The biggest difference can be seen in thickness of fat tissue measured in thigh ($9,27 \pm 3,63$ mm in women vs $5,67 \pm 1,53$ mm in man group $p = 0,019$), waist ($11,03 \pm 4,27$ mm in woman vs $6,77 \pm 1,8$ mm in man $p = 0,0018$) and triceps ($9,78 \pm 2,8$ mm vs $4,9 \pm 1,45$ mm $p < 0,00001$). Sexual differentiations were noted also in fat thickness in chest region ($6,0 \pm 1,61$ mm in women vs $4,78 \pm 1,32$ mm in man $p = 0,04$) and hips ($8,17 \pm 2,74$ mm in woman vs $5,9 \pm 1,37$ mm in man $p = 0,023$). The smallest differentiation were observed in subscapular region: ($6,68 \pm 2,74$ mm in woman vs $5,58 \pm 1,21$ mm in man $p = 0,11$).

Conclusions: Study shows the differentiation in fat tissue development due to anthropological adaptations. Results indicate that ultrasound method is useful for estimate regional tissue development in young population.

TITLE: PPV23 STIMULATION IMPACT ON SELECTED TLR EXPRESSION ON LYMPHOCYTES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL).

AUTHOR: Maciej J. Rutkowski

CO-AUTHORS: Anna Roszkowska, Łukasz Świerszcz, Anna Taracha, Anna Hymos

SUPERVISOR: assoc. prof. Ewelina Grywalska MD, PhD

AFFILIATION: Chair and Department of Clinical Immunology, Medical University of Lublin

Introduction: Chronic Lymphocytic Leukemia (CLL) is an example of secondary immunodeficiency. Toll-like receptors (TLR) are responsible for antigens recognition and activation of lymphocytes. TLR-2, TLR-4 and TLR-9 are particularly involved in the anti-infectious response. Leukemic lymphocytes may have altered expression of TLR and that can cause either lowered immune response or promotion of leukemic cells growth.

Aim of study: The objective of the research is the analysis of influence of PPV23 stimulation on TLR-2, TLR-4 and TLR-9 expression on mononuclear cells among patients with Chronic Lymphocytic Leukemia.

Material and methods: A study group of 15 subjects with CLL, with an average age of 64.9±9.1 years, patients of Immunology Dept. at Clinical Hospital No. 4 in Lublin was recruited. 10 of them was in stage 0 and 5 of them in stage 1 in Rai classification. None of them was taking immunosuppressive, immunomodulative or steroid treatment within the last 2 months and complained of ailments characteristic of the current infection. Blood sample (30 ml) taken from basilica vein into EDTA tubes was research material. Assessment of immunophenotype and isolation was performed immediately. Three-colour immunofluorescence analyses were performed using a FACS Calibur flow cytometer (Becton Dickinson) equipped with 488 nm argon laser. Statistical analysis was performed using Statistica 10.0 (Stat Soft Inc.) software. The Local Ethical Committee at the Medical University of Lublin approved the research and patients gave their prior written consent.

Results: There was significant lower amount of T CD3+TLR2+ lymphocyte after PPV23 stimulation after 72h (p=0.013), significant lower amount of B CD19+TLR2+ cells after 48h (p=0.008) and 72h (p=0.005) compared to non-stimulated cells. Lower amount of T CD3+TLR4+ after 24h (p=0.011) and 72h (p=0.004), B CD19+TLR4+ after 24h (p=0.005), 48h (p=0.01) and 72h (p=0.005) was observed. There was no significant differences in T CD3+TLR9+ and CD19+TLR9+ after stimulation.

Conclusions: Antigen stimulation lead to decrease number of T CD3+TLR2+ and T CD3+ TLR4+ compared to non-stimulated cells. This may suggest the stimulation of the immune response which is manifested by receptors internalization. Lowered amount of B CD19+TLR2+ and B CD19+TLR4+ may suggest PPV23 influence on those cells, however, it is smaller than in the case of T cells.

TITLE: PSYCHOACTIVE SUBSTANCE INTOXICATION AS A INCREASING PROBLEM IN LUBLIN PROVINCE IN A LAST YEARS.

AUTHOR: Jędrzej Tkaczyk

CO-AUTHORS: Klaudia Brożyna, Krystian Ciechański, Erwin Ciechański

SUPERVISOR: Michał Tchórz MD

AFFILIATION: Chair and Department of Toxicology and Cardiology, Medical University of Lublin

Introduction: Psychoactive drug, is a chemical substance that changes brain function and results in alterations in perception, mood, or consciousness. Psychoactive substances often bring about subjective changes in consciousness and mood that the user may find rewarding and pleasant or advantageous and are thus reinforcing. Substances which are both rewarding and positively reinforcing have the potential to induce a state of addiction. Number of hospitalizations after the use of these substances shows, how dangerous they can affect our organism. Especially dangerous, may be so called 'desinger drug' - a structural or functional analog of a controlled substance that has been designed to mimic the pharmacological effects of the original drug, while avoiding classification as illegal and/or detection in standard drug tests.

Aim of study: Aim of the study is to analyse tendency in a number of hospitalizations caused by psychoactive substances intoxication at the Department of Toxicology and Cardiology in Lublin in last years.

Material and methods: Data comes from yearly reports, made by the Department of Toxicology and Cardiology in Lublin.

Results: In last years, number of hospitalizations, after the use of psychoactive substances increased. In a year 2013 doctors at the Department of Toxicology and Cardiology had to deal with 133 cases of intoxication. In a 2016, total number of hospitalizations was 233. The most cases were registered in a year 2015 – 258 hospitalizations. Most of the patients were men – in a year 2016 190 patients were men and only 40 women. Percentage of men varied from 69 % in a year 2013 to 82 % in 2016. The percentage of a woman had decreasing tendency. Average age of a hospitalized patient had increasing tendency – in 2013 it stood at 24,3 and in 2016 at 25,7. Fortunately, percentage of underaged patients decreased from 31 % in 2013 to 7,8 % in 2016. The dominating age group was 8-25 years (45 to 51%) The age group in which we observed the biggest increasing tendency was 26-40 years (from 26 % in 2013 to 41 % in 2016).

Conclusions: Psychoactive substance intoxication is an increasing problem in our region. In a four years number of cases almost doubled. The substances that cause intoxication are 'typical' drugs, such as amphetamine, as well as 'designer drugs'. Designer drugs are a serious problem, because doctors don't know which substances contains a drug. There is no specific antidote, and the treatment is symptomatic. Positive conclusion from this study is that percentage of a underaged patients decreased in last years. Very important goal for the next years is to decrease total numbers of intoxications.

TITLE: THE INFLUENCE OF METFORMIN, AN ANTIDIABETIC DRUG, ON THE ANTICONVULSANT EFFECT OF VALPROATE IN PSYCHOMOTOR SEIZURE TEST IN MICE.

AUTHOR: Katarzyna Ligęza

CO-AUTHORS: Paweł Gryta, Paulina Chmielewska, Urszula Grudziń

SUPERVISOR: prof. Stanisław Jerzy Czuczwar MD, PhD, Barbara Miziak MD, PhD

AFFILIATION: Students Scientific Club of Pathophysiology, Medical University in Lublin

Introduction: Epilepsy is a chronic neurological disorder characterized by recurrent epileptic seizures. Seizure result from the excessive activity of neurons in the cerebral cortex. The causes of epilepsy can be different, usually have a structural, metabolic or genetic background. Furthermore, an oxidative stress is likely implicated in the initiation and progression of epilepsy. Around 50 million people are suffering from epilepsy in the world and about 400 thousand - in Poland. In spite of many modern drugs introduced into treatment of epilepsy, there are about 30-40% of patients whose epileptic seizures are not satisfactorily controlled. Consequently, experimental research is aimed at finding substances which are able to inhibit epileptogenesis. Metformin is a biguanide derivative, which is widely used in the treatment of type 2 diabetes mellitus. The recent data show that action of metformin is associated with AMP-activated protein kinase (AMPK). The expression level of AMPK is decreased in the brain tissue after acute and chronic seizures. There are also experimental data confirming that metformin has antioxidant properties. Therefore, this substance has received considerable attention in epilepsy treatment. Valproate is a first-generation antiepileptic drug. Valproate has a broad spectrum of anticonvulsant activity, although it is primarily used as a first-line treatment for many types of seizures. The mechanisms of actions are: a blockade of voltage-gated sodium channels, inhibition calcium T-channels and increase in brain levels of gamma-aminobutyric acid (GABA).

Aim of study: The aim of the present study was to evaluate the impact of metformin on the anticonvulsant activity of valproate.

Material and methods: This experiment was carried out on Swiss male mice. The rodents were divided into 2 groups. Both of them received valproate 30 minutes before the test. The experimental group additionally received metformin in a dose of 150 mg/kg, 60 minutes before the test. Metformin dose used in this study was determined in the convulsive threshold test. All drugs were administered intraperitoneally. After drug application, the psychomotor seizure test was carried out in mice. The seizure activity was induced by the stimulus delivered via corneal electrodes.

Results: Metformin in a dose of 150 mg/kg in combination with valproate led to the slight decrease of its anticonvulsant activity. However, this results was not statistically significant.

Conclusions: The results obtained indicate that metformin did not modulate the anticonvulsant effect of valproate. Thus, metformin can be used safely in the treatment of diabetes mellitus type 2 in patients with epilepsy, receiving valproate.

TITLE: QUANTITATIVE ASSESSMENT OF METAMORPHOPSIA USING MCHARTS IN PATIENTS WITH CENTRAL SEROUS CHORIORETINOPATHY.

AUTHOR: Agata Pietras

CO-AUTHORS:

SUPERVISOR: prof. Robert Rejdak MD, PhD, assoc. prof. Katarzyna Nowomiejska, Dominika Nowakowska MD, PhD

AFFILIATION: Department of General Ophthalmology, Medical University of Lublin

Introduction: Central serous retinopathy (CSR), is an eye disease which causes visual impairment, it is mostly temporary, usually in one eye. When active it is characterized by leakage of fluid under the retina, especially in macular area. The prognosis is good, most patients regain vision within 6 months, however reduced visual acuity may persist after the fluid has disappeared.

Aim of study: Quantitative assessment of metamorphopsia using M-charts in patients with central serous chorioretinopathy, based on 4 month study.

Material and methods: Studied group contains 19 patients. The average age was 31-years. Each patient had the following examinations performed: visual acuity for distance and near vision, M-charts – used vertically and horizontally and optical coherence tomography (OCT). All tests were carried out 3 times: immediately after first symptoms, after one month and after three consecutive months.

Results: Considerable improvement in the degree of metamorphopsia in both directions - the horizontal (0.45 to 0.26) and vertical (0.43 to 0.25) was noticed between the first and last examination. There has also been a significant reduction in retinal thickness of 386 to 290 micrometers.

Conclusions: 'M-charts' is the simple examination method used for metamorphopsia assessment in macular diseases. They can be used in the follow-up of patients with central serous retinopathy over a long period of time.

TITLE: Tγδ LYMPHOCYTES IN MULTIPLE SCLEROSIS – PRELIMINARY RESULTS

AUTHOR: Michał Zarobkiewicz

CO-AUTHORS: Wioletta Kowalska, Paweł Halczuk

SUPERVISOR: Agnieszka Bojarska-Junak MD, PhD¹, prof. Barbara Jodłowska-Jędrych MD, PhD²

AFFILIATION: ¹ Chair and Department of Clinical Immunology, Medical University of Lublin

² Chair and Department of Histology and Embryology with Experimental Cytology Unit, Medical University of Lublin

³ Chair and Clinic of Neurology, Medical University of Lublin

Introduction. Multiple sclerosis is a chronic inflammatory and demyelinating disease of central nervous system. Despite years of scientific efforts undertaken worldwide its pathology is still a mystery. Tγδ lymphocytes were found to be somehow involved in immunopathogenesis of multiple sclerosis, their potential in IL-17 (a primary cytokine linked to autoimmunity) production during disease course is unknown.

Aim. Assessment of Tγδ percentage and IL-17 production by Tγδ during multiple sclerosis.

Material and methods. 4 patients with relapsing-remitting multiple sclerosis and 6 healthy controls voluntarily donated peripheral blood sample. Samples were stained with anti-human TCRγδ FITC, CD3 PE-Cy5, RORγT PE, IL-17 PE and IL-23R PE antibodies and afterwards analysed by flow cytometry. This study utilised a standard, whole-blood assay with erythrocyte cell lysis for preparation of the peripheral blood specimens. Statistica 12 was used for statistical analysis.

Results. Tγδ comprised $3.03 \pm 1.64\%$ of total T lymphocytes in experimental while $5.16 \pm 2.60\%$ in control group. Among Tγδ $3.03 \pm 3.94\%$ expressed IL-17, $24.60 \pm 22.33\%$ IL-23R and $13.60 \pm 15.12\%$ RORγT while in control group it was respectively $3.87 \pm 6.28\%$, $3.19 \pm 1.33\%$ and $4.50 \pm 5.42\%$. Only expression of IL-23R differed significantly among groups with $p=0.02$ (U-Mann Whitney test).

Conclusions. Preliminary results indicate differences in functional state of Tγδ lymphocytes during multiple sclerosis. A larger number of samples is needed for definitive conclusion.