Setor 10. Câncer e Proliferação Celular/Cancer and Cell Proliferation

10.001

Evaluation of antitumor activity of crude extract of *Turnera ulmifolia L*. and interaction in biodistribution of technetium-99M. Barros ALS[1], Castro KC[1], Santiago-Sitonio MM[1], Moura SL[1], Silva TG[1], Catanho MTJA[2] [1]UFPE - Antibióticos, [2]UFPE - Biofísica e Radiobiologia

Introduction: The use of natural products increasing in the world in recent decades, because, has a great credibility as a result of the pharmaceuticals researches. The *Turnera ulmifolia L*. is a plant found in various parts of the world, in Brazil, it is known as *chanana* or *albina*. According to popular medicine it can be used to treat many kinds of diseases and especially in combating gastric ulcers. Phytochemicals tests showed the presence of flavonoids C-glucosyl in this plant. The aim of this work studying the antitumoral activity of aqueous extract of *Turnera ulmifolia L*. and the analysis of biodistribution of the technetium-99m in the treated animals. **Methods:** All procedures were approved by CEEA-UFPE (number of process: 008397/2007-84). To carry out the activity antitumor were used six mice by group, totaling three groups (50, 100mg/Kg weight and control). The implantation of the tumor mass was conducted through the transplant of sarcoma 180 of an animal donor. The tumor in ascetic form, was introduced in the hypoderm of the axillary region of the animals receiving in a concentration of 5 x 10^5 cells/mL. The treatment began 24 hours after the implantation of the tumors and the daily doses of the extract were administered by the intraperitoneal route for seven days, for the groups treated, while the control groups received saline 0.9% during the same period of time. The animals were sacrificed on the eighth day and the tumors were removed and weighed to obtain the rate of tumoral inhibition. In the study of biodistribution, were used two groups with six mice, a control group (0.9% saline) and a group treated with daily doses of *Turnera ulmifolia L.* for seven consecutive days. In the 8th day was 0.1mL of 99mTc administered intravenously (vein flow). After 30 minutes of the administration of technetium-99m, the animals were sacrificed and their organ and blood collected to verify the level of radioactivity. **Results and Discussion:** The analysis of the results, by the calculations of percentage of tumoral inhibition, showed that the *Turnera ulmifolia L*. in the concentrations of 100 and 50 mg/Kg of weight have respectively 70% and 21% of inhibitory activity on tumor growth when compared to the control group with $p \leq 0,05$ of statistical significance. The results concerning the biodistribution demonstrated that most of the organs had a decrease in capture of 99mTc significantly ($p \leq 0.05$), stand out among them the heart of 1.08 to 0.49, the stomach of 9.10 to 1,99, the liver of 1.72 to 0.58 and the right kidney of 1.51 to 0.94, and the muscle of 0.38 to 0.20. In the intestine the percentage of radioactivity increased significantly ($p \leq 0.05$) from 0.25 to 0.61. From these experiments that the crude extract of T. ulmifolia L. presents activity antitumoral in the dose of 100mg/kg, this action is probably related to presence of flavonoids C-glucosyl. The extract also modifies the transport of the 99mTc, probably for an action chelant of the extract or competition for specific connection sites in the biodistribution process, showing a reduced percentage of captation in most of the studied organs. **Funding**: CNPq

10.002

Biodistribuição do radiofármaco pertecnetato de sódio ($NA^{99M}TCO_4$) em camundongos portadores de carcinoma de Ehrlich. Castro KC[1], Barros ALS[1], Moura SL[1], Sitonio MM[1], Silva TG[1], Catanho MTJA[2] [1]UFPE - Antibióticos, [2]UFPE - Biofísica e Radiobiologia

Introdução: O tecnécio-99m (Tc-99m) tem sido um radionuclídeo com aplicação em muitos procedimentos clínicos da medicina nuclear. O elevado índice de utilização deste radionuclídio se deve as suas propriedades físicas e químicas ideais, tais como: meia-vida física de 6,01 horas; decaimento por emissão de radiação gama pura, fótons com energia de 140 keV e a praticidade da obtenção a partir de um sistema gerador de molibdênio-99/tecnécio-99m na forma de pertecnetato de sódio ($Na^{99m}TcO_4$). As características físico-químicas do radiofármaco determinam a sua farmacocinética, enquanto que as características físicas do radionuclídeo determinam a aplicação do composto em diagnóstico ou terapia. A própria solução de pertecnetato de sódio eluída do gerador constitui-se em um radiofármaco que se liga inicialmente às proteínas plasmáticas e tem maior afinidade por glândulas salivares, tireóide, estômago e rins, sendo 30% eliminado por via renal nas primeiras 24 horas. O $Na^{99m}TcO_4$ pode ser também utilizado em estudos de fluxo sanguíneo e pesquisas de sangramento oculto, entretanto, a grande utilidade deste composto está no seu uso na marcação de moléculas, resultando em diversos radiofármacos com especificidade por diferentes órgãos e sistemas do organismo. O objetivo desse trabalho foi avaliar a biodistribuição e captação do pertecnetato de sódio em animais sadios e portadores de carcinoma de Ehrlich. **Métodos:** Os procedimentos experimentais apresentados neste trabalho de pesquisa foram submetidos e aprovados pelo Comitê de Ética e Experimentação Animal da Universidade Federal de Pernambuco (n° do processo: 23076.002926/2009-06). Foram utilizadas células tumorais malignas (Carcinoma de Ehrlich) de animais portadores do tumor com 8 dias de implantação. Os animais doadores foram anestesiados para aspiração tumoral e, o tumor na forma ascítica, foi introduzido nos animais receptores numa concentração de 5×10^5 células/mL. Para o experimento foram utilizados 4 grupos de animais divididos em dois momentos experimentais (24h e 8 dias após o implante). A solução de pertecnetato de sódio foi administrada em grupos de animais sadios e portadores do tumor (0,1mL por via intravenosa) e após 30 minutos da administração os animais foram sacrificados e tiveram seus órgãos e uma amostra de sangue coletados para verificar o nível de radioatividade. **Resultados e Discussão:** Os resultados referentes à biodistribuição 24h após o implante do tumor não mostrou alterações significativas na captação tecidual do pertecnetato de sódio quando comparados a animais sadios. No estudo da biodistribuição 8 dias após o implante a captação tecidual no grupo de animais portadores do carcinoma de Ehrlich reduziu significativamente ($p \leq 0,05$) no sangue (de 0,7 ATI/g para 0,4), estômago (de 2,03ATI/g para 0,21), baço (de 0,16 ATI/g para 0,06), rins (de 0,3 ATI/g para 0,2), intestino delgado (de 0,29 ATI/g para 0,04) e tireóide (de 2,24 ATI/g para 0,05). As modificações da distribuição tecidual do radiofármaco observada nos resultados demonstram ter ligação direta com alterações no fluxo sanguíneo local e distúrbios metabólicos em inúmeros tecidos, característicos em estados avançados do câncer. Com base nos nossos resultados experimentais concluímos que o carcinoma de Ehrlich modifica a biodistribuição e captação do tecnécio-99m. **Apoio financeiro**: CNPq

10.003

Induction of apoptosis and cell cycle arrest in L1210 murine lymphoblastic leukemia cells by (2*E*)-3-(2-naphthyl)-1-(3'-methoxy-4'-hydroxy-phenyl)-2-propen-1-one. Pedrini FS[1], Chiaradia LD[2], Licínio MA[1], Moraes ACR[1], Curta JC[1], Costa A[1], Mascarello A[2], Pasa TBC[3], Nunes R[2], Yunes RA[2], Santos da Silva MC[1] [1]UFSC - Análises Clínicas, [2]UFSC - Química, [3]UFSC - Ciências Farmacêuticas

Introduction: Acute lymphoblastic leukemia is a malignant disorder resulting from a clonal proliferation of T or B cells progenitors. Approximately 80% of all children and 40% of adults with LLA present complete remission after therapy. Chalcones are precursors of flavonoids in higher plants and display a wide variety of pharmacological effects. Changes in chalcones molecules can result in different biological activities. New compounds with biological targets and smaller cytotoxicity to normal cells are necessary for the therapy against cancer. The aim of the present work was to investigate the cytotoxicity of ten synthetic chalcones derived from 2-naftaldehyde in murine acute lymphoblastic leukemia cells. **Methods:** Murine L1210 acute lymphoblastic leukemia cells were cultured in DMEM supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin and 10 mM HEPES, pH 7.4 at 37^{o}C in a 5% CO_2 humidified atmosphere. The cells were incubated with the compounds from 10 to 100 µM, for 12 to 48 hours, depending on the experiment procedures. Cell viability was assessed using MTT (3-(4,5-dimethiazol-zyl)-2-5-diphenyltetrazolium bromide) assay. The induction of apoptosis was assessed by the exposition of phosphatidylserine (PS) (ANNEXIN V-FITC®). The analysis of cell cycle phase was carried out by flow cytometry after propidium iodide staining. The analysis on the expression of p53, Bcl-2 and Bax was performed in flow cytometry FACSCalibur TM BD (Becton Dickinson Immunocytometry Systems) using the BD CELLQuestTM software for data acquisition. Immunoblotting analysis was performed to evaluate the expression of caspase-3. **Results and Discussion:** A preliminary screening of chalcones serie showed that chalcone **8** have the highest cytotoxic effect (IC_{50} of 54µM). The analysis of the exposure of fosfatidilserina by Anexina V-FITC showed that the chalcone 8 induced cell death by apoptosis. To investigate the mechanism of apoptosis, the cell cycle and p53, Bcl-2, Bax and caspase 3 expression was evaluated. The results showed that chalcones 8 increased p53 expression; involved mitochondrial apoptosis pathway, reducing the expression of Bcl-2 and increasing the expression of Bax; increasing the expression of active caspase 3 and blocked cell cycle progression on G2/M phase. More investigations are necessary in order to understand completely which mechanisms of that chalcone induce apoptosis, however, the results obtained are promising for the development of new antineoplasic drugs. **Acknowledgements**: ACRM is recipient of a Research Fellowship from CAPES (Brazil) and AC from FAPESC.

10.004

Evaluation of the antitumoral activity from new synthetic derivatives from isatin in ascitic tumor of Ehrlich. Zardo RS[1], Silva BV[2], Andrade SYR[1], Pinheiro MMG[1], Garden SJ[2], Violante FA[2], Matheus ME[1], Pinto AC[2], Fernandes PD[1] [1]UFRJ - Farmacologia Básica e Clínica, [2]UFRJ - Instituto de Química

Introduction: Isatin (1H-indole-2,3-dione) is a versatile molecule that present several biological activities, including anti-proliferative and apoptotic effect. It synthetic flexibility permits the synthesis of a great variety of derivatives. Ehrlich ascitic tumor (EAT) is a tumor derived from murine mammary adenocarcinome, with aggressive and rapid growth. The objective of this work was to evaluate an antitumoral activity of new isatins derivatives in a EAT. **Methods**: 0.5 x 10^6 EAT were intraperitonealy injected into Swiss mice (20-25g, n=5-8, ethical committee license #DFBC015). After 24h and during 9 subsequent days, mice received oral administration of vehicle (DMSO/TWEEN80), isatin derivatives: ISA003 (3-(2-oxopropyl)-3-hydroxy-2-oxindole), ISA147 (5-bromo-3-(2-oxopropyl)-3-hydroxy-2-oxindole) e ISA160 (5-fluoro-3-(2-oxopropyl)-3-hydroxy-2-oxindole), and ISA162 (5-metyl)-3-(2-oxopropil)-3-hydróxi-2-oxindole) (100 mg/kg), and vincristine (0,5 mg/kg, i.p). At 10^{th} day, mice were sacrificed, ascitic liquid collected and total cell count was performed in ascitic liquid (AL), blood (B), and medulla lavage (ML). Results are expressed as media ±DP. Statistical analyses was performed by ANOVA and Bonferroni's test (*p<0.001). **Results**: Oral administration of vehicle results in 9.5±0.2X10^7; 5.1±0.24; and 2.3±0.3 cell/mL in AL, B and ML, respectively. Vincristine reduced the counts to the values of: 0.6±0.09; 0.8±0.6; and 0.6±0.2 cell/mL, respectively. ISA003 inhibited total cells counts from AL in 36.8% (6.8±0.5*x10^7cell/mL); from B in 44.4% (2.5±0.1*x10^7cell/mL cell/mL), and ML from 36.2% (1.3±0.3*x10^7cell/mL cell/mL). ISA147 inhibited total cells counts from AL in 47.8% (5.0±0.2*x10^7cell/mL); from B in 65.3% (1.6±0.3*x10^7cell/mL cell/mL), and ML from 38.1% (1.3±0.3*x10^7cell/mL cell/mL). ISA160 inhibited total cells counts from AL in 43.2% (5.4±0.3*x10^7cell/mL); from B in 82.2% (0.8±0.1*x10^7cell/mL cell/mL), and ML from 81.9% (0.4±0.01* x10^7cell/mL cell/mL). ISA162 inhibited total cells counts from AL in 40.4% (6.6±0.3*x10^7cell/mL); from B in 82% (0.76±0.05* x10^7cell/mL cell/mL), and ML from 80.9% (0.4±0.04* cell/mL). **Discussion:** The new derivatives of isatin testes in this work demonstrated a significant antitumoral effect even when administered by oral gavage, in the ascitic tumor of Ehrlich. **Financial support:** CAPES, CNPq, and FAPERJ.

10.005

Evaluation of the antiproliferative activity from new synthetic derivatives from isatin. Zardo RS[1], Silva BV[2], Andrade SYR[1], Garden SJ[2], Violante FA[2], Matheus ME[1], Pinto AC[2], Fernandes PD[1] [1]UFRJ - Farmacologia Básica e Clínica, [2]UFRJ - Química

Introduction: Isatin (1H-indole-2,3-dione) is a versatile molecule that present several biological activities, including anti-proliferative and apoptotic effect. It synthetic flexibility permits the synthesis of a great variety of derivatives. Ehrlich ascitic tumor (EA) is a tumor derived from murine mammary adenocarcinome, with aggressive and rapid growth. The objective of this work was to evaluate the antiproliferative activity of new isatins derivatives in Ehrlich tumor cells *in vitro*. **Methods:** EA cells (EAC) were collected from male Swiss mice (25-28g), counted, plated at the density of 10^6 cells/mL in RPMI/10%fetal bovine serum, and incubated at 37^0C/5%CO_2. Cells were incubated with vehicle (DMSO/RPMI), isatin derivatives: ISA003 (3-(2-oxopropyl)-3-hydroxy-2-oxindole), ISA147 (5-bromo-3-(2-oxopropyl)-3-hydroxy-2-oxindole) and ISA160 (5-fluoro-3-(2-oxopropyl)-3-hydroxy-2-oxindole), and ISA162 (5-metyl)-3-(2-oxopropil)-3-hydroxi-2-oxindole) at 100 µM. After 6 and 12 h an aliquot was collected and cell count was performed by Trypan blue exclusion method (n=4, each one in triplicate). Results are expressed as media±DP. Statistical analyses was performed by ANOVA and Bonferroni's test (*p<0.001). **Results:** After 6 and 12h EAC growth in 121% and 93.5%, reaching 2.4±0.1x10^6 cell/mL and 1.9±0.05 x10^6 cell/mL, respectively. Incubation with ISA003 significantly reduced cellular proliferation in 8.3% (2.2±0.08 x10^6 cell/mL) and 2.1% (1.83±0.05 x10^6 cell/mL), for 6 and 12 hours, respectively. ISA147 reduced cellular proliferation in 40.4% (1.43±0.05*x10^6cell/mL) and 41.2% (1.1±0.08* x10^6 cell/mL). ISA160 reduced cellular proliferation in 1.23% (2.4±0.16 x10^6 cell/mL) and 25.1% (1.4±0.08*x10^6 cell/mL), and ISA162 reduced cellular proliferation in 31.3% (1.67±0.05* x10^6 cell/mL) and 15.2% (1.47±0.21x10^6cell/mL). **Discussion:** The new derivatives of isatin (ISA 147, 160, and 162) demonstrated the capacity to significantly reduce the proliferative activity of Ehrlich cells *in vitro* indicating that this isatins presents antiproliferative activity. **Financial support:** CAPES, CNPq, and FAPERJ.

10.006

Efeitos de diferentes chalconas derivadas da quinoxalina sobre a proliferação de células da linhagem C6 de glioma de rato. Mielcke,T.R.[1], Mascarello A[2], Calixto JB[3], Leal PC[4], Yunes RA[2], Battastini AMO[5], Morrone FB[6], Campos MM[8] [1]PUCRS - Farmacologia, [2]UFSC - Química, [3]UFSC - Farmacologia, [4]UFSC - QMC/CFM, [5]UFRGS - Bioquímica, [6]PUCRS - Farmácia, [8]PUCRS - Cirurgia-Odontologia

Introdução: O glioblastoma multiforme é um dos tipos de tumores cerebrais primários que mais comumente afetam adultos, apresentando um mau prognóstico. Os gliomas malignos recorrem cedo, levando a uma sobrevida média de menos de um ano (Holland, Nature, 2: 120, 2001). As chalconas são compostos precursores da via de biossíntese dos flavonóides, caracterizados por possuírem diversas atividades biológicas e farmacológicas (Ducki, IDrugs 10: 42, 2007; Kontogiorgis, Mini Rev Med Chem. 8: 1224, 2008). Considerando-se a gravidade dos gliomas e as poucas opções terapêuticas disponíveis, a identificação de novas alternativas de tratamento constitui um tema de grande relevância. Desta forma, o presente estudo teve por objetivo avaliar o efeito anti-proliferativo de uma série de chalconas derivadas da quinoxalina sobre linhagem de glioma C6 de ratos. **Métodos:** As células de glioma da linhagem C6 de rato foram cultivadas em garrafas com meio de cultura DMEM, suplementado com 5 % de soro fetal bovino (SFB). Após atingirem a confluência, as células foram semeadas em placas de 96 poços, na densidade de 1 x 10^3 células/poço (ensaio colorimétrico) ou em placas de 24 poços com densidade de 5 x 10^3 (contagem celular). Os efeitos da incubação de 9 chalconas diferentes derivadas da quinoxalina, denominadas de N2, N3, N4, N5, N7, N6, N9, N10 e N12, foram testados. Nos grupos-controle foi utilizado DMSO 0,01 %. Os experimentos foram repetidos 3 vezes e realizados em triplicata. As células foram incubadas a 37º C na presença das diferentes chalconas (0.1 µg/m a 10 µg/mL), durante um período de 48 h. A proliferação foi medida através da contagem celular em Câmara de Neubauer ou, indiretamente, através do método colorimétrico do 3-(4,5-Dimetiltiazol-2-il)-2,5-difeniltetrazólio brometo (MTT), para determinação da viabilidade celular. **Resultados:** O tratamento com as chalconas N2, N9, N10 e N12 produziu uma redução significativa e concentração-dependente da proliferação celular. Na contagem em câmara de Neubauer, as inibições máximas (Imax), observadas na concentração de 5 mg/ml, foram 90 ± 4 %, 85 ± 8 %, 82 ± 6 % e 67 ± 8 %, respectivamente. Além disso, no ensaio de MTT, as Imax observadas foram 47 ± 3 %, 50 ± 7 %, 49 ± 7 % e 48 ± 7 %, respectivamente. **Discussão:** Os resultados demonstram que as chalconas derivadas da quinoxalina, que apresentam o radical (-OCH_3) em posições alternadas, possuem um efeito marcante sobre a proliferação de células de glioma da linhagem C6 de rato. Este grupo de compostos poderia representar uma nova alternativa para o tratamento do glioblastoma multiforme. Estudos adicionais estão sendo desenvolvidos a fim de caracterizar os mecanismos de ação destas chalconas. **Apoio Financeiro:** CNPq, BPA-PUCRS.

10.007

Antineoplastic and antioxidant effects of a hydroalcoholic extract of Cat's Claw (*Uncaria tomentosa*) (Willd. Ex Roem. & Schult.). Dreifuss A[1], Bastos-Pereira AL[2], Ávila TV[2], Soley BS[2], Christoff AO[2], Acco A[2], Aguilar JL[1] [1]UPCH - Inmunología, [2]UFPR - Farmacologia

Introduction: Various properties of the amazonic vine *Uncaria tomentosa* (UT), commonly known as Cat's Claw, have been studied, especially as an anti-inflammatory, antioxidant and immunostimulant agent. Recent *in vitro* studies have also demonstrated a strong antineoplastic potential. The objective of the present work was to investigate the antineoplastic, antitoxic and antioxidant activity of an UT hydroalcoholic extract in the Walker-256 animal cancer model. **Methods:** All procedures were approved by both Universities' Ethics Committees registered with codes Nº 324 (UFPR) and 53973 (UPCH). Walker-256 cells were inoculated subcutaneously in the pelvic limb of male Wistar rats (10^7 cells/ rat), and treatment with UT extract or distilled water for the Control Group (*C*) commenced subsequently until 14 days afterwards. For some parameters, an additional group called Baseline (*B*) was added, which was composed of individuals not inoculated with tumor and treated only with distilled water. The hydroalcoholic extract of UT was administered daily by gavage in doses of 10, 50 and 100 $mg.kg^{-1}$. At the end of treatment, blood samples were collected for the determination of plasma urea concentrations and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LDH). Immediately after, animals underwent euthanasia and tumors were removed, as well as liver samples. Tumors were weighed and their volume calculated. The activity of enzymes catalase (CAT) and superoxide dismutase (SOD), as well as the lipid peroxidation rate (LPO), were measured both in liver and tumor samples. Additionally, glutathione-S-transferase (GST) activity was evaluated in the liver samples. The reactivity of the UT extract with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed *in vitro*. **Results and Discussion:** Treatment with UT successfully reduced tumor weight, while also reducing tumor volume by 46, 58 and 64% for the doses 10, 50 and 100 $mg.kg^{-1}$ respectively, compared to *C* Group. Treatment with all UT studied doses did not lead to any significant changes in AST levels, although it reduced the activity of ALT, which had been increased as a result of tumor inoculation, thus attempting to return it to normal levels (*B* Group). It did not reverse the increase of LDH and GGT plasma levels, however all doses of UT were remarkably effective in reducing urea plasma levels. An important free radical–scavenging activity was detected at various doses (1 to 300 $\mu g.mL^{-1}$) in the DPPH assay, confirming previous studies that had identified UT's antioxidant properties. Treatment also resulted in an increased CAT activity in the liver while decreasing it in tumor tissue, compared to *C* Group. As regards SOD, its activity was reduced in liver as well as tumor tissue, compared to *C* Group. No statistically significant differences concerning the activity of both GST and LPO, and GSH rates, were observed. Hence, the antioxidant and anti-proliferative potential of UT is confirmed, both *in vitro* and *in vivo* cancer model, associating the antitumoral activity to a modulation of the cellular oxidative mechanisms. **Financial support:** *PROF* (Programa de Pós-Graduação em Farmacologia - UFPR)

10.008

Antitumor property of proteolytic latex fraction from *Carica candamarcensis*, cytotoxic effect and cell migration. Dittz D[1], Viana CTR[1], Salas CE[2], Lopes MTP[1] [1]UFMG - Farmacologia, [2]UFMG - Bioquímica e Imunologia

Introduction: Recent studies of our research group demonstrated that P1G10, a fraction composed by cysteine proteases from the latex of Carica candamarcensis after column chromatography on Sephadex G10, has antitumor/antimetastatic activities on murine melanoma on B16-F1 and B16 - F10 models. The effects were evidenced by the reduction of mass tumor and decrease in the number of metastases (Figueiredo, C. et al. *39º Cong Bras Farm Ter exp. 2007*, 09119, Ribeirão Preto, Brazil.). Since then, we have evaluated the P1G10 antitumor activity in a murine cancer model (colon carcinoma-CT26.WT) as well as the fraction´s effect on tumor cells migration (CT26.WT and B16 F10) *in vitro*, as part of the study of P1G10 mode of action. **Methods and Results:** In the carcinoma tumor model (approved by CETEA/UFMG, protocol n º 103/2007), BALB/c mice (n=30) were inoculated with CT26.WT cells ($5x10^5$ cells/animal), below the subserosa layer in the *cecum* surgically exposed. After 15 days, we started daily treatment with P1G10 in doses of 5 and 10 mg/kg or saline (control), *s. c.*, for 15 days. After this period, the animals were sacrificed and the tumors weighed. Taking the tumor mass as parameter, there was a reduction in the group treated with 5 mg/kg (0.31±0.11g, $p < \pm 0.05$, ANOVA, Bonferroni *post test*) compared with control groups (1,75±0,71 g) and 10 mg/kg (1,52±0,57 g). In determining the cytotoxic activity in vitro, cells CT26.WT sown on plates of 96 wells were exposed to P1G10 (10^{-4} to $5x10^{-6}$ g/mL) for 72 h, when cell viability was determined by the method of MTT (IC_{50} = $1.23x10^{-5}$ g/mL). To evaluate the effect of P1G10 on cell migration, 10^6cels/mL (CT26.WT or B16-10 lines) were exposed for 2h to P1G10 (10^{-7}, 10^{-6} and 10^{-5} g/mL) or control solution. Then, the migration through a collagen membrane was determined using the QCMTM 24-well Collagen-based Cell Invasion Assay kit (Chemicon®). We observed an increase in the ability of migration after treatment with P1G10 for both cell lines, although such increase is significant only in the concentration of 10^{-5} g/mL for CT26.WT, when compared to control. **Conclusion:** The proteolytic fraction has no significant cytotoxic activity *in vitro*. However, *in vivo* antitumor effect was observed at doses well tolerated. The action of P1G10 doesn't seem to be directly on the tumor, since it was not cytotoxic and facilitated cell migration. **Financial Support:** CNPq, FAPEMIG and CAPES.

10.009

Effect of thalidomide on inflammatory corneal angiogenesis. Fechine FV, Fechine-Junior JU, Azevedo EG, Moraes MEA, Moraes MO UFC - Fisiologia e Farmacologia

Introduction: Previous studies demonstrated the inhibitory effect of thalidomide on corneal angiogenesis induced by specific factors, such as VEGF (vascular endothelial growth factor) and FGF-2 (fibroblast growth factor 2). However, its activity in corneal angiogenesis induced by inflammation, a nonspecific stimulus, has not yet been determined. Thus, the aim of this study was to evaluate the effect of thalidomide on inflammatory corneal neovascularization, using a model of corneal angiogenesis and a computational system for automated quantification of the neovascular response. **Methods:** All experiments were performed in accordance with protocol (Nº 07/03) approved by the Ethical Committee in Animal Research of UFC. Sixteen male New Zealand white rabbits were submitted to a punctual cauterization in the superior periphery of the right cornea using a circular piece of filter paper, 3 mm of diameter, soaked in NaOH 1M solution. The animals were randomly allocated into three groups: Control (cornstarch, n=6), Prednisone (2 mg/kg, n=5) and Thalidomide (200 mg/kg, n=5). Drugs were administrated orally in gelatin capsules once a day using an appropriate applicator during 21 days. Evaluations were done on days 3, 6, 9, 12, 15, 18, and 21, post cauterization. During these days, digital images of the cornea were captured in a standard fashion. Angiogenic response was measured using a software which was developed specifically for this purpose (SQAN – Angiogenesis Quantification System). It calculated the following parameters: Neovascularization Area (NA), Total Vascular Length (TVL), and Number of Blood Vessels (NBV). Based on NA parameter, it was calculated the Angiogenesis Mean Rate (AMR) and the Inhibitory Effect (IE) of each treatment in relation to Control on day 21. **Results and discussion:** In this model of angiogenesis, the neovascular response observed in Control group followed a biphasic pattern: proliferation (between days 0 and 12) and maturation (from days 12 to 21). During all evaluations, the three parameters NA ($P<0.001$), TVL ($P<0.01$), and NBV ($P<0.01$) measured on Prednisone group were significantly lower than Control. Thus, Prednisone fully inhibited inflammatory corneal angiogenesis with an AMR of 0.02 ± 0.02 mm^2/day which was significantly lower ($P<0.001$) than Control (0.14 ± 0.06 mm^2/day). However, the inhibitory effect of Thalidomide, considering the three parameters, started on day 12 (NA, $P<0.05$) and remained during the days 15 (NA, $P<0.05$), 18 (NA, TVL, NBV, $P<0.05$) and 21 (NA, TVL, $P<0.01$; NBV, $P<0.05$). Its AMR (0.06 ± 0.02 mm^2/day) was significantly lower ($P<0.05$) than Control, mainly caused by the reduction of vascular growth on the second half of the experiment. The parameter IE summarized the efficacy of the tested drugs. Thus, compared to Control group, IE of Prednisone and Thalidomide was 82.89% and 47.20%, respectively. **Conclusion:** Thalidomide inhibits partially inflammatory corneal angiogenesis, mainly during the second half of angiogenic process. **Financial support:** CNPq, CAPES, FINEP, DECIT/MS, InCB.

10.010

Obesity and insulin resistance influences tumor development: metformin effects. Fonseca EAI, Oliveira MA, Tostes RCA, Carvalho MHC, Zyngier SZ, Fortes ZB ICB-USP - Farmacologia

Introduction: Epidemiological studies have associated obesity with a wide variety of cancer. The insulin resistance and the hyperinsulinaemia can be the mechanisms by which obesity induces or promotes tumorigenesis. Metformin, an antidiabetic drug, can exert an antitumoral effect with the improvement of insulin sensitivity. Therefore, the objective of this study was to analyze the influence of obesity and insulin resistance in the tumor development and, the effect of metformin on it. **Methods:** Obesity was induced in rats by monosodium glutamate (MSG). Newborn male Wistar rats were subcutaneously injected with 400mg/kg MSG (obese) or saline (control) at 2,3,4,5 and 6 days of age. After 16 weeks, $5x10^5$ Walker-256 tumor cells were subcutaneously injected in the right flank of those rats and concomitantly the treatment with metformin 300mg/kg, *via gavage* started. After that rats were divided into 4 groups: Control tumor (CT), Control tumor metformin (CTM), Obese tumor (OT) and Obese tumor metformin (OTM). On the 18^{th} week, the obesity was characterized by Lee index (body weight $^{1/3}$ (g)/naso-anal length (cm)), periepididimal and retroperitoneal adipose tissues weight and lipid profile and, the insulin sensitivity was evaluated by plasma glucose disposal rate (Kitt; %/min). The percentage of tumor incidence, tumor relative weight and the percentage of cachexia incidence were also analyzed. **Results:** The tumor incidence (OT 82*** vs CT 55%),and the tumor relative weight (OT 8,9±0,9*** vs CT 5,5±0,5 g/100g body weight) were significantly higher in the OT. Both parameters were reduced by metformin treatment (CTM 56, OTM $59^{\#\#}$% and, CTM 4,1±0,5, OTM 5,1±0,$6^{\#\#}$g/100g weight , n=16, respectively). The cachexia incidence was higher in the OT group than in the other groups and the metformin did not correct this parameter (OT 90*** vs CT 50, CTM 50, OTM 100%, n=18). Metformin did not correct the insulin resistance in OT (Kitt OT 1,95±0,18*, OTM 2,43±0,18*, CT 3,07±0,28, CTM 3,45±0,27%/min, n=8), however it did correct the dislypidemia and reduced the periepididimal and retroperitoneal adipose tissues (OT 2,4±0,11*, OTM 2,15±$0,08^{\#}$, CT 1,32±0,05, CTM 1,00±0,05* g/100g weight, n=10 and OT 2,67±0,11*, OTM 2,14±$0,13^{\#}$, CT 1,17±0,08, CTM 0,58±0,065* g/100g weight, n=10, respectively). **Discussion:** Metformin was able to reduce the incidence and Walker-256 tumor development but not cachexia in MSG obese rats. The reduction occurred independently of the correction of insulin resistance, since insulin sensitivity was not improved by metformin treatment. ***p<0,001 vs CT, *p<0,05 vs CT, $^{\#}$p<0,05 vs OT e $^{\#\#}$p<0,0001 vs OT. Nº da Licença do Comitê de Ética: 007/04/CEEA. Financial support: CNPq (Brazil) and FAPESP (Brazil- Project No 2008/50933-5/ 2007/58311-0).

10.011

Efeitos da mistura dos triterpenos pentacíclicos alfa,beta-amirina no carcinoma oral de células escamosas. Zilberstein ACCV[1], Romanini J[2], Calixto JB[3], Battastini AMO[4], Campos MM[5], Morrone FB[1] [1]PUCRS - Farmácia, [2]PUCRS - Estomatologia, [3]UFSC - Farmacologia, [4]UFRGS - Bioquímica, [5]PUCRS - Cirurgia-Odontologia

Introdução: O carcinoma de células escamosas é o tipo mais comum de neoplasias que acometem a cavidade bucal e representa mais de 90% dos casos (INCA, 2002; Brinkman e Wong, Curr Opin Oncol, 18, 228, 2006). Nos últimos anos, tem havido um grande interesse em produtos naturais como fonte para a descoberta de novas terapias para o tratamento do câncer. As plantas pertencentes ao gênero *Protium* são utilizadas na medicina popular para o controle de alterações inflamatórias. Análises fitoquímicas do extrato desta planta revelaram a presença de uma série de triterpenos pentacíclicos, dentre eles uma mistura de dois isômeros, denominada alfa,beta-amirina (Recio et al., Planta Medica, 61, 182, 1995). O presente estudo teve por objetivo avaliar os efeitos *in vitro* da mistura terpênica alfa,beta-amirina sobre a viabilidade da linhagem de carcinoma oral de células escamosas, SCC 158, obtida de ratos. **Métodos:** As células da linhagem SCC 158 (JCRB, Japão) foram cultivadas em meio de Eagle modificado por Dulbecco (DMEM) com 2 mM de L-glutamina, suplementado com 10 % de soro bovino fetal, 150 U/ml de penicilina e 150 µg/ml de estreptomicina, mantidas em incubadora umidificada a 37^{o}C e 5 % de CO_2. O efeito da mistura terpênica alfa,beta-amirina sobre a viabilidade celular foi avaliado através do ensaio de MTT. Decorridas 24 e 48 h da adição do composto (100 - 400 nM), o meio de cultura presente nos poços foi desprezado e substituído por 100 ml de DMEM novo contendo 10% v/v de uma solução de MTT (5 mg/ml). A seguir, as células foram incubadas por 3 h em estufa apropriada. Após este período, o meio foi novamente desprezado e os poços preenchidos com 100 ml de DMSO, para dissolução dos cristais de MTT. A absorbância foi determinada em 595 nm. **Resultados:** Os resultados parciais mostram que a incubação da mistura terpênica alfa,beta-amirina, durante 24 h, produziu uma inibição significativa da viabilidade da linhagem celular SCC 158, que foi máxima com a concentração de 400 nM (39 ± 6 %). Resultados semelhantes foram observados quando a mistura triterpênica foi incubada por 48 h, produzindo uma inibição máxima de 35 ± 3 %, na concentração de 300 nM. **Discussão:** Os resultados apresentados permitem sugerir que o composto alfa,beta-amirina é capaz de reduzir a viabilidade da linhagem SCC 158 de carcinoma oral de células escamosas de rato. Estudos adicionais estão sendo realizados para avaliar os possíveis mecanismos de ação e a eficácia do composto *in vivo*. **Apoio Financeiro:** CNPq, BPA-PUCRS.

10.012

Ação hepática do extrato hidroalcólico da *Maytenus ilicifolia* (Espinheira-Santa) no tumor Walker-256 em ratos. Moyano R[1], Avila TV[1], Bastos-Pereira AL[1], Lugarini D[1], Christoff AO[1], Kassuya CAL[1], Iacomini M[2], Fernandez F[3], Acco A[1] [1]UFPR - Farmacologia, [2]UFPR - Bioquímica, [3]Universidad Nacional de Tucumán - Fisiologia Animal

Introdução: *A Maytenus ilicifolia* (espinheira-santa) ocorre predominantemente na região Sul do Brasil, suas folhas têm sido usadas popularmente como antiinflamatório e antiulcerogênico, além de ser indicada para o tratamento de câncer. Este estudo foi proposto para avaliar o desenvolvimento tumoral em ratos tratados com extrato de *Maytenus ilicifolia* e inoculados com tumor Walker-256 (W-256), através do peso e volume tumoral, correlacionando-o com marcadores de função hepática, renal e de estresse oxidativo hepático. Ratos machos adultos pesando entre 200 e 250 g foram utilizados. **Material e métodos**: Todos os procedimentos experimentais foram aprovados pelo comitê de ética em experimentação animal da UFPR sob o número 323. A manutenção das células W-256 foi feita através de passagens semanais por inoculação intraperitoneal de 10^7 células/animal. As células foram injetadas subcutaneamente ($2x10^7$células/rato) no membro pélvico esquerdo, e 24 horas após iniciou-se o tratamento oral com veículo (grupo controle) ou extrato de *M. ilicifolia* 100 mg/kg (grupo tratado). Ao final de 14 dias de tratamento os animais tiveram o sangue coletado para pra dosar ALT, AST e LDH e uréia, além dos níveis de peroxidação lipídica hepática e SOD para avaliar a condição de estresse oxidativo. Os animais foram eutanasiados e o tumor coletado para mensuracao. A análise estatística foi realizada utilizando-se ANOVA de uma via seguido do teste Tukey para múltiplas comparações, no programa Graph Pad Prism 5.0. Diferenças foram consideradas significantes quando $p<0,05$. **Resultados**: Através dos resultados, pode-se verificar uma diminuição do volume e do peso de tumor (53,97±4,704 cm³; 16,55±3,205 g) no grupo tratado, diferindo estatisticamente do controle (149±23,59 cm³; 31,15±4,253 g). O nível enzimático de AST do grupo tratado foi estatisticamente menor (45,06±7,09 U/mL) do que o do grupo controle (76,78±8,33 U/mL) e a atividade de ALT não diferiu (25,91±5,66U/mL) do grupo controle (30,68±6,18 U/mL). O mesmo ocorreu com a LDH, sendo os valores de 206,7±49,31 U/mL para o controle e 171,1±37,27 U/mL para o grupo tratado com *M. ilicifolia*. A dosagem de uréia (42,61±5, 828 U/mL) também não diferiu estatisticamente do controle (43,61±5,634 U/mL). Os níveis de peroxidação lipídica e SOD aumentaram consideravelmente no grupo controle (3,1±0,77 e 5,326±0,466) em comparação ao grupo basal (0,770±0,404 e 1,992±0,16 respectivamente). O tratamento com *M. ilicifolia* não reverteu o aumento da peroxidação lipídica, mas diminuiu a níveis basais os valores da atividade da SOD. **Discussão**: O extrato hidroalcoólico da planta demonstrou atividade antitumoral e hepatoprotetora, pois reduziu o crescimento do tumor Walker-256 e os níveis de AST no plasma, além de diminuir os níveis de SOD. Os mecanismos responsáveis por tais efeitos merecem investigação, uma vez que podem estar relacionados à atividade antiinflamatória, apoptótica ou antioxidante de componentes ativos do extrato da *Maytenus illicifolia*, que podem ser promissores na terapia do câncer. Apoio Financeiro: CAPES e CNPq

10.013

Antineoplastic activity of *Agaricus brasiliensis* in Walker-256 tumor-bearing rats. Bastos-Pereira AL[1], Jumes FMD[1], Lugarini D[1], Ávila TV[1], Christoff AO[1], Colauto, NB[2], Acco A[1]
[1]UFPR - Farmacologia, [2]Unipar - Agronomia

Introduction *Agaricus brasiliensis* is a mushroom studied for its medicinal proprieties. This study aimed to evaluate the influence of *Agaricus brasiliensis* extracts in rats bearing the W256 tumor to verify the antineoplastic activity and physiological parameters related to hepatic function and oxidative stress. **Methods** Procedures were approved by UFPR´s Ethics Committee and registered with code number 174. The extracts were prepared with *Agaricus brasiliensis* basidiocarps (from Molecular Biology Laboratory/Unipar), washed, dehydrated, triturated and stored at -70°C. Aqueous extract solution was prepared with triturated basidiocarp diluted in ultra pure water. To prepare the acid extract solution 1.0 M HCl was added till the pH reached 4.0, and for the alkaline extract solution 1.0 M NaOH was added until the pH was 8.0. All of the end solutions had the pH adjusted to 5.4. Dried and triturated basidiocarps were prepared without extraction to compare the efficiency of the solid particles. All solutions and solid particles were stored at -20°C. The maintenance of W256 cells was carried out by weekly intraperitoneal (IP) inoculation. W256 carcinoma cells were injected subcutaneously ($2´10^7$ cells), suspended in phosphate-buffered saline pH 7.4 to be injected into the right flank of the tumor-bearing rats. The rats (n=6) were divided into five groups, namely: Control: saline solution; Epure: saline solution added with pure mushroom powder (1:10); Eaque: mushroom aqueous extract; Eacid: acid extract from the mushroom powder; and Ealka: alkaline extract from the mushroom powder. The oral treatment started one day after cell implant, and continued for 14 days, at a dose of 136 mg.kg-1 in all groups. The body mass of the rats was evaluated during the treatment. On the last day of treatment the rats suffered anesthesia (thiopental 70 $mg.kg^{-1}$). The blood was collected for plasmatic glucose, albumin, ALT and AST determinations. Right after the blood collection, euthanasia was induced. The tumor was collected and weighed. Tumoral volume and inhibition growth rate were calculated. Enzymes related to oxidative stress (catalase, superoxide-dismutase, glutathione-S-transferase) were evaluated, using tumor and liver samples. Mean values ± standard error (SE) were calculated. Data were analyzed statistically by ANOVA and Tukey test for comparison of averages, significant when $P<0.05$. **Results and Discussion** All rats treated with the extract showed an increase in the body mass throughout the treatment, excepting the control group (-14,17 ± 24,8 g). Increase in body mass was less evident in the Epure group (32,65 ± 8,9 g), while the highest increase occurred in the Ealka group (54,69 ± 7,9 g). All the extracts significantly reduced the tumor growth when compared with the control group. The greater inhibition (92.02%) occurred in the rats treated with pure extract, while the minor rate was observed in the group treated with extract acid (74.18%). Plasmatic analysis showed a reduction in AST level and increased glycemia in the treated rats. Treatment with *A. brasiliensis* pure extract increased catalase activity in two-fold (123.7±15.37 µmol/min.mg $protein^{-1}$) compared with control rats (60.76±14.32 µmol/min.mg $protein^{-1}$). The treatment also elevated hepatic SOD (1.96 ± 0.14 UN SOD.mg $protein^{-1}$), raising it to the basal level (1.99 ± 0.16 UN SOD.mg $protein^{-1}$), while in the control group it was expressively reduced (1.58 ± 0.04 UN SOD.mg $protein^{-1}$). The data collected from the W256 tumor revealed the beneficial effects of *A. brasiliensis* in tumor treatment, an effect which could be partly related to antioxidant activity, reducing weight loss and tumor growth. **Financial support**: PROF (Pós-Graduação em Farmacologia)

10.014

Uso de painel de células tumorais humanas para triagem de frações ativas de *Psidium guajava* L. (nome popular: goiaba). Rizzo, LY[1], Ruiz AL[2], Tinti SV[3], Longato GB[4], Foglio M[5], Carvalho JE[3] [1]CPQBA-UNICAMP - Farmacologia e Toxicologia, [2]CPQBA-UNICAMP - Farmacologia, [3]CPQBA-UNICAMP, [4]UNICAMP - Biologia, [5]CPQBA - Fitoquímica

About 50% of currently used chemotherapics have some sort of origin from natural products. Nature continues to be the most important source of biologically active and diverse molecules, and the essential role played by natural products in the discovery and development of effective anticancer agents, together with the multidisciplinary collaboration for the creation of active leads cannot be taken for granted. This project evaluates the *in vitro* anticancer activity of active fractions from *Psidium guajava* L. (popular name: guava), traditionally used for its antiparasitic activity. The dry plant material (leaves) was submitted to the process of hot extraction with dichloromethane and ethanol (95%), leading to the obtention of crude extracts. The most active extract went through the 1st fractioning process through the filtrating column process. The 2nd fractioning process was held with the most active fraction, through the classic column method, and all the fractions obtained in this process were evaluated *in vitro* for its potential anticancer activity. The antiproliferative assay was done *in vitro* in nine human cancer lines, donated by *National Cancer Institute*, USA: K562 (leukemia), MCF-7 (breast), NCI/ADR-RES (breast cancer resistant to multiple drugs), NCI-H460 (lung), UACC62 (melanoma), PC-3 (prostate), HT-29 (colon), OVCAR-03 (ovary) and 786-0 (kidney). Each fraction was tested in 4 concentrations against the tumor cell lines: 0,25, 2,5, 25 and 250 µg/ml. The potency and selectivity of each fraction was evaluated through antiproliferative graphics (percentage of growth inhibition *versus* concentrations), and the *Total Growth Inhibition (TGI)* values were calculated for each sample. The *in vitro* assay against the panel of human cell line proved to be effective to choose the most active fractions obtained in the purifying phytochemical process. Therefore, it is an important tool when choosing the right samples to continue the research project and also for a pre-selection process for the *in vivo* assays, protecting against the use of unnecessary animals in inactive samples. *In vitro* experiments are particularly interesting to analyse big amount of samples in a short period of time, giving results in a relatively short term basis. This allowed the analysis of which fractions from *Psidium guajava* L., obtained from the phytochemical process were responsible for the anticancer activity. Besides, this method keeps track of were the active leads might be, to direct the use of the right fractions in *in vivo* models, and also to continue the purifying methodology, to try to elucidate the the chemical composition of each fraction. These experiments follow the protocol suggested by the *National Cancer Institute (NCI)*, USA and is part of a master project supported by Fapesp and Cnpq.

10.015

Investigação das propriedades antitumorais do elatol obtido da alga vermelha *Laurência microcladia*. Campos A[1], Siqueira Jr JM[1], Souza CB[1], Lhullier C[2], Schenkel EP[2], Ribeiro-do-Valle RM[1] [1]UFSC - Farmacologia, [2]UFSC - Ciências Farmacêuticas

Introdução: Pesquisas com algas do gênero *Laurencia* possibilitaram a descoberta de compostos biologicamente ativos com atividades antibacteriana, antifúngica e antitumoral. Este estudo teve como objetivo avaliar os efeitos do elatol, um sesquiterpeno extraído da alga *Laurencia microcladia,* através de modelos experimentais *in vitro* e *in vivo*. **Métodos:** A viabilidade celular das linhagens B16F10, C6, MCF7, DU145, A549 e L929 foi avaliada, pelo teste do MTT, utilizando elatol (0,1–100 µM). A proliferação celular de B16F10 foi analisada, pelo ensaio do MTT, com elatol (0,1–100 µM) em intervalos de 4–48 h. Ensaios de citometria de fluxo, pelo método do iodeto de propídio, e *Western blot* foram realizados para investigar o mecanismo de ação do elatol (10 e 50 µM) sobre B16F10. A análise do crescimento tumoral de células B16F10 em camundongos C57BL6, foi utilizada para avaliar o potencial terapêutico do elatol (3–30 mg/kg v.o. ou 1–10 mg/kg i.p.) e cisplatina (4 mg/kg i.p.). Os protocolos experimentais foram aprovados pelo Comitê de Ética para o Uso de Animais da UFSC (PP000102).**Resultados:** O teste do MTT permitiu observar significativa redução da viabilidade nas células B16F10, C6, MCF7, DU145, A549 e L929 (16,6; 28,3; 16,7; 10,9; 9,95 e 15,4 %, respectivamente, com elatol 100 µM). A proliferação celular de B16F10 apresentou diminuição significativa (67,1 % - 100 µM elatol) em 4 h, ficando ainda mais evidente ao final das 48 h (56,9; 16,3 e 14,4 % para 10 – 100 µM). Os ensaios de *Western blot* demonstraram que o elatol foi capaz de interferir na expressão de proteínas que participam da regulação do ciclo celular no início de G1, com leve redução na expressão nas ciclinas A, D1 e E e Cdk 2, 4 e 6. Também foi observada diminuição nas proteínas anti-apoptóticas Bcl-2 e Bcl-xl e aumento da p53. As análises de citometria de fluxo confirmaram aumento de células apoptóticas. Nos ensaios *in vivo*, após 10 dias de tratamento diário, o elatol (3, 10 e 30 mg/kg v.o. e 1, 3 e 10 mg/kg i.p.) e a cisplatina (4 mg/kg i.p.) reduziram o tamanho dos tumores em 50,4; 41,3; 61,2; 51,3; 61,4; 71,4 e 81,5 % respectivamente, em relação ao controle. **Discussão:** Os resultados demonstraram que o elatol apresentou significativo efeito citotóxico, reduzindo a viabilidade celular das diferentes linhagens testadas e a proliferação em B16F10. As análises de *Western blot,* em B16F10, demonstraram que este pode interferir no ciclo celular (promovendo um atraso na transição de G1/S e entrada em S) e acelerar o processo apoptótico, corroborando com os resultados de citometria de fluxo. Nos experimentos *in vivo*, tanto o tratamento v.o. como i.p. foram capazes de reduzir o crescimento tumoral. Estudos complementares estão em andamento para confirmar a eficácia e segurança do elatol, para assim, torná-lo um possível candidato a medicamento utilizado no tratamento do câncer. **Apoio Financeiro:** CNPq, Finep, Fapesc**.**

10.016

Influência de *(R)*- E *(S)*- goniotalamina na atividade estrogênica sobre a linhagem tumoral humana de mama MCF-7. Vendramini Costa DB[1], Ruiz, ALTG[1], Marquissolo, C[3], Pilli RA[3], Carvalho JE[1] - [1]CPQBA-UNICAMP - Farmacologia e Toxicologia, [2]UNICAMP - Química, [3]UNICAMP - Química Orgânica

Com o crescente entendimento da biologia do câncer surgem diversos tratamentos dessa doença, enfocando não somente o ciclo celular ou a reparação do DNA, mas também outros processos celulares. Antagonistas de receptores de estrógeno (como o Tamoxifeno) e drogas que bloqueiam a síntese de estrógeno são amplamente utilizadas na prevenção e retardo de cânceres de mama que expressam receptores desse hormônio[1]. *(R)*-Goniotalamina (1) é uma estiril-lactona comumente isolada de plantas do gênero *Goniothalamus*[2] e apresenta, bem como seu enântiomero *(S)*-Goniotalamina (2), pronunciada atividade antiproliferativa em linhagens de células tumorais humanas, com seletividade para as hormônio-dependentes de mama (MCF-7), ovário (OVCAR-03) e ovário com fenótipo de resistência a múltiplos fármacos (NCI-ADR/RES). Assim, este trabalho teve por objetivo avaliar a influência de (1) e (2) na atividade estrogênica em células MCF-7, adenocarcinoma mamário humano. **Métodos**: (1) e (2) foram obtidas, com 37% de rendimento global, a partir do *trans* cinamaldeído, por alilação catalítica assimétrica nas condições de Keck, seguida de acilação e metátese de olefinas para fechamento de anel, utilizando o catalisador de Grubbs de 1ª geração. As células foram plaqueadas na densidade de 4,0 $x10^4$ e após 24 horas foram expostas a concentrações crescentes de (1), (2) e de Tamoxifeno, na presença e na ausência de 17-ß estradiol (1nM). As células foram incubadas por 144 horas e a atividade determinada através do método da sulforrodamina B. **Resultados**: Concentrações maiores que 1 e 10 µg/mL de (1) e (2) respectivamente inibiram totalmente o crescimento das células incubadas na presença e ausência de 17-ß estradiol. Já concentrações menores não foram capazes de inibir o crescimento estimulado pelo hormônio, sugerindo mecanismo de ação independente dos receptores de estrógeno. O 17-ß estradiol estimulou o crescimento celular em até 100 %, porém tal atividade foi inibida quando as células foram expostas a concentrações maiores que 1 µg/mL de Tamoxifeno. Concentrações menores desse composto não influenciam o crescimento celular na ausência de 17-ß estradiol, porém limitam a atividade do hormônio sobre o crescimento das células, indicando uma influência dose-dependente sobre os receptores de estrógeno. **Discussão**: Como observado nesse trabalho o Tamoxifeno comprovou sua atividade antiestrogênica descrita anteriormente[3]. A ação independente de (1) e (2) sobre a linhagem permite concluir que nessas condições experimentais tais moléculas não atuam através da inibição da atividade estrogênica. Tratamentos antiestrogênicos não matam diretamente as células tumorais, mas previnem que o estrógeno promova sua proliferação[1]. De fato, relata-se que a ação de estiril-lactonas está relacionada à ativação das diversas vias apoptóticas levando à morte celular e não apenas a inibição de proliferação celular[2]. **Apoio**: FAPESP, CNPQ. [1]Alberts *et al*, *Mol Biol Cell*, 2008. [2] De Fátima *et al*, *Chem Bio Int*, 176 (2008) p. 146. [3] SOTO *et al*, *Environ. Health Perspect***.**, 103 (1995) p. 113

10.017

Antiproliferative activity of fractions obtained from *Calea pinnatifida* dichlorometane extract. Marchetti GM[1], Silva KA[2], Ruiz AL[1], Foglio M[2], Carvalho JE[1] [1]CPQBA-UNICAMP - Farmacologia, [2]CPQBA-UNICAMP - Fitoquímica

Introduction: Cancer is characterized by unstable cells that do not respond to outside stimulus that control proliferation, differentiation and death. Germacranolides are sesquiterpenes lactones with high potency against cancer cells. Phytochemical studies showed the presence of these compounds in *Calea pinnatifida* leaf extracts[1,2]. These compounds are frequently described as responsible for antiproliferative activities, with apoptosis induction[3]. Therefore, the study of anticancer activity of *Calea pinnatifida* extracts and substances became important. **Methods:** *C. pinnatifida* dried leaves were extracted with dicloromethane in Soxhlet during 48h resulting crude dichloromethanic extract (EBD). The EBD (m=16,5g) was submitted to pre-purification by column chromatography affording fractions that were bio-monitored by anticancer *in vitro* assay on human cancer cell lines. The most active fraction, Fr B3, was further purified by column chromatography, furnishing 6 new fractions. Among these fractions the most potent, B3.4 (m=850mg), was submitted to a new purification process by column chromatography providing 11 new fractions. Antiproliferative activity assay was evaluated in tumor cell lines of breast (MCF-7), lung (NCI-H460), melanoma (UACC-62), prostate (PC-3), kidney (786-0), colon (HT-29), ovary (OVCAR-03), multi drug resistance ovary (NCI-ADR/RES), leukemia (K562) and green monkey kidney epithelia (VERO). Samples were tested in a concentration range of 0.25 to 250 mg/ml and doxorrubicin was used as positive control. After 48h of treatment, the activity was measure by the sulforhodamine B method and total growth inhibition was calculated by non linear regression[4,5]. **Results:** Fraction B3.4 was the most potent obtained from EBD, with selectivity for melanoma (TGI: 1.50mg/mL), kidney (TGI: 3.71 mg/mL) and ovary (TGI: 3.92 mg/mL) and further purification of this fraction led to 11 new fraction with a good antiproliferative activity although with less potency than B3.4. Among the eleven fractions B3.4 III, had a similar phytochemical composition to FB3.4 with selectivity for the same cell lines; melanoma (TGI: 1.48mg/mL), kidney (TGI: 3.14 mg/mL) and ovary (TGI: 5.47 mg/mL). All these fractions also showed high antiproliferative activity to normal cell line. **Discussion:** The EBD of *Calea pinnatifida* had previously showed *in vivo* anticancer activity in Ehrlich ascite and solid tumor models[6]. Based on these results, isolation and identification of the active principles from EBD became important. The fractionation process show many active fractions, however with less potency. Therefore this study highlights the role of synergism presented in plants evidenced by the lost of potency when fraction FB3.4 was further purified, indicating that the different compounds have a role in promoting the anticancer activity observed. 1. Ferreira Z.S. *et al*. Ciência e Cultura (São Paulo) 32:83, 1980; 2. Gottlieb H.E. Phytochemistry 19:1481, 1980; 3. Nakagawa Y. *et al*. J Pharmacol Sci 97:242, 2005; 4. Skehan P *et al*. J Natl Cancer Inst 82: 1107, 1990; 5. Shoemaker RH. Nat Rev Cancer **6**: 813, 2006; 6. Marchetti G.M. Dissertação de Mestrado, IB-UNICAMP, 2008. **Financial Support**: Capes, Fapesp, CNPq.

10.018

In vitro study of the TK3 activity on tumor cell lines. Pott Jr. H[1], Prestes J C[2], Degasperi GR[3], Linarelli MCB[1] [1]PUCCamp - Medicina, [2]HC-UNICAMP - Oncologia, [3]UNICAMP - Medicina Experimental

Introduction: The interference of biomolecules present in foods and its consequences on the process of differentiation, growth and development of cells can be of great help in understanding its beneficial effects on health. The aim of this study is to evaluate the activity of a product made by a german biochemist in 1969, called "TK3" (association of tryptophan with thymine) on usual conventional *in vitro* culture of tumor cells and compare the results with the action of these substances alone, determining the effect on the induction of cell death. **Methods:** The cell lines used were: carcinoma of breast (MC7), colon (HT29), prostate (PC3) and hematopoietic system (K562). Cells (1 x 10^6/ml) were maintained for up to 48 hours in RPMI 1640 medium supplemented with 10% fetal bovine serum and gentamicin 1 µg / ml at 37 ° C and 5% CO_2. The cell lines were treated with TK3, thymine or tryptophan for 48 h. Every 12 hours an aliquot of each experimental condition was evaluated in a Neubauer chamber and optical microscope for analysis of cell viability. For the analysis of indices of cell death, was used anexin-V and propidium iodide (PI). **Results:** The TK3 was able to inhibit the growth of all cell lines at all times. After incubation, in the presence of TK3 the cells showed a pronounced reduction in growth ($p < 0.01$) when compared with the control in all cell lines, while thymine and mainly tryptophan, showed less effect after 48 hours. On the strains of MC7, the effect on inhibition of growth was more significant (95%), followed by K567 leukemic cells (73%). For all cell lines, we observed that cell death induced by thymine, tryptophan and TK3 was predominantly apoptosis. Furthermore, the results show that part of the cell lines HT29 and MC7 show susceptibility to necrosis and induction of necrosis increases with the combination of compounds in the form of TK3, a phenomenon observed especially in MC7 line that shows approximately 20% of cell death of type necrosis in the presence of thymine and tryptophan and 40% of death by necrosis in the presence of TK3. **Discussion:** The inhibition of cell growth induced by the nutritional supplement was higher with TK3, compared with the presence of thymine or tryptophan alone in all evaluation times and types of cell lines. The results suggest that treatment *in vitro* with thymine, in different tumor cell lines, was potentiated by the presence of tryptophan. Interestingly, the compound was able to induce cell death of tumor lines MC7, K567, PC3 and HT29, indicating a cytotoxic effect, which makes TK3 a potential association candidate in cancer therapy that deserves further investigation. The cellular signaling pathways responsible for the occurrence of programmed cell death are still targets of this study. **Financial support:** Lavilabor - Natural Products Ltda. Thanks to the Center for Multidisciplinary Chemical, Biological and Agricultural Research (CPQBA) at UNICAMP, for the tumor lines.

10.019

Murine melanoma progression: contribution of the kinin receptor B1. Dillenburg-Pilla P, Reis RI, Maria AG, Costa-Neto CM FMRP- USP - Bioquímica e Imunologia

Background: Melanoma is an aggressive, recurrent and frequently metastatic tumor with poor prognosis. Neoplasic tissues show high proliferation rate mainly due to up-regulation of survival and mitogenic pathways. It is also well established that an inflammatory microenvironment is crucial for tumor progression by secreting factors which stimulate malignancy growth. The Kallikrein-kinin-system (KKS) is classically involved in regulating vascular and inflammatory events. Such effects are mediated by Bradykinin (BK) and Kallidin (KD), peptides that act on the G-protein coupled receptor B2. Moreover, BK and KD are also cleaved by carboxypeptidases, generating desArg^{9}-BK (DABK) and desArg^{10}KD (DAKD), respectively, which have high affinity to the B1 receptor. **Objectives**: Investigate the role of the B1 receptor in murine melanoma progression. **Methods**: Tm5 melanoma cell line was cultured in RPMI medium supplemented with 5% FCS. *In vivo* experiments were carried out by injecting subcutaneously 3x10^{5} cells in C57/Bl6 mice. Malignancies were monitored for 14 days and then tumors were excised. Expression of KKS components were performed by RT-PCR. In *in vitro* experiments, cell viability was measured using MTT assay after 24, 48 and 72 hours of DABK stimulus; ERK1/2 phosphorylation was accessed by western blotting after 10, 30, 60 or 180 minutes of DABK stimulation; and intracellular calcium mobilization was evaluated using FLUO3/AM probe. All *in vivo* protocols have been approved by local ethical committee (protocol number 025-2007 from march 2007). **Results**: *In vitro* analysis demonstrated that the B1 receptor and angiotensin-converting enzyme (ACE) were constitutively expressed in Tm5 cells. On the other hand, this cell line did not express the B2 receptor. Tumor samples from mice showed that the B1 expression was increased by 4.5 -fold during tumor progression. Moreover, DABK-stimulated cells showed an increase in proliferation rate after 72h of agonist stimulation *in vitro*. Concerning intracellular pathways, we observed that ERK1/2 phosphorylation was increased from 10 to 60 minutes after DABK stimulus returning to basal levels after 180 minutes. However, calcium mobilization occurs immediately after agonist stimulation and is blocked in the presence of the B1 antagonist desArg^{9}-[Leu^{8}]-BK. **Conclusion**: Besides the classical involvement in vasodilatation and inflammatory processes, our results point to a participation of the kinin B1 receptor in melanoma progression, involving intracellular calcium mobilization, ERK1/2 phosphorylation, and modulation of cell proliferation rate. **Financial Support**: FAPESP, CNPq, CAPES, FAEPA.