1211 POSTER

Treatment of Solid Cancers Using a New Cationic Cytolytic Peptide

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Background: Known for their bacteriotoxic effects, many cationic peptides also exhibit cytotoxic activity against cancer cells, mostly binding rapidly to the slightly negatively charged plasma membrane of cancer cells and disrupting it. In this work we have identified novel cationic peptide of 27 amino acids (YGRKKRRQRRRGKTLRVAKAIYKRYIE) that interacts rapidly with the plasma membrane of cancer cells, showing potential therapeutic efficacy against a number of cancer cell types, both *in vitro* and *in vivo*.
***In vitro* Experiments:** MTT and Live/Dead cytotoxicity assays on various human tumour cell lines (5 osteosarcoma, 6 glioma ,4 mammary carcinoma and 4 melanoma cell lines) showed up to 95% peptide induced cell death at concentration of 10−35 μg/ml of peptide, whereas normal human fibroblast and osteoblast cell lines were considerably less affected. Surface plasmon resonance (SPR) experiments revealed that the peptide binds strongly to negatively charged liposomes at neutral pH. Fluorescence spectroscopy demonstrated that the peptide induces significant membrane leakage of liposome contents. Time lapse scanning confocal microscopy on dsRED transfected tumour cell lines, showed dsRED leakage from the tumour cells within 1hr after treatment. Electron microscopy and time- lapse confocal microscopy confirmed these findings.
***In vivo* Experiments:** Pharmacokinetic profiling *in vivo*, using i.v injection of I^{125}-radiolabelled peptide, showed a peptide half- life *in vivo* of 1hr with ensuing renal clearance. 4T1 murine breast carcinomas were xenografted in 16 BALB/c mice and treated by a single-shot local bolus injection of 600μg/100μl of peptide. This resulted in up to 50% reduction of tumour size within 2−3 days post injection and reduced tumour re-growth in the following 4 weeks. Moreover, HF1GFPLuc human melanomas were xenografted in 30 NOD.CB17.Prkdcscid mice and then either treated by a single-shot local bolus injection of 1000μg/100μl of peptide or by weekly treatment. Tumour growth was monitored both by calliper measurement as well as by optical imaging. Again, a notable reduction in tumour growth was observed. Detailed tumour histology revealed large areas of necrosis and numerous pyknotic cells. Apoptotic cells were also detected by means of various immunohistochemical staining methods as well as by Tunel assay.
Conclusion: In sum, these findings indicate that the peptide both causes tumour cell death by membrane disruption and by apoptosis induction.

1212 POSTER

Promising Anti-cancer Activity of a Novel Palladium (II) Complex on Human Breast Cancer Cells in Vitro and in Vivo

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Background: Treatment of breast cancer is still not satisfactory although new drugs have been introduced in recent years. Therefore, novel agents are required. Pd(II) complexes may be of importance for this aim.
Material and Methods: In this study, we synthesized ([Pd(sac)(terpy)](sac)-4H_2O) and tested its anti-cancer activities against human breast cancer cell lines, MCF-7 (estrogen receptor-positive) and MDA-MB-231 (estrogen receptor-negative). Anti-growth effect was assayed by the MTT and ATP assays, while the detection of programmed cell death (apoptosis) was performed by both caspase-cleaved cytokeratin 18 (M30-Antigen) and DNA-fragmentation assays *in vitro*. Apoptosis-related gene expressions were analyzed by RT-PCR and detection of protein expression levels by western blotting. Invasion capacity of cells was assayed by Matrigel® invasion assay. In addition, we investigated the anti-tumoral effect of the complex on Ehrlich ascites tumour (EAT) grown in female Balb-c mice *in vivo*.
Results: Results showed that the Pd (II) complex had a strong anti-growth effect on both cell lines in a time and dose dependent manner. IC_{50} values were 0.09 μM for MDA and 3.05 μM for MCF-7 cell line. The Pd (II) complex induced apoptosis at 3.12 μM in only MCF-7 cells. It was also effective in disrupting the formation of MDA-MB-231 tubules on matrigel, indicative of a putative anti-invasive activity. The gene expressions of cell death receptors of DR4 (TRAIL-R2) and DR5 (TRAIL-R1) were found to be induced by the complex. DR5 was also detected at the protein level. The anti-growth effect was confirmed by in vivo experiment in which the Pd (II) complex significantly inhibited the growth of the tumour.
Conclusions: The newly-synthesized Pd (II) complex has a strong anti-cancer activity against breast cancer cells by inducing apoptosis via cell death receptors *in vitro*. The complex is further able to significantly reduce the growth of tumour cells *in vivo* model. Taken together, the Pd (II) complex represents a potentially active novel drug for the breast cancer treatment.

1213 POSTER

The New Synthetic Compound Pterocarpanquinone LQB-118 Induces Apoptosis in Acute Myeloid Leukemia Cells Through Survivin and XIAP Downregulation

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Background: The development for novel compounds exhibiting pro-apoptotic potential to target simultaneously a diversity of drug resistant mechanisms is highly desirable in acute myeloid leukemia (AML). The pterocarpanquinone-LQB-118 is a hybrid between pterocarpans and quinones, a synthetic compound structurally related to lapachol [2-hydroxy-3-(3-methyl-2-butenyl)-1.4-naphthoquinone]. We have previously shown that this compound was very effective in inducing apoptosis in cells from chronic myeloid leukemia. The major aim of the present study was to analyze the effect of this compound to observe whether LQB-118 would be equally effective in AML cells.
Materials and Methods: The cells viability (by MTT assay), the apoptosis index (by Annexin V on flow cytometry) and the caspase-3, caspase-9, survivin and XIAP expressions (by Western blot), were analyzed before and after *in vitro* LQB-118 treatment in Kasumi-1 AML cell line and cells from AML 17 patients. P-glycoprotein, p53, and Bcl-2 expressions analyzed by flow cytometry were correlated with the apoptosis index induced by LQB-118.
Results: LQB-118 6 μM reduced the cell viability of the Kasumi-1 cells by about 40% ($p < 0.05$) after 48 h and 72 h incubation. The reduction of the cell viability with LQB-118 9 μM was markedly higher (70%), when cells were incubated after 48 h and 72 h incubation ($p < 0.001$). Apoptosis was observed when Kasumi-1 cells were incubated with LQB-118 3 μM, and 9 μM after 24 h (30.7%, $p > 0.05$ and 85% $p < 0.01$), and after 48 h (54.2%, $p < 0.01$ and 92% $p < 0.001$), respectively. After 48 h incubation, LQB-118 caused an increase in caspase-3 activation as well in caspase-9 activation. LQB-118 3 μM was capable to induce a median of 28% and a median of 25% apoptosis after 24h and 48h incubations, respectively, in AML samples from patients exhibiting or not multifactorial multidrug resistance (MDR) phenotype.
Conclusions: LQB-118 was effective in triggering apoptosis in AML cells through the increased activity of caspase-3 and -9 and downregulation of survivin and XIAP in cells from patients presenting multifactorial MDR phenotype. Taken together, these data indicate LQB-118 as a promising candidate for clinical tests in AML patients.
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1214 POSTER

Carbon Nanovector Used as Transfectional Agent in NIH-3T3 Cell Mice and Potential RNAi Carrier for Solid Tumours

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Background: Cancer is a major public health concern worldwide. In Brazil, the estimates for the years 2010/2011 are approximately 489,270 new cases per year, being the second leading cause of death. Nowadays, chemotherapy, radiotherapy and surgery are used to treat cancer. Despite of being effective, some problems continue to occur, such as, antitumour agents nonspecific systemic distribution, low tolerance of some patients to treatment, high cytotoxicity to normal cells and multiple drug resistance development. It is extremely important that innovative technological methodologies can be designed to delineate tumour margins, separate cancer cells from normal, also identifying micrometastasis and if the tumour was completely removed. Within this perspective, many researchers in the

oncology field turned their attention to nanotechnology, with the aim of finding more effective and efficient methodologies to fight and cure cancer. Among all the nanoparticles tested, one that proved to be the most effective was the carbon nanoparticle. Carbon nanotubes (CNT) consist exclusively of carbon atoms arranged in a series of benzene rings condensed and wrapped in a tubular way. Due to their physicochemical properties, they are internalized by endocytic cells. Moreover, they are capable of carrying drugs and organic molecules such as DNA, RNA and proteins. In this work, we report on the fabrication of carbon nanofibers by a synthesis polymer (poly-ethylene glycol) combined method using metal catalysts and rapid immersion in a hot filament system fed with ethanol highly diluted in hydrogen and argon. Additionally, these nanotubes were dissolved in P85, conjugated with PIRES plasmid and compared to the comercial transfection kit Effectene (Qiagen). The results showed that carbon nanovectors are more effective than Effectene in transfection assays, suggesting their utilization as RNAi, proteins or drugs carriers in a near future.
Material and Methods: Carbon Nanotube: The carbon nanovector has been made and characterized in the Departamento de Semicondutores UNICAMP. **Functionalization**: Carbon nanotube was suspended P85 buffer (10% w/v). **Cell line:** NIH/3T3 mice fibroblast cells were acquired from the National Institutes of Health. ***In vitro* cytotoxicity:** Cells were incubated with carbon nanovectors without the polymer in concentration range of 0.008 to 1 mg/mL for 12, 24, 48 and 72 h in 96-well plates. **The MTT assay**: was performed according to manufacturer's data. **Celular Transfection:** was in accordance with Effectne (Qiagen) guide.
Results: The carbon nanotube produced by this method and suspended in P85 copolymer solution was able to carrier pIRES into the cells showing higher fluorescent intensity than Effectene. The carbon nanotube produced was not cytotoxic to tested cell.
Conclusion: The carbon nanotube is not cytotoxic and has proved to be an excellent carrier of genomic material. This carbon nanovector is going to be used in future experiments with RNAi carrier in solid tumours.

1215 POSTER

Glyco-PEGylated R-metHuG-CSF (XM22/Lipegfilgrastim) − a Novel Long-acting Once-per-cycle Filgrastim: Pharmacokinetics and Pharmacodynamics for Body Weight Adjusted Doses and a 6 mg Fixed Dose in Healthy Volunteers

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Background: Long-acting filgrastim offers the advantage of less dosing intervals when used for reduction of neutropenia and incidence of febrile neutropenia (FN) in patients treated with cytotoxic chemotherapy for malignancy. A novel once-per-cycle filgrastim (XM22/INN lipegfilgrastim) for fixed dose administration to prevent FN was designed by attaching a 20 kD PEG to glycan at natural O-glycosylation site.
Materials and Methods: The Glyco-PEGylated r-metHuG-CSF (GPF) (XM22/INN lipegfilgrastim) was intensively studied in non-clinical models. The first clinical use was in healthy volunteers in order to study pharmacokinetic and pharmacodynamic parameter as well as safety of XM22. A single dose, dose-escalating study was performed in healthy volunteers using a body weight adjusted dosing for XM22 (25, 50 and 100 μg/kg). In another cohort of healthy volunteers a single fixed dose of 6 mg lipegfilgrastim was compared versus a single fixed dose of 6 mg pegfilgrastim using a parallel group design. Pharmacokinetic and pharmacodynamic parameters were studied after all single dose s.c. administrations. Safety was studied for all healthy volunteers. Blood samples were drawn and serum levels of XM22 were detected and measured using an immunological assay based on the Mesoscale Discovery Platform and individual and mean concentration-time profiles were plotted per treatment group. Relevant pharmacokinetic parameters are described such as area under the curve (AUC), maximal serum concentration (Cmax) and half-life for XM22. The mean ANC (absolute neutrophile count) response per treatment group was chosen as pharmacodynamic parameter.
Results: A dose dependent increase in bioavailability was observed and ANC increased comparable to the comparator long acting filgrastim pegfilgrastim. Pharmacokinetic parameters for XM22 at the dose level of 100 μg/kg and for 6 mg fixed dose showed a higher bioavailability of XM22 compared to an equivalent dose of the clinically used long acting filgrastim pegfilgrastim/Neulasta® (about 60% higher AUC). Furthermore, a higher pharmacodynamic effect of XM22 was found compared to the equivalent dose of the comparator long-acting filgrastim pegfilgrastim (about 30% higher ANC response). XM22 was well tolerated in all healthy volunteers treated.
Conclusions: The clinical data on pharmacokinetics and pharmacodynamics confirm that the novel long-acting filgrastim XM22 − developed by using a glyco-PEGylation platform technology − is suitable to be further studied for a once-per-cycle fixed dose use to prevent FN in patients. Pharmacokinetic and pharmacodynamic characteristics demonstrate a dose-dependent increase of the bioavailability of lipegfilgrastim and consequently a dose-dependent increase of the ANC. Based on this study a dose of 100 μg/kg lipegfilgrastim was selected as the optimal dose level for further studies.

1216 POSTER

Albumin-fusion R-metHuG-CSF (Balugrastim) − a Novel Long-acting Once-per-cycle Fixed Dose Filgrastim: Pharmocokinetics and Pharmacodynamics in Breast Cancer Patients

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Background: Long-acting filgrastim offers the advantage of less dosing intervals when used for reduction of neutropenia and incidence of febrile neutropenia (FN) in patients treated with cytotoxic chemotherapy for malignancy. A novel once-per-cycle filgrastim (INN balugrastim) for fixed dose administration to prevent FN was developed using an albumin-fusion platform technology by fusing r-metHuG-CSF to human serum albumin.
Materials and Methods: The recombinant albumin-fusion protein balugrastim − composed of filgrastim and human serum albumin − was intensively studied in non-clinical models. The first clinical use was performed in breast cancer patients to study pharmacokinetic and pharmacodynamic parameters as well as observe the safety of balugrastim. In a first pilot phase a cohort of 13 breast cancer patients received escalating doses of balugrastim (50, 150, 300 and 450 μg/kg) 14 days prior to their doxorubicin + docetaxel chemotherapy. Pharmacokinetic and pharmacodynamic parameters were studied after a single dose s.c. administration. Safety was studied for all patients. Blood samples were drawn for the measurement of balugrastim serum levels. Relevant pharmacokinetic parameters are described such as area under the curve (AUC), maximal serum concentration (Cmax) and half-life for balugrastim. The mean ANC (absolute neutrophile count) response per treatment group was chosen as pharmacodynamic parameter.
Same pharmacokinetic and pharmacodynamic parameters were checked later on in cycle 1 in breast cancer patients receiving an active chemotherapy treatment of doxorubicin and docetaxel.
Results: A dose dependent increase in bioavailability was observed and ANC increased comparable to historical data for the comparator long acting filgrastim pegfilgrastim. Pharmacokinetic parameters for balugrastim at the dose level of 450 μg/kg were comparable to 6 mg fixed dose s.c. pegfilgrastim. Balugrastim was well tolerated in all patients treated.
Conclusions: The clinical data on pharmacokinetics and pharmacodynamics confirm that balugrastim − a novel recombinant albumin fusion long-acting filgrastim − is suitable for a once-per-cycle fixed dose use to prevent FN in patients. Pharmacokinetic and pharmacodynamic characteristics demonstrate a dose-dependent increase of the bioavailability of the drug and consequently a dose-dependent increase of the ANC. Based on this study a dose of 450 μg/kg was selected and recommended for the phase II study for balugrastim.

1217 POSTER

Discovery and Evaluation of 3-phenyl-1H-5-pyrazolylamine-based Derivatives as Potent, Selective and Efficacious Inhibitors of FMS-like Tyrosine Kinase-3 (FLT3)

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Preclinical investigations and early clinical trial studies suggest that FLT3 inhibitors offer a viable therapy for acute myeloid leukemia. However, early clinical data for direct FLT3 inhibitors provided only modest results because of the failure to fully inhibit FLT3. In this study, we have designed and synthesized a novel class of 3-phenyl-1*H*-5-pyrazolylamine-derived compounds as FLT3 inhibitors which exhibit potent FLT3 inhibition and high selectivity toward different receptor tyrosine kinases. The structure-activity relationships (SARs) led to the discovery of two series of FLT3 inhibitors, and some potent compounds within these two series exhibited comparable potency to FLT3 inhibitors sorafenib and ABT-869 in both wt-FLT3 enzyme inhibition and FLT3-ITD inhibition on cell growth (MOLM-13 and MV4;11 cells). In particular, one selected compound exhibited the ability to regress tumours in mouse xenograft models using MOLM-13 and MV4;11 cells.