247 EFFECT OF OLIGOELEMENTS AND PHOSPHOLIPASE A_2 ON TUMOR CELL GROWTH AND INDUCTION OF APOPTOSIS. *IN VITRO* STUDIES.

E.Crescenti, M. Croci, R. Bergoc*, A. Crescenti, E. Rivera*. *Fundación 'Dr. Ernesto Crescenti', *Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, ARGENTINA.*

Aim : In the present work we investigated the direct action of A_2 phospholipase and the oligoelements Zn, Se and Mn (O-PA2) on *in vitro* cell proliferation and in the induction of apoptosis in malignant cells. We also compared this action with the effect produced in normal cell lines.

Methods: Cellular suspensions from chemically induced mammary carcinomas were cultured in soft agar and the effect of different doses of O-PA_2 were tested by evaluating colony formation. In other set of experiments, cell lines derived from diverse human neoplasias as well as normal, not transformed epithelial cells, were challenged with O-PA_2 at concentrations from 10^{-3} to 10^{-12} g/l and proliferation was evaluated by different methods. Induction of apoptosis was investigated in experimental carcinomas developed in control animals and in tumors from rats treated *in vivo* with O-PA_2. The proportion of apoptotic cells was evaluated by employing immunohistochemical methods.

Results: Data obtained *in vitro* with all neoplastic cells indicate that O-PA_2 produced a dose-dependent inhibition on cell proliferation. The maximal inhibitory response observed was 50% ($p < 0.01$ versus control cell cultures) with slight differences among the different cell lines. When normal cells were tested, although not significant, an increase in cell proliferation was obtained with lower O-PA_2 doses. In the experimental carcinomas, studies of apoptosis indicated a significant higher number of apoptotic cells in all the tumors from rats pretreated with O-PA_2. On the contrary, in tumors from control rats, only a low number of apoptotic cells were observed and exclusively in the stroma.

Conclusions: These results clearly demonstrate that O-PA_2 treatment exerts a direct action on tumor cells. The final response of this effect is a significant inhibition on cell proliferation that may be consequence of an induction of apoptosis and/or cell differentiation. This effect proved to be a differential action on transformed cells, not observed in normal cell lines.

248 EFFECT OF OLIGOELEMENTS AND PHOSPHOLIPASE A_2 ON TUMOR CELL GROWTH. *IN VIVO* STUDIES.

E. Crescenti, M. Croci, E. Rivera*, A. Crescenti, R. Bergoc*. Centro de Investigaciones Medicas, Fundacion Dr. Ernesto Crescenti, *Facultad de Farmacia y Bioquímica, Universidad de Buenosa Aires, ARGENTINA.

Aim: a) To investigate the chemopreventive effect of *in vivo* treatment with (O-PA_2) combination of oligoelements Zn, Se, Mn (1,5 µg/kg) and A_2 phospholipase (0,1 µg/kg) on the induction of mammary tumors in rats; b) To determine *in vivo* effect of treatment with O-PA_2 on rats bearing tumors.

Methods: Mammary tumors were induced by ip injection of three doses (50 mg/Kg BW) of N-Nitroso-N-methylurea (NMU) to inbred Sprague-Dawley female rats aged 50, 80 and 110 days old. Experiments were carried out under two different conditions: a) Ten days before the first NMU injection, rats were randomly divided in two batches, treated and control. Batch treated rats was daily sc injected with O-PA_2 and this treatment continued during entire carcinogenesis process. Batch control daily received sc 0.9% NaCl. For malignant carcinomas, the following parameters were determined: Latency period, LP (days); mean tumor number per rat, n/r, and tumor incidence, TI (%); also, mean survival (days) of animals was calculated. b) Rats bearing ip-NMU induced mammary tumors were sc daily injected with O-PA_2. Control group received daily sc 0.9% NaCl. Tumor growth rate and mean survival were determined in O-PA_2 treated and control rats.

Results: a) LP did not show significant differences for both batches: (126 ± 14); n/t decreased significantly (1 versus 4.2 ± 0.8, $p < 0.004$) and TI decreased dramatically (11.1 versus 91.2, $p < 0.001$) in treated versus control rats, respectively. Mean survival of treated batch increased significantly (384.5 and 205, respectively, $p < 0.0004$). b) Growth tumors rate in treated animals was slower than in control ones. Mean survival of treated rats was significantly higher than control (265 versus 205, $p < 0.0012$). Histopathological studies showed an important quantity of mast cells majority degranulated and the presence of lymphocytes, macrophages and eosinophyles cells. Immunohistochemical results indicate in all treated tumors (a and b conditions) an important increase in the immune response, with intratumoral presence of "T" Helper lymphocytes with respect to other lymphocytes subpopulations.

Conclusions: *In vivo* O-PA_2 treatment during initiation/promotion/progression steps exerts an inhibiting effect on chemical carcinogenesis. For established tumors, growth rate was low in animals under O-PA_2 treatment. In both experimental conditions a significant increase in survival time and an important clinical improvement in O-PA_2 treated animals were evident.

249 ANTITUMORAL ACTIVITY OF A NEW SERIES OF 5,6-DIHYDROBENZO(A)CARBAZOLES

G. Martin, C. Cocca, E. Rivera, G. Cricco, A. Segall, H. Pappa, R. Casaubon, R. Caro, M. T. Pizzorno, R. M. Bergoc. *Faculty of Pharmacy and Biochemistry. Buenos Aires University. Buenos Aires. Argentina.*

Aim: To synthesize drugs with antitumoral activity. The overlapping of three-dimensional structures of 2,8-dihydrobenzo(a)carbazole (DHBC) derivatives over the structure of 4-hydroxytamoxifen (4-OH-TAM), by means of the MDL CHEMLAB 11.0 computational program, shows a reasonable structural and spatial resemblance. This finding raised the hypothesis of their possible anti-tumoral activity, similar to that of tamoxifen (TAM).

Methods: A number of DHBCs (9 in all) with an alkyl chain and a second basic nitrogen as substituent were synthesized in our laboratory and their possible anti-tumoral activity tested by means of: 1) competitive radioligand assays to determine relative drug binding affinity for the estrogen receptor of post-partum rabbit uterus (RBA); 2) *in vivo* studies, giving the synthetic drugs subcutaneously (1 mg/kg day) to Sprague-Dawley rats with NMU-induced mammary tumors; and 3) *in vitro* tumor cell proliferation experiments employing the soft agar clonogenic technique.

Results: 1) RBA for the estrogen receptor were similar to that of TAM (0.04 ± 0.02) for all compounds; 2) only three synthetic structures showed important antitumoral activity and induced tumoral regression similar to TAM (33, 44, 42 versus 48%, respectively, p: NS); and 3) these three compounds also produced *in vitro* inhibitory effect on cell proliferation ($p < 0.05$ versus control). Tumor growth rate of regressed and non regresed tumors was similar that TAM. Survival of these treated rats was similar to obtained for TAM. Histopathological studies demonstrated intratumoral necrosis and absence of hepatic and renal injury or other toxic effects.

Conclusion: Though all the compounds of the series of synthesized DHBCs showed affinity for the estrogen receptor similar to TAM, the results of *in vivo* and *in vitro* experiments confirmed the crucial role of hydroxyl groups in the molecule and of the interatomic distance between them, similar to that of estradiol, as well as the necessary presence of the aminoalkyl chain on the annular N atom. Further investigations would be important in view of a promisory future for mammary tumor therapy of the three DHBCs derivatives with antitumoral activity.

250 TRENDS IN SMOKING PREVALENCE AND LUNG CANCER MORTALITY IN POLAND

Witold Zatoński, Jerzy Tyczyński, Krzysztof Przewoźniak. *Center of Oncology, Department of Epidemiology and Cancer Prevention, Warsaw, Poland*

Aim: To evaluate trends and patterns in lung cancer mortality in Poland and to explain their possible association with changes in tobacco use indicators.

Methods: Analysis of annual standardized and age-specific lung cancer mortality rates by sex and of smoking prevalence rates by sex for specific age groups from the nation-wide randomized surveys carried out in adult population.

Results: Lung cancer is still leading cancer in male population in Poland and the second frequent cancer site in women. An overall lung cancer mortality in males in Poland was increasing from the begining of 1960s to the end of 1980s. In 1990s no further increase was observed. Some decrease of cancer mortality has been recorded in young adults (aged 20-44) since the beginning of 1980s. In the middle-aged males (45-64), increase of mortality has been stopped by end of 1980s. Lung cancer mortality rates for older males (65 and over) have grown in the entire time period. Positive changes in lung cancer mortality have not been noted in female population. Trends in lung cancer mortality reflect changes in tobacco smoking in Poland. The following changes in smoking behaviours among men are considered to explain the halting of increase in lung cancer mortality in young and middle-aged men: (1) almost two-fold increase in percentage of never smokers, (2) substantial increase in ex-smoking rates, (3) considerable decrease in daily smoking rates, especially among young age groups. Although there are no significant changes in smoking attitudes in all women (beside increase in ex-smoking rates), we have observed decrease of daily smoking in young women. It can influence upon lung cancer mortality in this population in coming years.

Conclusion: Comparison with other CEE countries shows that implementing of the national tobacco control programs and policy can be effective method to stop the epidemic of tobacco-related cancer in CEE in coming decade.

160 ACTIVITY AND FAVORABLE TOXICITY OF STEALTH LIPOSOMAL DOXORUBICIN (DOXIL) IN A POPULATION OF HEAVILY PRETREATED PATIENTS WITH SOLID TUMORS.

M. Jahanzeb, C. Frankel, M. Elkersh, A. Koletsky, P. Radice and C. Vogel. The Comprehensive Cancer Research Group, Inc., Miami, FL 33169

The goal of encapsulating chemotherapeutic agents in liposomes is to increase their circulating half-lives and lessen their toxicities. One vital addition which differentiates the STEALTH liposome Doxil (Sequus Pharmaceuticals, Menlo Park, California) from other conventional liposomes is the addition of a coating of methoxypolyethylene glycol (MPEG) to the surface of the liposome. This coating provides a steric stabilization effect therefore allowing the STEALTH liposome to bypass the body's immune system. This prolongs the circulation time of the STEALTH liposome by as much as ten times that of a conventional liposome. Over the last 14 months 28 patients with metastatic disease, refractory to conventional therapy (16 breast, 3 prostate, 2 ovarian, 2 lung, 1 mesothelioma, 1 endometrial, 1 colon, 1 pancreas, and 1 hepatoma) were treated with Doxil 40 - 50 mg/mg^2 every four weeks. Complete blood counts were followed weekly for 5 weeks then monthly and as needed. 20 patients received two courses or more and were considered evaluable for response. 7 patients were chemo naive (5 breast, 1 mesothelioma, 1 hepatoma) the others had received 1 - 8 courses. 4 patients had received Doxorubicin as prior therapy. Median number of metastatic sites was 2 (1 - 4 range). Toxicity data is available for 73 cycles of treatments in 28 patients (range 1 - 8 cycles). Leukopenia was mild with no grade 4, 8 grade 3, 16 grade 2, and 8 grade 1, toxicities with a delayed nadir (median time to nadir 3 weeks) range 1 - 4 weeks. No grade 4 anemia and only 2 grade 3 anemias were observed. There was no grade 3 or 4 thrombocytopenia. There was no alopecia. Four grade one and one grade two palmer plantar erythrodysesthesia was observed. Five patients had nausea, three patients experienced emesis, of which two were delayed. Five patients experienced mild mucositis, 3 patients experienced constipation and 2 experienced diarrhea. There was one allergic reaction, and one rash observed. Two patients complained of lethargy, 2 of weakness and 1 of fatigue. Other infrequent side effects included peripheral neuropathy, hypotension, shortness of breath, chest pain, cough, and leg cramps. Of 20 evaluable patients we saw 3 partial responses, 3 patients demonstrated a clinical benefit response, 10 had progressive disease, and 4 are too early for assessment. Patients with stage four disease, refractory to many therapies, showed clinical improvement and mild toxicity while on Doxil. In the future and after further study Doxil may prove to be more effective without the troubling side effects as the parent compound Doxorubicin.

161 ACTION OF OLIGOELEMENTS AND PHOSPHOLIPASE A_2 ON THE ENHANCED TOLERANCE TO TREATMENT WITH HIGH CYTOSTATIC DOSES.

E. Crescenti, M. Croci, E. Rivera*, A. Crescenti, R. Bergoc*. Centro de Investigaciones Medicas, Fundacion Dr. Ernesto Crescenti, *Facultad de Farmacia y Bioquimica, Universidad de Buenosa Aires, ARGENTINA.

Aim: To evaluate *in vivo* and *in vitro* tolerance to treatment with chemotherapeutic drugs used for human breast cancer combined with different doses of oligoelements Zn, Se, Mn and phospholipase A_2 (O-PA_2).

Methods: For *in vivo* experiments male Sprague-Dawley rats were employed. Cyclophosphamide, methotrexate and 5-fluoruracyl (CMF) action was evaluated in rats treated with sc daily administration of O-PA_2 versus control (daily sc NaCl). Range of CMF doses tested in different batches covered from 1 to 20-fold the dose used in human patients with breast cancer. For *in vitro* experiments, different cell lines derived from human pancreatic carcinoma and from normal mammary epithelium were employed. Cells were cultured in appropiate suplementary medium and grew in suspentions or monolayers in accord with its characteristics. 5-fluoruracyl (5-FU) was tested in differents doses.

Results: In vivo results indicate a significantly higher survival in treated rats versus control. Even with very high doses of CMF (14 to 18-fold dose used in humans), data indicate a significant difference versus control non O-PA_2 treated rats ($p < 0.002$). Macroscopic observation of collateral effects, as fur discoloration and gut hemorrhage, were milder in all O-PA_2 treated animals. Histological studies disclosed that renal tubule lesions, centrolobular hepatic steatosis and diverse degrees of bone marrow aplasia were present in rats and were invariably milder in O-PA_2 treated animals. Treated rats also showed less severe bone marrow aplasia, sepsis and incidence of malignant secondary tumors, as leukemias. *In vitro* results demonstrated that in both, normal and tumor cells, 5-FU produced a dose-dependent effect on cell proliferation with an EC_{50} value of 10 µM. This cytostatic effect was significantly enhanced by O-PA_2 pre-treatment in transformed cells and was observed at lower 5-FU doses. In contrast, O-PA_2 exerted on normal cells a protective effect. At lower doses cells showed the same pattern growth as control, while at 100 µM a significant number of viable cells remained after 5-FU treatment.

Conclusions: In vivo studies demonstrate the protective immunostimulant action of O-PA_2 on rats receiving large doses of combined chemotherapeutic drugs with an important enhancement of toxic tolerance. *In vitro* results confirmed this effect and demonstrate that the mechanism of this action is also due to direct action on normal and neoplasic cells. O-PA_2 acts magnifying the cytostatic effect on tumor cells and stimulating the proliferation of normal cells.

162 ANTITUMORAL ACTIVITY OF A NEW SERIES OF 5,6-DIHYDROBENZO(A)CARBAZOLES

G. Martin, C. Cocca, E. Rivera, G. Cricco, A. Segall, H. Pappa, R. Casaubon, R. Caro, M. T. Pizzorno, R. M. Bergoc. *Faculty of Pharmacy and Biochemistry. Buenos Aires University. Buenos Aires. Argentina.*

Aim: To synthesize drugs with antitumoral activity. The overlapping of three-dimensional structures of 2,8-dihydrobenzo(a)carbazole (DHBC) derivatives over the structure of 4-hydroxytamoxifen (4-OH-TAM), by means of the MDL CHEMLAB 11.0 computational program, shows a reasonable structural and spatial resemblance. This finding raised the hypothesis of their possible anti-tumoral activity, similar to that of tamoxifen (TAM).

Methods: A number of DHBCs (9 in all) with an alkyl chain and a second basic nitrogen as substituent were synthesized in our laboratory and their possible anti-tumoral activity tested by means of: 1) competitive radioligand assays to determine relative drug binding affinity for the estrogen receptor of post-partum rabbit uterus (RBA); 2) *in vivo* studies, giving the synthetic drugs subcutaneously (1 mg/kg day) to Sprague-Dawley rats with NMU-induced mammary tumors; and 3) *in vitro* tumor cell proliferation experiments employing the soft agar clonogenic technique.

Results: 1) RBA for the estrogen receptor were similar to that of TAM (0.04 ± 0.02) for all compounds; 2) only three synthetic structures showed important antitumoral activity and induced tumoral regression similar to TAM (33, 44, 42 versus 48%, respectively, p: NS); and 3) these three compounds also produced *in vitro* inhibitory effect on cell proliferation ($p < 0.05$ versus control). Tumor growth rate of regressed and non regresed tumors was similar that TAM. Survival of these treated rats was similar to obtained for TAM. Histopathological studies demonstrated intratumoral necrosis and absence of hepatic and renal injury or other toxic effects.

Conclusion: Though all the compounds of the series of synthesized DHBCs showed affinity for the estrogen receptor similar to TAM, the results of *in vivo* and *in vitro* experiments confirmed the crucial role of hydroxyl groups in the molecule and of the interatomic distance between them, similar to that of estradiol, as well as the necessary presence of the aminoalkyl chain on the annular N atom. Further investigations would be important in view of a promisory future for mammary tumor therapy of the three DHBCs derivatives with antitumoral activity.

163 Imexon Decreases Levels of Glutathione Reductase (GSH-R) activity, Glutathione (GSH) and Glutathione Disulfide (GSSG) Levels in Human 8226 Myeloma Cells In Vitro

K.Dvorakova[1], R.T.Dorr[1], W.Remers[2]
Arizona Cancer Center, Department of Pharmacology,[1] and College of Pharmacy,[2] The University of Arizona, Tucson, AZ U.S.A.

Aim: Imexon, an aziridine compound originally studied for immune-enhancing effects on lymphocytes, has demonstrated antitumor activity in human multiple myeloma cell lines in vitro (Salmon et al: J Natl Cancer Inst 86(3):228-230, 1994). The antitumor activity of imexon was associated with DNA strand breaks, no alkylation in vitro nor myelosuppression in vivo, and an alternate hypothesis that imexon may inhibit GSH-R and decrease the GSH pool by binding to sulfhydryl groups.

Methods: Human 8226 myeloma cells, which are markedly sensitive to imexon were incubated with imexon for 24 hours at different concentrations at 37°C and cell lysates were assayed for GSH, GSH-R, GSH-peroxidase and GSSG. GSH-peroxidase and GSH-R were measured by spectrophotometry; GSH and GSSG by HPLC. Imexon (1 mM) and GSH (1 mM) were also incubated in vitro at 37°C and the formation of any GSH-imexon adducts were followed by HPLC.

Results: In myeloma cells treated with imexon (0.09 mM or 0.9 mM), GSH-reductase activity decreased by 30% and 80%. GSH decreased by 60% and 100%, and GSSG levels decreased by 70% and 100%, respectively. GSH-peroxidase activity was not changed in cells exposed to 0.09 mM imexon and levels increased by 200% with exposure to 0.9 mM imexon. A putative GSH-imexon adduct was detected by HPLC after 2 hours of incubation at 37°C. Adduct formation increased at 24 hours with a concomitant decrease in GSH levels.

Conclusion: These results suggest that imexon can bind to sulfhydryl groups in GSH and GSH-reductase. This reduces the activity of GSH-R and decreases the cellular pool of GSH. This mechanism of action may be responsible for imexon-induced DNA single strand breaks and antitumor cytotoxicity.