observed in tumours. In particular, we demonstrated that patients at the stage $T_3N_0M_0$ were characterized by highly elevated levels of expression of LMP7 (β5i), LMP2 (β1i) and 19S activator (up to 4 times in last two cases) in the tumours in contrast to normal tissues and tumour samples at stage $T_2N_0M_0$. We have also identified a population of immune cells penetrating the tumour, which were also characterized by increased expression of LMP2 (β1i) and LMP7 (β5i), but not 19S activator. Low expression of 19S activator was revealed in stromal cells.
Conclusions: Taken together on the basis of obtained data we can conclude that the accumulation of 19S activator in tumour cells is associated with tumour progression. In this regard we assume that the utilization of 19S activator as a possible antitumour drug target could become a promising approach in thyroid cancer therapy.

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VEGF/VEGF-R Blockade Modulates Tumour-induced Immunosuppression in Colorectal Cancer

M. Terme[1], S. Pernot[1], E. Marcheteau[1], F. Sandoval[1], E. Tartour[1], J. Taieb[2]. *[1]INSERM U970, PARCC- Hôpital Européen Georges Pompidou − Université Paris-Descartes, Paris, [2]INSERM U970 and Service d'Hépatogastroentérologie et d'oncologie digestive, PARCC- Hôpital Européen Georges Pompidou − Université Paris-Descartes, Paris, France*

Background: Anti-angiogenic molecules targeting Vascular endothelial growth factor (VEGF)-A (bevacizumab) or its receptors (sunitinib, axitinib, sorafenib. . .) are routinely used as first or second line treatment of cancer patients. Anti-angiogenic molecules act not only on the vascular/endothelial system, but also seem to have an impact on immune escape mechanisms. We and others have recently shown that regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) decrease after Sunitinib administration in mice and metastatic renal cancer patients (Adotevi et al., J Immunother 2010; Ko et al., Clin Cancer Res). However, Sunitinib is a multi-target inhibitor specific of VEGF, KIT, PDGF, SCF, Flt3-L receptors, and signalization pathway(s) involved in immunomodulation induced by anti-angiogenic molecules is/are unidentified.
Methods: To better understand the role of the VEGF blockade in these immune phenomenon, we administered sunitinib or the mouse ortholog of bevacizumab (anti-VEGF antibody) to colorectal tumour-bearing mice (CT26 tumour model). The CT26 tumour cell line was chosen because of the known efficacy of sunitinib and anti-VEGF in this model and the use of anti-VEGF therapy in colorectal cancer patients. We analyzed tumour-induced immunosuppressive cells such as Treg, MDSC and PD-1 expressing T cells.
Results: In CT26 tumour model, Treg, MDSC, and PD-1 expressing T cells were significantly decreased after Sunitinib treatment in spleen and tumours, but also after anti-VEGF antibody administration. This decrease was not correlated with tumour size suggesting an immunomodulatory effect independent of a direct anti-tumour effect. Though Treg numbers were decreased after anti-VEGF treatment their regulatory functions were not altered by any of the treatment used. Moreover the use of masitinib, a tyrosine kinase inhibitor acting on KIT, PDGFR and FAK but not on the VEGF/VEGF-R pathway, was not able to modulate these different immunosuppressive cell populations. Finally, Treg, MDSC and $PD1^+$ T cells were also reduced in the peripheral blood of colorectal cancer patients after bevacizumab therapy.
Conclusion: Our results suggest that the blockade of the VEGF/VEGF-R pathway is sufficient to inhibit the induction of immunosuppressive cells by the tumour in mice and humans with colorectal cancer. This new property of anti-VEGF antibody opens perspectives for the use of such a molecule in association with anti-tumoral vaccination strategies in the future.

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A Pilot Study on the Efficacy of RGTA OTR4120, a Family of Regenerating Agents, on the Restoration of Bone Microarchitecture of the Mandible and Nasomaxilla in a Murine Model

U. Djasim[1], L. Spiegelberg[1], J.W. van Neck[2], E.B. Wolvius[1], K.G.H. van der Wal[1]. *[1]Erasmus MC, Oral and Maxillofacial Surgery, Rotterdam, [2]Erasmus MC, Plastic and Reconstructive Surgery, Rotterdam, The Netherlands*

Background: RGTA (ReGeneraTing Agent) comprises a family of heparan sulphate mimetics, and are considered to be able to stimulate repair and regeneration in various tissues, such as skin, muscle, and bone. The purpose of this pilot study was to investigate the effects of RGTA OTR4120 on the bone microarchitecture in the irradiated murine mandible and nasomaxilla.
Materials and Methods: Mice received either radiotherapy only or radiotherapy followed by weekly RGTA injection until sacrifice at 2, 6 and 10 weeks after radiotherapy. Mandibles and nasomaxillas were harvested for microcomputed tomographic analysis. Bone volume, trabecular pattern factor, trabecular thickness, trabecular separation, and trabecular thickness were quantified and compared.
Results: Generally, there seemed to be no effect of RGTA-treatment compared to the RT-only group, although incidental positive effects were observed in trabecular separation and trabecular number.
Conclusion: RGTA has been reported to be a promising healing agent that can be effective in tissue repair and regeneration in various tissue defects. However, based on the current results, no positive effects of RGTA OTR4120 on repair and regeneration of irradiated bone tissue could be identified, although additional research is needed to further explore and determine the role that the relatively new healing agents of the RGTA-family can play in the repair and regeneration of irradiated bone tissue.

1082 POSTER

Nigella Sativa Oil Ameliorates Methotrexate-induced Liver Toxicity

R. Labib[1], H. Hafez[1], O. Badary[2]. *[1]National Cancer Institute, Pharmacology, Cairo, [2]Faculty of Pharmacy, Pharmacology, Cairo, Egypt*

Backgound: Methotrexate (MTX), a chemotherapeutic agent, is used to treat many types of cancer. However its use is limited by its side effects. We studied the use of Nigella sativa L (N. sativa) oil, a natural antioxidant, as a protective agent against MTX-induced liver toxicity.
Materials and Methods: Twenty-four male albino rats were divided into four groups: saline, N. sativa oil (10 ml/kg), saline plus MTX (20 mg/kg, ip single dose) or N. sativa oil plus MTX. Blood samples were collected for hematological assessment of hemoglobin (Hb%), RBCs, WBCs and platelets, and also to determine serum MTX levels of the two groups receiving MTX. All rats were then sacrificed; a section from liver was removed for pathological examination and another was homogenized for analysis of liver enzymes.
Results: Body weight loss in N. sativa oil plus MTX treated group compared to MTX group was (12.7% versus 29.4%, $P < 0.05$). N. sativa oil showed significant decrease in SOD content which was elevated in case of MTX ($P < 0.05$). GSH was significantly decreased by 53.75% ($P < 0.05$) in MTX group compared to combination group. Furthermore histologically, severe degeneration of the liver parenchyma which was observed in MTX-treated group was improved by N. sativa oil. There were alterations in MTX-treated rat group including dilated congested portal vein and central veins, marked lymphocytic infilteration in the portal area. Furthermore, many binucleated hepatocytes were seen. Degeneration of hepatocytes in the form of vacuolation of cytoplasm, pyknosis of nuclei and fatty degeneration of some cells were also observed. As well as, intrahepatic haemorrhage, areas of necrosis and marked periportal and porto-portal fibrosis were also observed. Addition of N. sativa oil, caused improvement in the lymphocytic infiltration, no porto-portal fibrosis, some binucleation, some vacuolation of cytoplasm, less congestion in the portal vein and less extent periportal fibrosis were all observed. However, there was still degeneration of hepatocytes in the form of vacuolation of cytoplasm and few pyknosis of nuclei. Moreover, addition of N. sativa oil did not significantly affect the therapeutic level of MTX ($P > 0.05$).
Conclusion: Administration of N. sativa oil before and after MTX injection ameliorated MTX-induced liver toxicity and maintained its structure through anti-oxidant activity. These results can lead to further clinical applications for prevention of MTX-induced liver toxicities.

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The Effects of Heparan Sulfate Mimetic RGTA-OTR4120 on Radiation-induced Salivary Gland Dysfunction in Mice

L. Spiegelberg[1], U.M. Djasim[1], J.W. van Neck[2], E.B. Wolvius[1], K.G.H. van der Wal[1]. *[1]Erasmus MC, Oral and Maxillofacial Surgery, Rotterdam, [2]Erasmus MC, Plastic and Reconstructive Surgery, Rotterdam, The Netherlands*

Background: To study the effects of the heparan sulfate mimetic RGTA-OTR4120 on the salivary glands of mice that have been irradiated in the head and neck region.
Methods and Materials: Female C3H mice were irradiated with a single dose of 15 Gy in the head and neck region. RGTA-OTR4120 was injected 24 hours after radiotherapy, followed by weekly injections. At 2, 6 and 10 weeks after radiotherapy, salivary flow rates were measured and animals were sacrificed to obtain parotid and submandibular glands for histology. Periodic acid Schiff stain was performed to visualize mucins that are produced by acinar cells. Amylase and total protein content were measured in saliva samples.
Results: Salivary flow rates were increased at 2 and 6, but not at 10 weeks after radiotherapy with RGTA-OTR4120 administration, compared to irradiated controls. Two and ten weeks after radiotherapy, the mucin production activity of acinar cells was increased under influence of RGTA