Table. 5-HMT PK Following Administration of Fesoterodine Alone or Coadministration of Fesoterodine and Fluconazole			
	Fesoterodine 8 mg + Fluconazole 200 mg BID (Test)	Fesoterodine 8 mg Alone(Reference)	Test/Reference Ratio* (90% CI)
AUC0-∞, ng.h/mL	62.8	49.5	126.8 (118.3, 135.9)
Cmax, ng/mL	5.27	4.42	119.2 (110.8, 128.3)
Tmax, h	5.0 (2.1–6.0)	5.0 (3.0–6.0)	ND
t½, h	8.05 (35)	7.83 (40)	ND
CI=confidence interval; BID=twice daily; ND=not determined. AUC0-∞ and Cmax presented as adjusted geometric means, Tmax presented as median (range), and t½ presented as arithmetic mean (% coefficient of variation).*Ratio of adjusted geometric means.			

275 ALTERED DETRUSOR FUNCTION IN FEMALE CAVEOLIN-1-DEFICIENT MICE

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Introduction & Objectives: Caveolae are omega shaped membrane organelles that have been shown to sequester and regulate important signalling intermediaries. Caveola biogenesis depends on proteins from the caveolin and cavin families and on membrane cholesterol. Caveolin-1-deficiency is associated with substantial urogenital alterations in male mice. These changes include prostate hypertrophy, distension of the seminal vesicles, disturbed erectile function and impaired muscarinic contractility of isolated detrusor strips in vitro. Whether the contractile changes in the bladder are secondary to outflow obstruction caused by the hypertrophic prostate or reflect a primary role of caveolae in detrusor signalling is not known. This was addressed by making a detailed functional characterization of detrusor contractility in wild type (WT) and caveolin-1-deficient (KO) female mice.
Material & Methods: Detrusors from 32 week old KO and congenic WT female mice were examined in vitro using myography. Diuresis was estimated by water consumption. Length-tension relationships were generated and contractility in response to the muscarinic agonist carbachol was determined at the optimum length (L0) for active force. Western blotting was used to assess contents of caveolin-1, cavin-1, and muscarinic M3 receptors.
Results: KO mice consumed significantly less water per day suggesting reduced diuresis. The bladder to body weight ratio was not changed in KO mice. The passive length-tension relationship of the KO bladders was left-shifted in comparison with WT bladders. Maximal carbachol-induced force at L0 was increased in KO bladder when normalized to high-K^+ contraction.
Conclusions: Genetic ablation of caveolae in the female mouse leads to substantial functional changes in the bladder, ruling out obstruction related hypertrophy as a contributing factor. The left-shift of the passive length-tension relationship in the KO bladder may reflect adaptation to reduced diuresis or increased tissue stiffness. Carbachol contraction is increased in the caveolae-deficient female detrusor suggesting that the presence of caveolae suppresses muscarinic signalling in the female mouse bladder.

276 CHANGES IN BLADDER WALL PERFUSION IN THE OVERACTIVE OBSTRUCTED BLADDER

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Introduction & Objectives: Basal animal and human studies suggest that in overactive and obstructed bladder ischemia occurs. Ischemic damage to bladder muscle- and nerve cells add to the bladder dysfunction. In the follow-up of patients with overactive/ obstructive bladder monitoring of ischemia is not applied as the exact relation between bladder function and bladder blood oxygen supply is not known and the means are lacking. We now measured bladder wall oxygenation in vivo in an animal model of bladder obstruction to establish the exact relation between tissue oxygenation and bladder function.
Material & Methods: Oxygenation and blood volume of the bladder wall was measured in vivo by differential path-length spectroscopy (DPS) using glass-fibers in guinea pigs; sham operated (n=10) and urethrally obstructed (n=10). Measurements were performed mainly on the bladder top during multiple voiding/ filling cycles; before surgery, 4 weeks after (2 SHAM and 2 obstructed) and eight weeks after (8+8). Urodynamic investigations during the 8 week period provided data on bladder-pressure, -compliance, -contractility and –overactivity and urine flow-rate.
Results: Before surgery in both groups and 8 weeks after SHAM surgery, oxygen saturation in the bladder wall was above 90% during filling. It dropped during voiding (lowest value 80%) and returned to >90% within 30 seconds. Eight weeks after obstruction saturation was significantly ($p<0.05$, unpaired student t-test) lower compared to the sham group both during filling (lowest value 38%) and voiding (lowest value 12%). The decrease was positively related to bladder pressure both during filling and voiding and was strongest when overactivity was present. This pattern of changes was also found after 4 weeks. In the 5 seconds before voiding blood volume was found to increase significantly. This occurred before >85% of the recorded voidings.
Conclusions: A normal functioning bladder maintains a high oxygen saturation level. Bladder obstruction compromises this ability especially when it involves overactivity. Monitoring bladder oxygen saturation will be of clinical value. It can be performed with the DPS technique using glass fibers that fit in the working channel of a cystoscope. The question is raised how the oxygen saturation responds to treatments aiming specifically at bladder function or those aiming to improve bladder blood circulation. The changes in blood volume that occurred before voiding can have two explanations. First, bladder contraction starts at the base and blood is propelled to the top (our measurement site) before total bladder contraction is obtained and pressure rises or blood supply and bladder voiding are regulated simultaneously.

277 THE INHIBITORY ROLE OF MELATONIN ON ISOLATED RAT URINARY BLADDER CONTRACTION: THE NOVEL PATHWAY FOR THE PHARMACOLOGICAL MANAGEMENT OF NOCTURIA

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Introduction & Objectives: Nocturnal melatonin levels may affect bladder activity. Here, we tested the effects of melatonin on detrusor muscle contraction of isolated rat bladder.
Material & Methods: We evaluated the effects of melatonin on the contractions induced by phenylephrine (PE), acetylcholine (ACh), betanechol (BCh), KCl, and electrical field stimulation (EFS) in 20 detrusor smooth muscles of Sprague-Dawley rats. To determine the mechanisms of the inhibitory responses after melatonin, melatonin pretreated muscle strips were reacted with the calcium channel antagonist (verapamil), the potassium channel blockers [tetraethyl ammonium (TEA), 4-aminopyridine (4-AP), and glibenclamide], direct voltage dependent calcium channel opener (Bay K 8644), and KN-93 [specific calcium/calmodulin-dependent kinase II (CaMKII) inhibitor].
Results: Melatonin pretreatment (10^{-8}~10^{-6} M) dose-dependently decreased the contractile responses of PE (10^{-9} ~ 10^{-4} M) and Ach (10^{-9} ~ 10^{-4} M). Melatonin (10^{-7} M) also blocked contraction induced by high KCl ([KCl]ECF ; 35 mM, 70 mM, 105 mM and 140 mM) and EFS. Verapamil potentiated the relaxation response of melatonin-treated strips (10^{-7} M), but other potassium channel blockers did not. Melatonin pretreatment significantly decreased the contractile responses of Bay K 8644 (10^{-11} ~ 10^{-7} M). KN-93 enhanced melatonin-induced relaxation.
Conclusions: The present results suggest that melatonin can inhibit bladder smooth muscles as a voltage dependent calcium antagonistic effect and calmodulin/ CaMKII system inhibition. Clinical significance will be studied in the future.

Oral Session 5
PROSTATE CANCER STEM/INITIATING CELLS AND THE MICROENVIRONMENT
Sunday, 18 April, 12.15-13.45, Amsterdam Room

278 ROLE OF STROMAL FIBROBLASTS INTERACTION WITH PROSTATIC CANCER CELLS IN ACHIEVEMENT OF METASTATIC PHENOTYPE

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Introduction & Objectives: Tumoral micro-environment, also called “reactive stroma”, plays an important role in prostate cancer progression and in the achievement of a metastatic phenotype. Particularly, activated fibroblasts (AF) with an ex novo expression of α-SMA protein (smooth muscle actin), are supposed to enhance tumoral cell aggressive characters and metastatic capacity. Aim of our study was to analyze the biunivocal interaction between fibroblasts and prostatic cancer cells and to establish how tumoral cells achieve the aggressive and metastatic phenotype.
Material & Methods: We performed cultures of fibroblasts, untransformed prostatic epithelial cells (PNT-1) were cultivated in RPMI medium and prostatic cancer

cells (PC-3) were cultivated in DMEM medium to observe cell to cell interaction between those cells placed in contact among them. After 24 hours we separated the 2 cellular lines on the basis of their different times of plate adhesion. We used Boyden chamber for the tridimensional test of cells migration in DMEM 4500 medium (chemoattractive). Migrated cells that were found in filter pores, fixed and inked, were counted at microscope. Cellular invasion was evaluated by Boyden chamber with a filter containing matrigel to copy extracellular matrix.
Results: We isolated among cancer-activated fibroblasts (CAF) the myofibroblasts, in addition to α-SMA negative fibroblasts (PC-AF) with a remarkable ability to enhance migration and invasiveness of PC3. Effects induced by MF and PCAF on invasive capacities of PC3 resulted different: MF induces a uPA/uPAr system mediated invasiveness, while PC-AF promotes a metalloprotease dependent invasiveness. IL-6 resulted to be able to induce trans-differentiation of fibroblasts in PC-AF. Our results show that activated fibroblasts determine an increase of invasiveness of PC3, while don't affect tumorigenesis. In primary cultures of prostatic fibroblasts, these can be activated either by TGFβ in MF, and much more by IL6 in PCAF. Analysis of cancer associated fibroblasts (CAF) allowed us to confirm their facilitator role in tumour cell invasiveness.
Conclusions: In our results we highlights the existence of a biunivocal cooperation between fibroblasts and prostate cancer cells, which was implemented by IL6 or TGFβ dependent manner, in promoting a metastatic and aggressive phenotype. Indeed, PC3 induce fibroblasts activation, with or without expression of αb-SMA, through the production of TGFβ or IL6. These activated fibroblasts increase the aggressiveness of PC3 inducing a pro-invasive phenotype through the system dependent on uPA/uPAr or MMP.

279 STROMA REACTION IN MOUSE XENOGRAFT MODELS OF PROSTATE CANCER BONE METASTASIS

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Introduction & Objectives: Analysis of the stromal-epithelial interaction in cancer is problematic. We present an experimental method of global gene expression analysis in bulk tissue specimens of bone metastasis able to dissect the cancer cell-specific from the stromal cell-specific transcriptome induced by the stromal-epithelial interaction without prior cell separation. This method is based on species-specific (mouse and human, respectively) cDNA gene arrays where the stroma-induced response in the mouse tibia bone marrow to xenografted prostate cancer (PC) cell lines is compared to normal bone.
Material & Methods: The human osteoinductive PC cell line C4-2B4 was xenografted into the tibia of SCID mice. Mice were sacrificed when the osteoblast response was evident at radiography, and RNA was extracted from the tumour bearing bone shaft. RNA extracted from an equivalent portion of intact tibiae of age-matched mice served as a "mouse-only" control, and from cultured C4-2B4 cells as a "human-only" control. Each probe was hybridized onto the whole human (U133A 2.0, Affymetrix) and mouse (430A 2.0, Affymetrix) genome array. The cDNA probe sequences were compared by bioinformatics analysis and cross-hybridizing probes were excluded from analysis by computational mask.
Results: A limited number of genes were excluded because of cross-hybridization between human and mouse (7.7% and 6.3%, respectively). In the bone stromal compartment 77 genes were up-regulated at least 8-fold in presence of PC cells. A literature analysis confirmed that their expression is confined to the stroma and not to the cancer cell component of various cancers. As genes encoding extracellular matrix (ECM) proteins overexpressed by the stroma reactive to cancer cells may represent putative biomarkers of prostate cancer bone metastasis, 3 of these highly up-regulated ECM proteins, namely periostin (POSTN), asporin (ASPN), hevin (SPARCL1), were further analyzed. Real time RT PCR assays with mouse (stroma) specific probes validated the stromal expression of the 3 genes of interest (GoI) and their overexpression in presence of osteoinductive PC cells. Immunohistochemistry performed on tumour xenografted mouse bone, primary PC, benign prostate hyperplasia (BPH) and bone metastasis samples further confirmed that the protein products of these 3 GoI are exclusively localized in the stroma. mRNA expression levels of POSTN, ASPN and SPARCL1 in murine primary calvaria osteoblasts in co-cultures with C4-2B4 cells are significantly increased after 24h compared to the expression levels in absence of C4-2B4.
Conclusions: Species-specific microarrays are able to dissect reliably the stroma derived gene expression from the cancer derived gene expression. Cancer cells induce expression of ECM proteins in bone marrow stroma. Genes up-regulated as the result of the cross talk between the cancer cells and bone marrow stroma may represent potential markers for bone micro-/macro-metastasis.

280 ARACHIDONIC ACID PRIMES HUMAN BONE MARROW STROMA FOR PROSTATE CANCER METASTASIS

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Introduction & Objectives: Prostate cancer preferentially metastasizes to the bone and we have previously shown that the poly-unsaturated fatty acid arachidonic acid is a potent stimulator of CaP invasion. Here we present that arachidonic acid also promotes prostate cancer invasion by inducing bone marrow adipocyte formation.
Material & Methods: Boyden invasion chambers were used to assess the ability of dietary oils and their poly-unsaturated fatty acid components to induce PC-3 invasion. Adipocytes loaded with specific poly-unsaturated fatty acid, generated from primary human bone marrow mesenchymal stem cells, were used as invasion stimulants in Boyden chamber assays and in co-culture experiments with the human bone metastatic prostate PC-3 cell line. Lipid transfer and metabolism was followed using deuterated poly-unsaturated fatty acid and time-lapse Fourier Transformed Infrared Spectroscopy.
Results: Dietary oils unlike their poly-unsaturated fatty acid components do not induce PC-3 invasion suggesting that it is the composition of the oils that is of importance. Poly-unsaturated fatty acids induce bone marrow adipocyte differentiation with arachidonic acid inducing higher levels of bone marrow adipocyte differentiation as compared with other poly-unsaturated fatty acids (3998514.4 vs. 932265.8; p=0.00002). Arachidonic acid pulsed adipocytes stimulated greater prostate epithelial cell invasion than free arachidonic acid (22408.5607.4 vs 16236313.9; p=0.01111) or adipocytes generated in the presence of other poly-unsaturated fatty acids. In the co-culture model of bone metastasis, PC-3 and bone marrow adipocyte interactions result in direct uptake and metabolism of arachidonic acid by PC-3 cells, destruction of the adipocyte and subsequent formation of a bone metastasis.
Conclusions: The data supports the hypothesis that arachidonic acid not only promotes CaP invasion, it also prepares the "soil", making it more supportive for implantation and propagation of the migrating metastatic cell.

281 NOGGIN CONTRIBUTES TO THE OSTEOLYTIC RESPONSE IN BONE METASTASIS OF PROSTATE CANCER (CAP)

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Introduction & Objectives: Members of the bone morphogenetic protein (BMP) and of the wingless (Wnt) protein family play a relevant role in the osteoblast response to CaP bone metastasis. Extracellular antagonists are crucial for the modulation of their biological activity. We have reported that the lack of expression of the BMP antagonist noggin by cancer cell lines is a determinant of their osteoinductive activity in vivo. In contrast, osteolytic cell lines express noggin constitutively. The Wnt-antagonist dickkopf-1 (DKK-1) has been shown to participate in the osteolytic process of multiple myeloma (MM) by repressing bone formation, thus, adding an osteopenic component to the lesion. Noggin may contribute by an equivalent mechanism to osteolysis in bone metastasis of CaP. Therefore, we utilized a RNA silencing strategy to investigate whether the constitutive noggin expression by an osteolytic CaP cell line plays a role in the osteolytic response induced by its bone metastasis.
Material & Methods: Stable noggin-knock down (Nog-KD) was achieved by transfection of multiple shRNA vectors into a luciferase-expressing PC-3 cell clone (PC-3/luc). A vector encoding a non-targeting shRNA (mock) was used as negative control. Silencing efficiency was monitored at the mRNA and protein level by real time PCR and immunoblotting, respectively. Several Nog-KD and mock clones were xenografted into the tibia of immunocompromised mice. Their intra-osseous growth and osteolytic effect were monitored by bioluminescent imaging (BLI) and radiography, respectively. 3-D images of the xenografted tibiae were generated by micro-tomography (μCT). Bone structural parameters were analyzed by peripheral quantitative computerized tomography (pQCT) and histomorphometry.
Results: Significant reduction of noggin mRNA and protein expression was achieved in several Nog-KD clones. Their growth rate or expression in vitro of the osteolytic cytokines parathyroid hormone-related protein and interleukin-8, and of DKK-1 were not markedly affected. Tumour growth in bone xenografts of different mock and Nog-KD clones was only marginally or not affected. Radiographic and mCT analysis showed that the bones xenografted with the Nog-KD clones have structural modifications indicative of bone formation/repair activity, which could not be observed in parental PC-3 cells and mock clones. Bone histomorphometry and pQCT have further corroborated these findings.
Conclusions: The shRNA-mediated suppression of noggin expression in the osteolytic cell line PC-3 may have restored the bone formation that normally follows bone resorption, as an effect of the "coupling phenomenon". Conversely, noggin expression by CaP may contribute to the induction of an osteolytic lesion by adding an osteopenic component, similarly to the mechanism shown to operate in MM. Noggin suppression may be an additional therapeutic option for the treatment of osteolytic bone metastases by solid cancers.