Heart Failure 2009

Den årlige *Heart Failure*-kongressen som arrangeres av *Heart Failure Association of the European Society of Cardiology* ble i år avviklet i Nice i Frankrike 30. mai til 2. juni. Byen som ligger i regionen Provence-Alpes-Côte d'Azur på den franske rivieraen ved Middelhavet, var et hyggelig møte med sin flotte strandpromenade og sin livlige gamleby med mange restauranter, barer og små butikker. Kongressen dekket de fleste emner innen hjertesvikt. Det var 8 forskjellige sesjoner med foredrag: *Main Sessions* (essensielle emner valgt av *Executive and Scientific Committees of the Heart Failure Association*), *Educational Sessions* (praktisk kliniske kasus-baserte og "how-to"-sesjoner), *Joint Sessions* (foredrag fra relaterte disipliner samlet i én sesjon), *Translational Sessions* (klinisk relevant forskning som potensielt har betydning for dagens praksis), *Nursing Sessions, French language Sessions* (fransk-språklige sesjoner for sykepleiere og allmennpraktikere), *Basic Science* ("state-of-the-art"-presentasjoner) *og Oral Abstract Sessions* i tillegg til poster-presentasjoner og firma-sponsede satellittsymposier. Neste Heart Failure-kongress vil bli arrangert i Berlin i mai/juni 2010.

Årets kongress hadde 3800 deltagere som var en økning i forhold til fjordårets kongress i Milano der det var 3458 deltagere. I år var det 1097 innsendte abstrakter som var en 18 % økning i forhold til i fjor. Det var i år en klar økning i antallet abstrakter fra Norge. På fjorårets kongress var det ingen norske abstrakter på de orale abstrakt-presentasjonene og bare 4 blant poster-presentasjonene, mens det i år var 2 norske orale abstrakt-presentasjoner i tillegg til 25 poster-presentasjoner. Av de 2 norske orale abstrakt-presentasjonene var én i kategorien "Best across topics" (Røsjø et al) og én i kategorien "Judges Choice Clinical" (Øie et al).

Her presenteres studiene fra *Late Breaking Trials*, en kort kommentar vedrørende data som ble lagt fram om kardiell resynkroniseringsterapi, en presentasjon av 3 nye hjertesvikt-medikamenter i horisonten samt de norske abstraktene

Erik Øie, stedlig redaktør

Late Breaking Trials

Marit Aarønæs, Hjertemedisinsk avdeling, Rikshospitalet, Oslo universitetssykehu

Congestive Heart failure: A multidisciniplinary Non-pharmacological approach for Changing in rE-hospitalisation and prognosis (CHANCE)

V. Mareev, Moskva, Russlan

Studiens formål var å kartlegge om en multidisiplinær tilnærming er bedre enn standard oppfølging av pasienter innlagt med forverret hjertesvikt. 745 pasienter innlagt med hjertesviktforverring (NYHA III-IV) ble randomisert etter vellykket stabiliserende behandling til videre optimal medisinsk behandling samt opplæring før utskrivning samt oppfølging etter utskrivning, bl.a. telefonisk (n=360) eller standard oppfølging (n=385). Den multidisiplinære tilnærmingen hadde overraskende god effekt med ca. 40 % reduksjon i ny hospitalisering, reduksjon i tid til første hospitalisering, færre nye liggedøgn og ikke minst 40 % reduksjon i mortalitet (p<0,05). Det ble konkludert med at multidisiplinær tilnærming gir gode resultater og at en slik tilnærming er kostnadseffektivt. Disse resultatene er overraskende positive, spesielt sett i lys av tidligere lignende studier med nokså skuffende resultater

B-convinced

G. Jondeau, Paris, Frankrik

Studiens hovedformål var å undersøke om betablokkade kan kontinueres eller må seponeres ved innleggelse for akutt forverrelse av kronisk hjertesvikt. 160 pasienter ble screenet og 147 inkludert i studien. 69 pasienter ble randomisert til kontinuering av betablokkade til tross for forverret hjertesvikt, mens 78 pasienter ble randomisert til seponering av betablokker. Endepunkt var hjertefrekvens, symptomer og tegn på hjertesvikt, mortalitet, varighet av sykehusinnleggelse og BNP-konsentrasjon ved 7 dager og etter 3 måneders oppfølging. Hos pasienter med kontinuert betablokkade var hjertefrekvensen signifikant lavere ved begge kontrolltidspunkt (p<0,01), mens det var ingen signifikant forskjell på noen av de andre endepunktene på noe tidspunkt. Konklusjonen er at betablokkade trygt kan kontinueres ved akutt forverrelse av kronisk hjertesvikt

Chronic Heart failure Assistance by Telephone (CHAT)

H. Krum, Melbourne, Australi

Studien undersøkte om mulighet for systematisk telefonkontakt med en automatisert hjertesviktinformasjonstelefon for hjertesviktpasienter i grisgrendte strøk, her i "the outback" i Australia, ville bedre livskvalitet og redusere mortalitet og hospitalisering. Inklusjonskriterier var hjertesvikt i NYHA-klasse II-IV, LVEF ≤40 % eller hjertesvikt med bevart ejeksjonsfraksjon (HFPEF). 218 pasienter ble randomisert til vanlig hjertesviktomsorg ved sin fastlege, mens 188 ble randomisert til telefonisk kontakt i tillegg til vanlig fastlegeomsorg. Oppfølgingstiden var 12 måneder. Primært endepunkt som var et kombinert endepunkt (*Packer clinical composite score*), var ikke signifikant forskjellig mellom gruppene. Et kombinert endepunkt av død av alle årsaker og innleggelse av alle årsaker var signifikant redusert (p<0,01), og innleggelse for alle årsaker var også signifikant lavere hos dem med telefonisk oppfølging (p<0,001), mens antall sykehusinnleggelser for forverrelse av hjertesvikt ikke var signifikant redusert, og mortaliteten var lik i gruppene etter 1 års oppfølgin

Beta-blocker tolerance in elderly patients with heart failure: The Cardiac Insufficiency Bisoprolol Study in Elderly (CIBIS-ELD) Trial

H-D. Duengen, Berlin, Tysklan

Denne studien sammenliknet effekt av to ulike betablokkere, karvedilol og bisoprolol, på eldre hjertesviktpasienter ≥65 år. 1876 pasienter ble randomisert 1:1 i to grupper som fikk opptitrert dose med betablokkade justert etter bivirkninger. Primært endepunkt var oppnådd måldose av medikamentet som er karvedilol 25 mg x 2 (>85 kg 50 mg x 2) og bisoprolol 10 mg x 1, evaluert etter 12 måneder. Kun 11,5 % av pasientene nådde primært endepunkt, og det var ingen signifikant forskjell mellom medikamentgruppene. Sekudære endepunkt var bedret ejeksjonsfraksjon, bedret NYHA-klasse, bedret 6 minutters gangdistanse og bedret livskvalitet.

Alle parametre var signifikant bedret og det var ikke forskjell mellom gruppene. Hjertefrekvensreduksjonen var størst i bisoprololgruppen, og bradykardi var mer uttalt i denne gruppen. Lungestuvning og anemi var mer uttalt i gruppen som fikk karvedilol, mens FEV1 var lik i begge grupper. Studien viser problemet med å nå angitt måldose også hos eldre pasienter

Execise training in heart failure with preserved ejection fraction (Ex-DHF)

B. M. Piesker, Graz, Østerrike

Studien undersøkte effekt av trening hos 67 hjertesviktpasienter med bevart ejeksjonsfraksjon (HFPEF). Pasientene ble randomisert 2:1, trening mot kontroller. Primær endepunkt var endring i maksimal VO_2. Det var en signifikant bedring i treningsgruppen på maksimal VO_2 etter 6 måneder ($p<0,001$). Parametre som livskvalitet, gangdistanse, maksimal belastning og endring i E/E', ble også signifikant bedret av trening sammenliknet med kontroller. Studien bekrefter at også hjertesviktpasienter med bevart ejeksjonsfraksjon kan ha effekt av systematisk og regelmessig fysisk trening

Signal-HF - A Randomized Study of NT-proBNP guided Management of Heart failure in Elderly Primary Care Patients

H. Persson, Stockholm, Sverig

Formålet med denne svenske studien var å sammenlikne effekt etter 9 måneder på serumkonsentrasjoner av NT-proBNP ved å behandle eldre hjertesviktpasienter i primærhelsetjenesten med ejeksjonsfraksjon ≤50 % med økte serumnivåer av NT-proBNP etter gjeldende retningslinjer eller ved å bruke repeterte BNP-målinger som veiledning under medisinering og oppfølgning (mål å redusere NT-proBNP med >50 % i forhold til "baseline"). 237 pasienter i NYHA-klasse II og III ble randomisert, 80 % hadde atrieflimmer. Etter 9 måneders oppfølgning var det ingen forskjell mellom gruppene på overlevelse, innleggelsesfrekvens, livskvalitet, eller NYHA-klasse. Endringer i NT-proBNP var lik i begge gruppene (10 %). *Signal-HF* er en ny skuffende studie når det gjelder effekten av å bruke proBNP/BNP som veiledning for behandling av hjertesvikt

The selective myosine activator, CK-1827452, increases systolic function in heart failure

J. J. V. McMurray, Glasgow, Storbritanni

Dette medikamentet har lovende effekter med gunstig effekt på slagvolum, LVESV og LVEDV. Denne studien blir nærmere presentert i avsnittet "Nye medikamenter i horisonten"

Pre-Relax-AHF

M. Metra, Brescia, Italia

Svangerskapshormonet relaksin er også lovende som ny behandling ved hjertesvikt. Fase II-studien *Pre-Relax*-AHF presenteres også i avsnittet "Nye medikamenter i horisonten"

Kardial resynkroniseringsterapi

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Det ble ikke presentert nye store studier inne dette feltet ved kongressen. Man avventer MADIT-CRT-resultatene som forventes senere i år der man har implantert resynkroniseringspacemaker med defibrillator (CRT-D) på hjertesviktpasienter i NYHA-klasse I og II og sammenlikner mot implanterbar defibrillator (ICD) alene. REVERSE-studiens europeiske forlengelse på 263 pasienter bekrefter effekt på revers remodellering og funksjonsevne, samt forsinkelse av sykdomsutviklingen hos sviktpasientene med QRS-bredde ≥120 ms og i NYHA-klasse II som fikk CRT sammenliknet med optimal medikamentell behandling. Oppfølgingen har nå vart 24 måneder.

Nye medikamenter i horisonten

Kaspar Broch, Hjertemedisinsk avdeling, Rikshospitalet, Oslo universitetssykehu

På nittitallet kom en rekke epokegjørende studier innen behandling av hjertesvikt (1-3). Dagens retningslinjer reflekterer funnene i disse studiene (4). De siste ti årene er det imidlertid ikke introdusert nye medikamentgrupper som har fått noen etablert plass i hjertesviktbehandlingen. En egen sesjon i Nice tok for seg nye, lovende medikamenter. Blant de mest interessante er relaksin, CK 1827452 og CD-NP

JR Teerlink fra San Fransisco Veterans Affairs Medical Center i USA foredro om potensiell bruk av relaksin ved hjertesvikt. Relaksin er et peptidhormon som produseres under graviditeten. Det formidler mange av de kardiovaskulære endringene som ses da, bl.a. vasodilatasjon. Preliminære studier har vist lovende effekter ved hjertesvikt. Bl.a. ses reduksjon i perifer arteriell motstand, fall i sirkulerende pro-BNP-nivåer og økt hjerteminuttvolum (5). Hormonet må gis intravenøst. Under "Late Breaking Trials" ble resultater fra pre-RELAX-AHF lagt frem. Dette er en randomisert, placebokontrollert fase IIb-studie, som ble publisert i *Lancet* i april (6). 234 pasienter innlagt med akutt hjertesvikt (definert som dyspné i hvile eller ved minimale anstrengelser, stuvningstegn på røntgen thorax og forhøyet serumkonsentrasjon av pro-BNP) ble innen de første 16 timer etter innleggelse randomisert til relaksin eller placebo intravenøst. Pasientene hadde normalt eller forhøyet blodtrykk (>125 mmHg systolisk) og nedsatt nyrefunksjon med estimert GFR mellom 30 og 75 ml/minutt. Pasienter med hjerteinfarkt eller pågående inotrop eller vasodilaterende behandling ble ekskludert.

Ulike doser av relaksin ble sammenlignet med placebo i fem armer. Studiens endepunkter inkluderte semiobjektiv vurdering av dyspné og symptomer på hjertesvikt, forverring av nyrefunksjon, varighet av opphold i sykehus, antall døgn i live og utenfor sykehus etter 60 dager, død og reinnleggelse etter 60 dager og kardiovaskulær død etter 180 dager.

Studien var ikke utformet for å kunne vise effekt på noe enkelt endepunkt. Relaksin syntes dog å være assosiert med bedring i kliniske markører for hjertesvikt, mens nyrefunksjonen tenderte til å være dårligere. Ingen bekymringsfulle bivirkninger ble avdekket. Spesielt i dosegruppen 30 µg/kg/døgn syntes pasientene å profittere på behandling med relaksin. Det var dog vanskelig å se noen konsekvent dose-respons-sammenheng over de ulike armene i studien. En større klinisk studie med relaksin i ovennevnte dose er under utarbeiding.

CK 1827452 er en kardial myosin-aktivator som fasiliterer aktin-myosin-interaksjon og hemmer ikke-produktivt ATP-forbruk. I motsetning til "klassiske" inotrope medikamenter virker stoffet direkte på myosin og ikke via cAMP og økt intracellulær kalsiumkonsentrasjon. J. Cleland fra universitetet i Hull (Storbritannia) presenterte en studie på hunder med hjertesvikt og kunne demonstrere at systolisk ejeksjonstid, kontraktilitet og hjerteminuttvolum øker når CK 1827452 gis, uten at kardialt oksygenforbruk går opp. Samtidig synker høyre- og venstresidige fyllningstrykk, perifer arteriell motstand og hjertefrekvens, mens middelarterietrykk forblir uendret. Hos friske, frivillige forsøkspersoner synes doser opp til 0,5 mg/kg/time å gi økt systolisk ejeksjonstid og økt ejeksjonsfraksjon i venstre ventrikkel (LVEF) uten økning i QTc-tid og uten signifikante bivirkninger (7).

Preliminære data fra den pågående fase II-studien CY 1121 ble lagt frem. Her deltar pasienter med stabil hjertesvikt og LVEF <40 %. Flere doseregimer prøves ut. Data fra de første tre kohortene foreligger. Her gir CK 1827452 økt systolisk ejeksjonstid, fall i hjertefrekvens og økt hjerteminuttvolum på doserelatert vis. Ingen alvorlige bivirkninger har vært observert så langt. For tiden arbeides det med å utvikle et preparat for per oral bruk, og man har planlagt å starte fase III-studie(r) (7).

J. C. Burnett fra Mayo-klinikken i Rochester, Minnesota (USA) holdt foredrag om et nytt, kimerisk natriuretisk peptid kalt CD-NP. CD-NP er skapt ved å kombinere humant C-type natriuretisk peptid (CNP) med *Dendroaspis* natriuretisk peptid (DNP) ekstrahert fra giften til grønn mamba. CNP er en naturlig forekommende venedilatator, som virker på natriuretisk peptid-reseptor B (i motsetning til atrialt natriuretisk peptid og BNP som virker på A-type reseptorer). I bedøvede hunder har CD-NP vist seg å gi nedsatte fylningstrykk i hjertet, økt diurese og suppresjon av renin-angiotensin-aksen uten eksessiv hypotensjon (8).

En studie på friske, frivillige forsøkspersoner ble nettopp publisert i *Journal of Clinical Pharmacology* (9). Fire, fire og fire personer ble gitt CD-NP i doser på henholdsvis 10, 25 og 17,5 ng/kg/minutt. I den høyeste dosegruppen opplevde to av fire personer bivirkninger i form av symptomatisk, ortostatisk hypotensjon. Dessuten ble det observert flushing, svimmelhet, rask puls, parestesier og dyspné ved denne doseringen. 17,5 ng/kg/minutt syntes å representere høyeste tolererte dose, og man randomiserte så 10 pasienter til henholdsvis placebo eller CD-NP ved denne doseringen. CD-NP ga signifikant økning i plasma cGMP, diurese og natriurese. Middelarterietrykk falt noe uten at dette ga symptomer eller fall i glomerulær filtrasjonsrate. Aldosteronnivået falt, mens angiotensin II-konsentrasjonen i serum ikke var signifikant forskjellig i de to gruppene. På bakgrunn av lovende funn er en multinasjonal klinisk studie av CD-NP ved hjertesvikt under planlegging.

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Norske abstrakter

34 Increased levels of cytokines, vasoactive peptides, and growth factors in alveolar macrophages in heart failure

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Purpose: Pathophysiologic interactions between the heart and the lungs in heart failure (HF) are well recognised. Increased circulating levels of vasoactive peptides and cytokines known to be synthesised in the heart in HF may directly affect the lungs and potentially also vice versa. The purpose

of the present study was to investigate whether expression of different factors known to be increased in the myocardium and/or the circulation in HF is also increased in alveolar macrophages in HF.

Methods: Twenty-two non-smoking HF patients (NYHA functional class II-IV) and 16 healthy controls were included in the study. Lung function and diffusion capacity were investigated by spirometry and DLCO, respectively. Induced sputum was performed after inhalation of hypertonic saline, and alveolar macrophages were isolated from the sputum by use of magnetic microbeads. Gene expression was examined in alveolar macrophages and in peripheral blood by real-time RT-PCR.

Results: Lung function and diffusion capacity were reduced in HF patients compared to controls with significantly lower FVC (88±4 vs. 112±3% of predicted value), FEV1 (84±4 vs. 104±3% of predicted value), and DLCO (69±4 vs. 101±3% of predicted value) ($P<0.05$ for all). Real-time RT-PCR demonstrated increased mRNA levels of several important cytokines, chemokines, vasoactive peptides, and growth factors in alveolar macrophages from HF patients compared to controls ($P<0.05$): endothelin-1 (1.8-fold), adrenomedullin (10-fold), TNFα (2.3-fold), IL-1β (3.9-fold), IL-6 (12-fold), MCP-1 (2.2-fold), IL-8 (4.2-fold), activin A (10.5-fold), and CTGF (3.2-fold). MIP-1α mRNA levels were not altered in HF. A similar increase in mRNA levels was not found in peripheral blood, indicating that the increase in gene expression is taking place in the lungs and is not a result of induction in monocytes in the circulation before entering the pulmonary compartment. mRNA levels of adrenomedullin, IL-6, MCP-1, IL-8, and CTGF in alveolar macrophages from HF patients displayed a negative correlation to left ventricular ejection fraction ($P<0.05$).

Conclusions: Several important cytokines, chemokines, vasoactive peptides, and growth factors are induced in alveolar macrophages in human HF. Further studies should clarify whether this induction affects pulmonary remodelling and whether the increased synthesis of these factors is reflected by increased release to the circulation and thus potentially may affect the failing myocardium.

119 Low total mortality rates due to extended use of optimal medical treatment and CRT-D in a primary prophylactic ICD population

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Introduction: Total mortality is still considerable among high risk heart failure patients despite optimal medical treatment (OMT) and implantable cardiac defibrillators (ICD). In major primary prophylactic ICD-trials total mortality varies between 10 and 16% at 22 months follow-up (MADIT II, SCD-Heft, COMPANION). This heterogenous outcome may depend on variations in defining OMT and simultaneous use of cardiac resynchronization therapy (CRT).

Methods: From our in-hospital primary prophylactic ICD-registry we obtained total mortality and rate of appropriate ICD-interventions as well as use of heart failure drugs in 106 consecutively recorded patients from start of 2004 to end of 2006. These data were compared with similar data reported in MADIT II, SCD-Heft and COMPANION.

Results: Our registry demonstrated that among 106 consecutive patients with a median follow-up (FU) of 24 months all patients used betablockers, 98% an ACE-I or ARB and 50% an aldosterone-antagonist. The use of such drugs in the trials varied from 68 to 70% for betablockers, 68-94 for ACE-I or ARB and 10-55% for aldosterone antagonists. In our registry 68% of patients received CRT-D. Among these 2/3 were patients in New York Heart Association (NYHA) class III-IV° compliant with AHA guidelines for CRT and 1/3 in NYHA class II compliant with AHA guidelines for ICD and accepted for CRT by inclusion in the REVERSE-study. In MADIT II and SCD-Heft 40 to 50% of the patients had resting ECG with QRS width >120 ms. According to protocol all these patients had just an ICD. In COMPANION the patients in the intervention groups had CRT or CRT-D.

Twenty-two months FU demonstrated a total mortality of 7.6% in our registry, 14.2% in MADIT II, 9.7% in SCD-Heft and 16% in COMPANION. Rate of appropriate ICD interventions were respectively 19.8%, 27%, 10% and 19.3%. The rate of appropriate ICD interventions in our registry dem-

onstrates that our patients are as high risk patients as in the major studies.

Conclusion: Our registry demonstrates that heart failure patients selected according to guidelines for primary prophylactic ICD treatment obtain a even greater benefit than reported from major randomized studies when drug treatment is really optimized and cardiac resynchronization therapy a tool at hand.

183 General and specific markers of inflammation are associated with advanced cardiac allograft vasculopathy and an increased necrotic core component determined by virtual histology analysis

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Objectives: We evaluated an extensive profile of clinical variables and immune markers to assess the inflammatory milieu associated with advanced cardiac allograft vasculopathy (CAV) assessed by intravascular ultrasound (IVUS) and plaque tissue composition determined by virtual histology (VH).

Background: A range of inflammatory mediators are likely to be responsible for the development of CAV, but a broad characterization of these markers in relation to IVUS and VH endpoints has not been performed previously.

Methods: In total, 101 heart transplant (HTx) recipients were included and all patients underwent IVUS/VH examination and plasma sampling for measurement of the following immune markers: C-reactive protein (CRP), soluble tumor necrosis factor receptor-1 (sTNFR-1), interleukin-6 (IL-6), osteoprotegerin (OPG), soluble gp130, von Willebrand factor (vWf), vascular cell adhesion molecule-1 (VCAM-1) and neopterin.

Results: Mean Percent Atheroma Volume (PAV) was 32.4±9.5% and median necrotic core component (NCC) was 3.7 (1.6-5.3)%. Multivariate regression analysis revealed that CRP >1.5 mg/L (adjusted OR 4.5, $p<0.01$), VCAM-1 >391 ng/mL (adjusted OR 3.2, $p=0.04$) and neopterin >767 nmol/L (adjusted OR 3.8, $p=0.02$) were independently associated with PAV >32%, whereas only CRP >1.5 mg/L (adjusted OR 4.2, $p<0.01$) was independently associated with NCC >3.7%

Conclusions: Advanced CAV is associated with an inflammatory signature comprising of elevated CRP, VCAM-1 and neopterin and elevated CRP is also associated with an increased NCC. Forthcoming studies should clarify if routine measurements of these markers can allow accurate identification of HTx recipients at risk of developing advanced CAV and potentially vulnerable lesions with an increased necrotic component.

284 Female and male rat hearts are equally affected by chronic exogenous angiotensin II (ang II)

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Purpose: Increase in circulating ang II is part of the proposed mechanism behind pathological heart hypertrophy. Gender-dependent difference in heart hypertrophy due to pressure overload has been established. In the present study we wanted to examine if chronic ang II overexposure leads to a milder response in female compared to male hearts with respect to gene expression changes and ischemic injury.

Methods: 16 female and male age and litter matched rats (Fischer 344xBrown Norway) received ang II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) 200 ng/kg/min (miniosmotic pumps) or sham treatment. After 14 days hearts were excised, Langendorf perfused under isovolumetric conditions and subjected to 30 minutes ischemia and 30 minutes reperfusion. Left ventricular developed pressure, coronary flow and hearts rate were monitored. Hearts were harvested for expression of 18 genes related to hypertrophy, apoptosis, inflammation and interstitial fibrosis using quantitative RT-PCR. Statistics: TwoWay ANOVA, $p\leq0.05$, mean ± SD.

Results: Heart to body weight ratio increased significantly from 5.33±0.55 in sham to 7.16±0.98 g/kg after ang II in females. Corresponding values were 4.72±0.51 in sham and 5.76±1.0 after ang II in males (n.s.). Hearts treated with ang II had significantly higher LVDP prior to ischemia (sham 124±32 vs ang II 170±36 mmHg) and significantly higher end diastolic pressure at the end of reperfusion (26±17 in sham vs 68±22 mmHg in ang II). No significant gender difference was detected in heart function. Ang II increased expression of hypertrophy related genes ANF, 3β MHC, RD1 (AT-2, BNP unchanged); fibrosis related genes Col I, Col III, FN, TIMP; apoptosis related genes

TP53, casp 3 (Bcl-2 unchanged); inflammation related genes IL1, TNF (IL6, iNOS, eNOS unchanged).

Conclusion: Chronic exogenous ang II leads to significant increase in gene expression related to hypertrophy, interstitial fibrosis, inflammation and apoptosis and to increase in contractile force and reduced tolerance to ischemia in the heart. In rat heart, female gender seems not to protect against these changes.

Treatment induced change in gene express				
Corresponding protein	**One week**		**Two week**	
	TAC	**gender**	**TAC**	**gender**
ANF	increase	n.s.	increase	sign p<0.014
BNP	increase	sign p<0.016	increase	(p<0.06)
Akt-2 and MLC-1	decrease	n.s.	n.s.	n.s.
VDAC3	decrease	(p<0.063)	n.s.	n.s.
Ankyrin-like repeat prot	increase	interaction	increase	(p<0.088)
SERCA2	decrease	n.s.	n.s.	n.s.
iNOS	decrease	n.s.	n.s.	n.s.
ET-1	n.s.	n.s.	increase	n.s.

RT-PCR, TwoWay ANOVA analysis.

286 Increased gene expression diversity between female and male mice hearts after transverse aortic contriction (TAC)

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Purpose: Gender-dependent difference in heart hypertrophy due to pressure overload has been widely recognized using different experimental models and in clinical studies. The present study examines how increase in afterload affects gene expression in female hearts compared to male hearts.

Methods: 32 female and male balb/c mice were randomized to TAC or sham operation using minimal invasive procedure. After 1 week hearts were subjected to gene expression analysis (32K mouse genome survey v2.0 ABI microarrays) and at 1 and 2 weeks RT-PCR for genes selected based on previous reports and pilot studies.

Results: Heart/body wt was significantly increased by TAC and male gender (3.2±0.3 and 4.3±0.6 in sham versus 4.3±0.6 and 6.3±0.5 g/kg in TAC, females and males respectively). Microarrays revealed a total of 851 genes differently expressed ($p<0.05$) by gender or TAC. Only 6% of the differently expressed genes were associated with gender difference in sham. In contrast, 40% were differently expressed in male vs female TAC hearts. The rest (54%) were common genes regulated in both gender due to TAC. Gender difference in TAC induced gene expression was sorted according to biological processes. Male hearts showed changes in gene expression related to apoptosis, carbohydrate and nucleic acid metabolism, and protein transport whereas gene expression related to these processes appeared unaltered in female hearts. Female hearts showed changes in cell adhesion genes, whereas these appeared unaltered in male TAC hearts.

Conclusion: Increase in afterload leads to significant diversity in gene expression between female and male mice hearts.

369 Chromogranin B is regulated in the myocardium and circulation during heart failure development; a novel marker of cardiac disease

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Purpose: Chromogranin B (CgB) is a 50 kDa member of the granin protein family. A recent study found CgB closely associated with development of myocardial hypertrophy in vitro. However, whether CgB levels in the myocardium and circulation are regulated in proportion to markers of disease severity during heart failure (HF) development is currently unknown.

Methods: In a post-myocardial infarction (MI) HF mouse model animals were evaluated by echocardiography before being sacrificed one week post-MI. CgB gene expression was measured

by qRT-PCR and protein levels were assessed by Western blotting. Localization of myocardial CgB production was examined by immunohistochemistry. Circulating CgB and chromogranin A (CgA) levels were measured with RIA. Patients (n=80) were recruited mainly from an out-patient HF clinic and were compared to age- and gender-matched healthy control subjects (n=20).

Results: Myocardial CgB gene expression was 5.2 fold increased in the non-infarcted part of the left ventricle (LV) in HF animals compared to sham operated animals ($p<0.001$), and CgB mRNA levels in HF animals correlated significantly with animal lung weights ($r=0.74$, $p=0.04$). CgB protein levels were also markedly increased in both the non-infarcted (110%) and infarcted part of the LV (70%) compared to normal myocardium. In contrast, CgB levels were unaltered in pulmonary tissue, spleen, liver, gastrointestinal tract and skeletal muscle during HF development, indicating that the LV may be an important source of circulating CgB during HF development. By immunohistochemistry we localized myocardial CgB production to cardiomyocytes. Circulating levels of CgB were also higher in mice with HF than in sham operated animals (1.44 ± 0.12 vs. 1.02 ± 0.07 nmol/L, $p=0.003$), and in patients with established HF of mainly moderate severity compared to controls (1.69 ± 0.03 vs. 1.52 ± 0.05 nmol/L, $p=0.007$). Circulating CgB levels correlated with indices of disease severity in HF as levels in patients increased according to NYHA functional class (test for trend: $p=0.03$). Circulating CgA and CgB levels were only modestly correlated in HF patients ($r=0.31$, $p=0.005$), indicating that CgB may be regulated differently during HF development than CgA, another granin protein and established HF biomarker. Comparing the accuracy of circulating CgA and CgB for diagnosing HF, CgB was superior to CgA (ROC-AUC 0.70 vs. 0.61).

Conclusion: Myocardial production and circulating concentrations of CgB are increased in proportion to disease severity during HF development, indicating that CgB may represent an interesting novel marker of cardiac disease.

522 Cardiac Resynchronization Therapy (CRT) reduces and delays progression of inflammation in heart failure

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Purpose: CRT improves functional capacity and survival and causes reversed myocardial remodeling in patients with advanced heart failure (HF) and dyssynchrony. The aim of the present study was to investigate the short and long term effect of CRT on neurohormonal and cytokine levels in HF.

Material and methods: 81 consecutive patients all on optimal medical treatment, in NYHA-class III and IV, left ventricular ejection fraction (LVEF)≤35% and QRS-width≥120 ms, underwent successful implantation of a CRT-system. 15 were female (19%), mean age was 64(±10) years, and 52% had DCM. Mean QRS duration was 174±22ms. Plasma levels of NTproBNP, CRP, osteoprotegrin (OPG) 1, soluble (s) TNF receptor 1 (TNF-R1), IL1-receptor antiagonist (IL-Ra), monocyte chemoatractant protein (MCP)-1, von Willebrand factor (vWF), interleukin (IL)-6 and soluble CD40 ligand (sCD40L) were evaluated at baseline and 6 and 12 months after implantation. Response to CRT was a combined end-point of improvement of NYHA-class≥1 or improvement in peak oxygen consumption of ≥1 ml/kg/min and reduction of LV end systolic volume≥10%.

Results: At 6 months and 12 months 52% and 59% were responders, respectively. CRT resulted in significant reduction of levels of NTproBNP and IL-1-RA in both responders and non-responders ($p<0,05$). The level of IL-6 decreased in responders after 6 months, while remained unchanged during the whole follow up in non-responders. sTNF-R1 concentration remained unchanged in responders, while it increased in non-responders. The other measured markers did not change significantly after CRT in either group.

Conclusion: CRT improves cardiac function and functional capacity in 52% and 59% of patients after 6 and 12 months. Plasma levels NT-proBNP and IL-1RA were markedly reduced by CRT, demonstrating reverse remodeling and decreased myocardial inflammation.

535 Efficacy of cardiac resynchronization therapy depends on outcome definitions

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Purpose: The reported response to cardiac resynchronization therapy (CRT) is variable ranging from 50-90%, and we hypothesized that this was due to the response criteria used. We therefore evaluated response to CRT at 6 and 12 months and tested a novel response criterion.

Methods: 81 patients were enrolled. Pre- and postoperatively at 6 and 12 months we evaluated the following definitions of response in our material: 1. NYHA-class (increase by≥1); the patients physical and metal function i.e. quality of life, evaluated by the questionnaire short form (SF)-36 (improvement cut-off an increase>3 points), 4. 6 min walk test (increase≥10%), 5. peak VO2 (increase≥1 ml/kg/min), 6. left ventricular end systolic volume (LVESV) (reduction≥10%), 7. left ventricular ejection fraction (LVEF)(increase≥5%), and 8. n-terminal pro B-type natriuretic peptide (NT-proBNP) (reduction≥20%).

Results: Employing different criteria, response ranged from 31-79% (table). A combined definition of response to CRT was predefined as 1) a reduction of LVESV≥10% and 2) either an improvement in NYHA class by 1 or more or an increase in peak VO2≥1 mL/kg/min occured in 42 (52%) and 48 patients (59%) after 6 and 12 months of CRT, respectively.

Response to CRT by different cirteria		
Parameter	% responders at 6 months	% responders at 12 months
NYHA-class improved≥1	70	79
ΔPCS>3 points	60	63
ΔMCS>3 points	51	59
Δ6 min hall walk test≥10%	33	31
Increased peak VO2≥1/ml/kg/min	53	56
Reduction in Δ LVESV≥10%	61	74
Increased LVEF≥5%	52	55
ΔNTproBNP≥20%	62	67

NYHA - New York Heart Association, PCS - physical combined score, MCS - mental combined score, LVESV - left ventricular end systolic volume, LVEF - left ventricular ejection fraction, NTproBNP - N-terminal pro B-type natriuretic peptide.

Conclusions: 52% and 59% were responders to CRT at 6 months and 1 year given a predefined novel endpoint. Different response criteria to CRT gave response rates ranging from 31 to 79%.

540 Long interventricular mechanical delay (IVMD) and a high physical combined score (PCS, SF-36) predicts response to cardiac resynchronization therapy

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Purpose: CRT improves functional capacity and survival in patients with moderate to severe heart failure and dysyssynchrony. Non-responders to CRT is however an important issue. The purpose of this study was to evaluate predictors of respons to CRT.

Methods and results: 81 consecutive patients all on optimal medical treatment, in NYHA-class III and IV, left ventricular ejection fraction (LVEF)≤<35% and QRS-width ≥120 ms, underwent successful implantation of a CRT-system. 15 were female (19%), mean age was 64(±10) years, and 52% had DCM. Mean QRS duration was 174±22ms. A combined definition of response to CRT was predefined as 1) a reduction of LV

end-systolic volume of ≥10% and 2) either an improvement in NYHA class by ≥1 or an increase in peak oxygen consumption (VO2) of ≥1 ml/kg/min. After 6 and 12 months of CRT, 42 (52%) and 48 patients (59%) were responders, respectively. To identify possible selection criteria preoperatively, we tested in a univariate analysis the following baseline parameters as predictors of response: Rhythm (sinus rhythm/atrial fibrillation), etiology, QRS-width, IVMD, quality of life by short form (SF)-36 (QoL), NT-proBNP, peak VO2, NYHA-class, LV-volumes, and tissue doppler longitudinal septal to lateral wall delay (TDI sep-lat delay)≥65ms. We then included the parameters that had a p-value<0.1 in a forward stepwise multiple logistic regression analysis, and found a large pre-operative IVMD and a high PCS to be the only predictors of response to CRT at six and twelve months ($p<0.05$).

Conclusion: A large pre-operative interventricular motion delay (IVMD) and a high physical combined score (PCS) were predictors of response to CRT.

578 Dietary red palm oil concentrate protects the heart against the cyto-toxic effects of chemotherapy

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Purpose: Anti-cancer drug in clinics today induce apoptosis in cancer cells, lipid oxidation and protein aggregation. Strong anti-neoplastic anthracyclines like Daunorubicin (DNR) has a high efficacy against systemic neoplasm and solid tumors. However, their clinical use is hampered by the induction of toxicity in healthy tissue, most notably in the form of chronic cardio-myopathy and congestive heart failure. Previous studies have shown that dietary supplementation with red palm oil (RPO) can protect the heart against the consequences of ischaemia. We therefore hypothesize that dietary supplementation with red palm oil during chemotherapy may protect the heart against the cyto-toxic effects of DNR treatment.

Methods: In the control group rats were fed a standard rat chow diet (SRC) for 4 weeks. In the experimental group the SRC diet was supplemented with concentrated RPO (SRC+RPO) for 4 weeks. Both groups were injected with DNR every second day for 12 days at the end of 4 weeks on their respective diets. Hearts were excised and mounted on a working heart perfusion model. The hearts were normally perfused. Heart function was measured by monitoring aortic output. Biochemical samples were collected at 35 minutes of perfusion to perform biochemical analysis.

Results: Control hearts (SRC) treated with DNR showed a significant reduction in aortic output compared to the experimental hearts (SRC+RPO). In the presence of dietary RPO concentrate supplementation before and during treatment with DNR, aortic output was significantly increased from 31.73±2.28 ml/min to 37.05±2.67 ml/min at 35 min of perfusion. Biochemical analysis of Akt, Akt (Ser473), Akt (Thr308), Caspase-3 and PARP by western blot showed no significant changes between groups.

Conclusions: RPO concentrate supplementation improved the aortic output in hearts after a 12 day period of chemotherapy with DNR, compared to chemotherapy treated hearts not supplementation with RPO concentrate. Results suggest that the protection is not associated with the Akt or the apoptotic pathway.

610 TTA - the first PPAR ligand shown to increase fatty acid oxidation in normal and diabetic myocardium when administered in vivo

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Diabetic cardiomyopathy is associated with increased myocardial fatty acid oxidation (FAox) due to activation of the transcription factor PPARα by circulating FA. Pervious studies have shown that in vivo administration of PPARα ligands in animal models of diabetes, on the other hand, reduced myocardial FAox, most likely as a secondary effect of their lipid-lowering action, which will attenuate the FA supply and thus PPARα activity in the heart. In the present study we examined cardiac function and metabolism in diabetic (db/db) and non-diabetic (db/+) mice following 8 days treat-

ment with a novel pan PPAR ligand, tetradecylthioacetic acid (TTA, 0.5% dietary supplement).

Hearts from db/db mice exhibited increased rates of FAox (measured ex vivo using radioactive isotopes), reduced ventricular function, reduced tolerance to low-flow ischemia and reduced cardiac efficiency (assessed by measuring the relationship between cardiac P-V work and myocardial oxygen consumption, MVO2).

TTA-administration reduced plasma lipids both in db/+ and db/db mice. Despite this reduction in lipid supply to the heart, PPARα target genes in the heart (real time PCR) were unregulated and myocardial FAox was increased. The cardiometabolic effect of TTA was, however, abolished in PPARα KO mice. TTA-induced increase in FAox was associated with reduced cardiac efficiency in non-diabetic (db/+) mice. In diabetic mice, on the other hand, cardiac efficiency was unaltered, and ventricular function following low-flow ischemia was significantly improved following TTA-treatment.

Thus, TTA is the first ligand which increases FAox (both in normal and diabetic myocardium) following in vivo administration. The fact that the increase in FA oxidation (1) occurred in the face of reduced circulating lipids and (2) that it was abolished in PPARα KO mice, indicates that TTA directly activates PPARα in the heart. Apparently, TTA-treatment also confers cardiac protection in hearts from type 2 diabetic db/db mice, although the mechanism for this finding is not known.

843 Natriuretic peptides increase beta-1-adrenoceptor mediated signalling through inhibition of phosphodiesterase 3 in failing hearts

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Purpose: Whereas natriuretic peptides are known to increase cGMP levels with presumed beneficial vascular and cardiac effects through cGMP-dependent protein kinase in heart failure, we found an unexpected cardio-excitatory component of CNP and higher concentrations of BNP through natriuretic peptide receptor B (NPR-B) stimulation in failing cardiac muscle and explored the mechanism.

Methods: Congestive heart failure was induced in male Wistar rats by coronary artery ligation and adult ventricular cardiomyocytes were isolated by enzymatic digestion of the failing heart 6 weeks post infarction. Contraction studies were performed in left ventricular muscle strips. PDE activity was assayed using a standard two-step procedure and cyclic nucleotides were measured by radioimmunoassay. Apoptosis was determined by Annexin-V/propidium iodide staining and protein phosphorylation was measured by Western blot

Results: We demonstrate that NPR-B accounts for the majority of the natriuretic peptide-dependent guanylyl cyclase activity in failing cardiomyocytes and NPR-B stimulation enhances beta-1-adrenoceptor mediated contractile responses through cGMP-mediated inhibition of phosphodiesterase 3 (PDE3). CNP also enhanced beta-1-adrenoceptor mediated increase of cAMP levels to the same extent as the selective PDE3 inhibitor cilostamide and increased beta-1-adrenoceptor-evoked PKA activity, as demonstrated by increased phospholamban and troponin I phosphorylation. In primary cultured cardiomyocytes 20h inhibition of PDE3 by cilostamide or CNP promoted cardiomyocyte apoptosis in the absence and presence of beta-1-adrenoceptor stimulation by noradrenaline, indicative of adverse effects of NPR-B signalling in failing hearts.

Conclusions: Thus, the NPR-B-cGMP-PDE3 inhibitory pathway may in the long-term be detrimental to the failing heart through mechanisms similar to those operating during treatment with PDE3 inhibitors or during chronic beta-adrenergic stimulation. Administration of recombinant BNP to heart failure patients may stimulate NPR-B and elicit detrimental PDE3-inhibitory effects counteracting the presumed beneficial clinical effects expected of this treatment.

865 Intermittent claudication as a new predictor of outcome in heart failure: evidence from the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA)

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Purpose: Patients ≥60 years with NYHA class II to IV, low ejection fraction (EF) heart failure (HF) of ischemic etiology were enrolled in CORONA. Rosuvastatin did not reduce all-cause mortality. Intermittent claudication (IC) is an important predictor of clinical outcome in patients with coronary heart disease but its prognostic importance in HF has not been studied.

Methods: To determine whether IC is an independent predictor of mortality in ischemic, systolic, HF we built a multivariable model, first using demographic/clinical variables (step 1), then adding biochemical measures (step 2) and finally incorporating high-sensitivity C-reactive protein (hsCRP) and N-terminal pro B-type natriuretic-peptide (NT-pro BNP) – 3342 patients had all variables measured.

Results: 637 patients in CORONA had IC. 38% of patients with IC died compared to 28% of those without. There were 934 deaths overall. The table shows the top ten predictors of death at step 1, ranked according to the Wald X^2 (total model X^2 343). Creatinine was most predictive at step 2 (overall X^2 440). Log NT-pro BNP was the most powerful of the 14 independently predictive variables at step 3 (overall X^2 600). IC remained an independent predictor of mortality at all three steps.

Multiple Cox regression: all-cause mortality			
Variable	Hazard (95% CI)	X^2	p-value
Age/10	1.44 (1.31,1.58)	56	<0.0001
Ejection fraction ×100	0.97 (0.96,0.98)	44	<0.0001
BMI	0.95 (0.93,0.96)	42	<0.0001
Diabetes Mellitus	1.44 (1.25,1.66)	25	<0.0001
Sex	0.67 (0.57,0.80)	22	<0.0001
NYHA	1.40 (1.21,1.62)	20	<0.0001
Intermittent claudi.	1.40 (1.18,1.67)	14	0.0002
Heart rate/10	1.11 (1.05,1.17)	12	0.0005
SBP/10	0.94 (0.90,0.98)	10	0.0020

Conclusion: IC is a previously unrecognized independent predictor of outcome in HF.

948 Is the Na+, Ca2+ exchanger (NCX1) phosphorylated by cAMP-dependent protein kinase A (PKA)?

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Several studies have indicated that NCX1 is phosphorylated by the cAMP-dependent protein kinase A (PKA) in vitro and its activity is increased in response to PKA activation. However, no phosphorylation site has so far been reported and it remains unclear whether PKA actually does phosphorylate NCX1. Using bioinformatic analysis and peptide arrays we have analysed NCX1 for PKA phosphorylation sites. Although several NCX1 synthetic peptides were phosphorylated by PKA in vitro, only one putative PKA site was identified after mutational analysis. Immunoblotting experiments with different phospho-specific antibodies were performed to investigate phosphorylation of endogenous NCX1. No increase in phosphorylation level of full length NCX1 protein was detected after forskolin treatment of total heart homogenate. Phospho-NCX1 levels were also not increased in isolated neonatal cardiomyocytes after treatment with forskolin or isoproterenol. To further examine whether NCX1 can be PKA phosphorylated, alanine substituted NCX1-GFP-fusion proteins expressed in HEK293 cells were generated and are currently be tested. Although our data so far suggests that NCX1 might not be directly phosphorylated by PKA, we cannot exclude that PKA phosphorylates other protein partners and thus, has an indirect effect on NCX1 function.

949 Beta2-adrenoceptor-mediated inotropic and lusitropic responses are highly compartmentalized by PDEs; differential role of PDE3 and PDE4 in failing and non-failing rat myocardium

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β-adrenoceptors (-ARs) play a major role in regulating the myocardium function through cAMP/PKA-dependent pathway. Phosphodiesterases (PDEs) hydrolyze cAMP and thus compartmentalize cAMP-dependent effects. We explored the involvement of PDEs in limiting the β2-AR mediated positive inotropic (PIR) and lusitropic (LR) responses in sham and failing rat hearts. In rat left-ventricular strips, stimulation of β2-AR with (-)-adrenaline (300nM CGP 20712A present) exerted a small PIR and LR. In sham hearts β2-AR-mediated responses were increased by selective inhibition of either PDE3 (1μM cilostamide) or PDE4 (10μM rolipram). These responses were further enhanced when PDE3 and PDE4 were inhibited simultaneously, amounting to β1-AR-mediated responses. In failing rat hearts PDE3 inhibition enhanced the functional responses to both β1- and β2-AR stimulation while PDE4 inhibition had no effect on the functional responses despite a significant increase in cAMP levels. However, combined PDE3/4 inhibition in failing hearts further enhanced the functional responses as in the sham hearts. In conclusion, the β2-AR-mediated functional responses are limited by both PDE3 and PDE4, playing primary roles in non-failing hearts. In failing hearts PDE4 plays only a secondary role in limiting both β1- and β2-AR-mediated response, along with a reduction in total PDE4 activity. PDE3 and PDE4 to some extent regulate separate cAMP pools. During PDE inhibition, and especially in heart failure, the myocardial β2-ARs play a significant functional role.

951 Levosimendan exerts its inotropic effect mainly or exclusively by inhibition of PDE3

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Levosimendan is a novel inotropic drug used in the treatment of decompensated heart failure, and elicits inotropic responses by two mechanisms; calcium-sensitization and phosphodiesterase (PDE) 3 inhibition. Characterizing the relative importance of these two mechanisms in respect to contractility is relevant, as agents that elevate cAMP levels, such as milrinone and dobutamine, increase mortality in longterm treatment of HF patients. Thus the aim of the study was to single out each component and to characterize their relative importance in generating a positive inotropic response.

Concentration-response curves of levosimendan on failing human ventricular strips, revealed a positive inotropic response, with a maximum increase of 26% above control at 10-5 M levosimendan. In addition, lusitropic effects were elicited, a typical finding for PDE3 inhibition accentuating the cAMP-PKA-pathway In the presence of the PDE3 inhibitor cilostamide, the positive inotropic effect of levosimendan was nearly abolished, indicative of the importance of the PDE3-inhibitory component of levosimendan in generating a positive inotropic response.

Further experiments on the beta-adrenergic system in human and rat ventricular strips showed that levosimendan caused a significant shift of the concentration-response curve of isoproterenol to lower concentrations of agonist. In experiments done in the presence of cilostamide, levosimendan failed to cause a further shift to lower concentrations of isoproterenol. Thus, the main component responsible for the shift is the PDE3 inhibition by levosimendan.

The results demonstrate a dominating PDE-inhibitory mechanism of levosimendan with respect to increased contractility.

1248 Aquaporins: new players in heart physiology and ischemia-reperfusion injury

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Aquaporins (AQPs) mediate transport of water and some small molecules across cell membranes. They are expressed in the heart, but their function there is unknown. We investigated: 1. The role of AQP-4 in global ischemia-reperfusion injury in isolated, perfused hearts of AQP-4 knockout (KO) and wild-type mice; 2. Expression of AQP-1 and some protective protein kinases in AQP-4 knockout mice (real time PCR and Western blots); 3. Effect of perfusion and ischemia on expression of AQP-1 and -4 in mouse hearts and in humans during heart surgery.

Results: AQP-4 KO had reduced infarct size (34±17% vs. 50±23%, p=0.05) and improved post-ischemic left ventricular function (p=0.04). No difference was found in tissue water content. In AQP-4 KO hearts AMP-kinase expression was reduced by 15±13% (p=0.045), phosphorylation of AKT kinase decreased by 41±16% (p=0.0025), and AQP-1 mRNA increased ($p \leq 0.05$). No difference in ERK, JNK, PKC-epsilon and P38 expression and phosphorylation was found. Perfusion per se reduced AQP-1 and -4 mRNA ($p \leq 0.05$) levels, without any additional effects of ischemia-reperfusion. AQP-1, but not AQP-4 mRNA, decreased in patients after cardioplegia ($p \leq 0.05$).

Conclusion: AQP-4 may play a detrimental role in acute tolerance to ischemia. AQPs may be new targets in therapy of the heart

1264 Inhibitory properties of Sildenafil and Cilostamide on cGMP phosphodiesterases in failing cardiac tissue

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During heart failure circulating levels of CNP and BNP are elevated, leading to an increase in intracellular cGMP in cardiac tissue. Phosphodiesterases (PDEs) are the only enzymes that hydrolyze cAMP and cGMP to 5'AMP and 5'GMP, respectively. PDEs play an important role in regulation and compartmentalization of the second messenger cAMP in cardiac tissue but less is known about the role of PDEs in cGMP signalling in cardiovascular homeostasis.

The second messenger cGMP is generated by particulate guanylyl cyclase (pGC) and soluble cytoplasmic guanylyl cyclase (sGC). These two enzymes are activated by natriuretic peptides (NP's) and nitric oxide (NO), respectively. During heart failure, circulating levels of both NO and NP's are elevated, leading to increased intracellular levels of cGMP in cardiac tissue.

We have characterized cGMP-PDEs in normal and failing myocardium by using selective inhibitors, such as W7 (PDE1 inhibitor), EHNA (PDE2 inhibitor) cilostamide (PDE3 inhibitor) and sildenafil (PDE5 inhibitor).

Results show that the cGMP-PDE activity in normal hearts is about 50% higher than in failing hearts. Further, CNP stimulation of failing hearts increases the cGMP-PDE activity to nearly match the cGMP-PDE activity of a normal heart.

We demonstrate that PDE2 (EHNA-sensitive) accounts for about 50% of the total cGMP-PDE activity in both normal and failing myocardium and most of the remaining cGMP-PDE activity (about 40% of total) is abolished both by sildenafil and by cilostamide. Clearly, the additive activity of PDE2, 3 and 5 is apparently far beyond 100% of the total activity. This may be due to insufficient selectivity of the PDE3 and PDE5 inhibitors, cilostamide and sildenafil, respectively.

Upon clarifying their selectivity, we will know which PDEs play an important role in the hydrolysis of cGMP, and how the activity of the PDEs is altered from a normal to a failing myocardium. It is a special challenge to clarify if PDE5 in fact is

responsible for as much as 40% of cGMP-PDE activity in normal myocardium, as our results thus far could indicate, or if this finding is due to lack of selectivity of sildenafil.

1294 Adrenomedullin enhance proliferation and angiogenetic potential of late outgrowth endothelial progenitor cells

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Purpose: Endothelial progenitor cells (EPCs) represent a potential treatment option in cardiovascular diseases through its ability to replenish damaged endothelium and enhance neovascularisation at sites of ischemia. The utility of therapeutic EPC transplantation is potentially hampered by their low number in the circulation and inhibitory factors in the recipient causing low levels of engraftment and reduced survival of the transplanted cells. These obstacles can possibly be circumvented by ex vivo priming of harvested cells or co-treatment with pro-angiogenetic cytokines and peptides.

The endogenous vasoactive peptide adrenomedullin (AM) has been found to promote angiogenesis in various experimental models. Therefore, we sought to investigate the in vitro effects of AM on EPCs in culture.

Methods: To obtain EPCs, peripheral bood MNCs from healthy donors were plated on collagen coated culture plates with endothelial growth media (EGM2, 10% FBS). Colonies with cobblestone morphology appeared within 3 weeks and cells displayed high levels of proliferation after replating. Phenotypic characterisation was done by real-time PCR gene expression analysis of EPC markers. Proliferation was assessed using a colorimetric MTS cell proliferation assay and absorbance (optical density, OD) was measured at 490nm. Also, cells were seeded onto a Growth Factor Reduced Matrigel Matrix to allow differentiation and formation of tubular structures in order to assess the angiogenetic potential of EPCs stimulated by either AM or VEGF (vascular endothelial growth factor).

Results: Cultured cells expressed VEGFR-2, VE-cadherin, CD31, CD146, but were CD45 negative, confirming the phenotypic properties of EPCs. They also expressed the AM1 receptor (CLRL/RAMP2) mRNA. Addition of AM (10-7 M) stimulated EPC proliferation compared to control (OD 0.535, SE 0.035 vs. 0.456, SE 0.027, p=0.006). VEGF (50 ng/ml) increased growth compared to both control and AM alone (OD 0.636, SE 0.047, p=0.037 and 0.003). AM and VEGF combined (OD 0.676, SE 0.054) yielded an additional effect on proliferation compared to AM alone (p=0.002), but not compared to VEGF. Both AM and VEGF increased tubule formation on matrigel by respectively 48% (p=0.001) and 54% (p=0.016). The addition of both agents resulted in a more pronounced increase compared to control 105% (p=0.004).

Conclusion: This study shows for the first time that late outgrowth EPCs express the adrenomedullin receptor (AM1) gene. In addition, AM stimulates EPC proliferation and in vitro differentiation into tubular structures suggesting a pro-angiogenetic effect on EPCs similar to that of VEGF.

1370 Adrenomedullin-epinephrine cotreatment: an inodilator treatment approach that increases cardiac output without causing hypotension

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Purpose: The vasoactive peptide adrenomedullin (AM) has been shown to reduce mortality in the acute phase of experimental murine myocardial infarction. However, the hemodynamic profile of AM is potentially deleterious in human AMI and cardiogenic shock as the peptide is mainly vasodilative with no clear inotropic effects. We hypothesized that cotreatment with AM and low-dose epinephrine would increase cardiac output while maintaining perfusion pressure.

Methods: 15 open-chest pigs were employed. Left ventricular (LV) function was measured using a pressure-volume catheter, transit-time flow probes and coronary sinus blood sampling. LV energetics was analyzed in the PVA-MVO2 framework. After baseline recordings, the AM group (n=9) received a continuous infusion of 15 ng kg-1 min-1 AM while time-matched controls (TMC; n=6) received

saline as vehicle. The second set of measurements was obtained at steady-state after 2 h. Epinephrine (50 ng kg-1 min-1) was then added in both groups and the final recordings were carried out at steady-state.

Results: An increase in CO (16±13%; mean±SD) and heart rate (25±16%) with a parallel reduction in systemic vascular resistance by 14±12% was evident following 2 h of AM infusion (p<.01). The TMC group was unaffected. Compared to baseline, AM combined with epinephrine increased CO, heart rate, and coronary blood flow by 48±26, 57±35, and 67±27% (p<.01) vs. 13±12, 19±17, and 18±31% by epinephrine alone (NS). LV contractility, measured as PRSWi and dP/dtmax, were increased by 34±23 and 29±12% by the combined treatment (p<.01) vs. 23±27 and -2±14% by epinephrine alone (NS). Interestingly, MAP was maintained in the AM group (93±10 vs. 91±16; baseline vs. cotreatment; mmHg) while MAP in the TMC group tended to decrease (89±10 vs. 78±6; baseline vs. epinephrine; mmHg; NS). Y-intercept and slope of the PVA-MVO2 relationship were unaffected across all conditions.

Conclusions: Low-dose AM is inotropically neutral and increases CO primarily through chronotropic and vasodilative mechanisms. Cotreatment with low-dose epinephrine induces the hemodynamic profile of an inodilator with LV function moderately augmented beyond that achieved by epinephrine alone. This could possibly be related to interactions with common mediators such as cAMP.

1538 Isoform-specific activation of NFAT signalling in ANGII and NA-stimulated neonatal cardiomyocytes of mice

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A fundamental mechanism involved in cardiac hypertrophy and subsequent heart failure is sympathetic adrenergic hyperactivity accompanied by activation of the renin-angiotensin system, in which noradrenaline (NA) and angiotensin II (ANGII) are primary effectors mediating hypertrophic, apoptotic and fibrotic events in the heart. As NA and ANGII have been shown to regulate intracellular calcium in cardiomyocytes, we hypothesize that the calcium-sensitive calcineurin-Nuclear Factor of Activated T-cell (NFAT) signalling pathway is activated downstream of these factors. More specifically, our aim was to investigate isoform-specific activation of NFATs in ANGII and NA-mediated hypertrophy. The NFAT transcription factors have previously been shown to be important in the regulation of pathological hypertrophy in cardiomyocytes, and it is likely that each of the four isoforms, termed c1-c4, play specific roles in this regulation. However, little is known about the endogenous protein expression of the NFATs in cardiomyocytes, their differential regulation or their possible role in ANGII or NA signalling. Previous results show that stimulation of neonatal rat cardiomyocytes with ANGII or NA induces important characteristics of hypertrophy in addition to an increase in cell size. We have stimulated neonatal ventriculocytes from C57/B6 mice for 5, 10, 15 or 30 minutes or 24 hours with 1 µM ANGII or 100 µM NA. NFAT activity was quantified on Western blots using specific antibodies against the phosphorylated, inactive form of the isoforms.

Our results show that the level of pNFATc4 was reduced by 26% (n=4) and pNFATc1 by 12% (n=3) after 24 hours of stimulation with ANGII. Similarly, NA stimulation for 24 hours reduced the level of pNFATc4 by 22% (n=4) and pNFATc1 by 12% (n=3). Neither ANGII nor NA regulated the activity of NFATc2 or NFATc3 in our system. Preliminary results on the immediate response of these transcription factors showed that ANGII activated NFATc4 already after 5 minutes of stimulation, and NFATc1 after 30 minutes. Conversely, short-time NA stimulation did not affect the activity of any of the four NFAT isoforms. To our knowledge, we are the first to show isoform-specific activation of endogenous NFATs in isolated cardiomyocytes and we here demonstrate that the NFAT signalling system is controlled by both ANGII and NA. As the main therapies for heart failure aim at antagonizing the adrenergic and renin-angiotensin systems, understanding their molecular mechanisms of action is of clinical importance, and our data indicate that ANGII and NA act partly through activation of the NFAT signalling system.

1539 Increased Gi activity does not mediate the reduced beta-adrenergic inotropic effect in failing rat ventricle but Gi is necessary to maintain low basal contractility

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Purpose: Beta-adrenergic (βAR) inotropic effects are attenuated and muscarinic receptor-mediated inhibition thereof is enhanced in chronic heart failure. It has not been determined conclusively whether increased Gi activity contributes to the attenuated βAR-mediated inotropic effect in the failing heart. We determined whether increased Gi activity contributes to the reduced βAR-mediated inotropic effect through regulation of adenylyl cyclase (AC).

Methods: Contractility was measured in ventricular strips and AC activity in ventricular membranes from rats with post-infarction heart failure (failing) or sham-operated controls (Sham).

Results: The maximal βAR-mediated inotropic effect of isoproterenol was reduced by ~70% but potency was increased (~1 log unit) in failing vs. Sham (inotropic effect of 45% and 140% above basal respectively). In failing hearts, basal, βAR- & forskolin-stimulated AC activity was significantly lower than in Sham. Maximal muscarinic inhibition (by carbachol) of the βAR-mediated inotropic effect was larger in failing vs. Sham despite a 30% reduction in the ability of carbachol to inhibit βAR-stimulated AC activity in failing vs. Sham. Pertussis toxin inactivation of Gi did not increase the maximal βAR-mediated inotropic effect in failing or Sham, but the potency of isoproterenol was increased only in Sham (~0.5 log unit). In failing heart, the attenuated basal, βAR- & forskolin-stimulated AC activity was not restored by pertussis toxin inactivation of Gi. In failing ventricular strips pre-treated with pertussis toxin, simultaneous inhibition of phosphodiesterases (PDE) 3 and 4 alone produced a larger inotropic effect (by ~67%) than the maximal βAR-mediated inotropic effect in failing ventricle not pre-treated with pertussis toxin (75% and 45% above basal respectively). In contrast, in the absence of pertussis toxin pre-treatment, PDE 3 and 4 inhibition evoke negligible inotropic effects in failing ventricle.

Conclusions: These data indicate that the reduced βAR-mediated inotropic effect and AC activity do not result from increased Gi activity alone. However, Gi is critical for maintaining a low basal level of contractility through chronic inhibition of adenylyl cyclase in failing ventricle. Furthermore, a large inotropic response to PDE3 and 4 inhibition after pertussis toxin treatment in failing ventricle, indicates that increased PDE activity may also contribute to reduced βAR-mediated inotropic effects in failing ventricle.

1540 The inotropic effects of prostanoids are attenuated in failing human and rat ventricular myocardium

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Purpose: Non-selective COX-inhibitors and COX-2 inhibitors are deleterious in patients with heart failure (HF). Despite favorable hemodynamic effects, treatment of HF patients with epoprostenol was terminated due to increased mortality. Prostanoids increase cardiac output in HF patients, but it is unknown if it results from a direct inotropic effect on the myocardium. Knowledge of prostanoid inotropic effects in human ventricle is relevant, since most inotropic agents acting through elevating cAMP levels increase mortality in HF patients. In rat ventricle, stimulation of the prostanoid FP receptor (FPR) causes a large inotropic effect. Our objectives were 1) Characterize the inotropic effects of prostanoids in human ventricle 2) Determine if the FPR-inotropic effect in rat is mediated by increased phosphorylation of myosin light chain-2 (MLC-2) 3) Elucidate the signaling pathway(s) activated by FPRs.

Methods: Contractile force was measured in ventricular strips from non-failing or HF human and Wistar rat hearts. Muscles were also snap frozen and the ratio of phosphorylated protein was quantified by Western blotting for both MLC-2 and myosin phosphatase targeting subunit-1 (MYPT-1).

Results: Several prostaglandins evoked a positive inotropic effect in human ventricle from donor hearts (non-failing), whereas only negative inotropic effects were observed in terminally HF human hearts to all prostaglandins tested. Similarly, the FPR-inotropic effect was reduced (~50%) in HF rats. FPR activation increased phosphorylation of MLC-2 through both activation of MLC

kinase and inhibition of MLC phosphatase. The FPR-inotropic effect was modestly affected by the IP3 receptor blocker 2-APB, but not by the protein kinase C inhibitor bisindolylmaleimide. FPR signaling mechanism(s) regulating MLC-2 phosphorylation likely extend beyond those classically established for Gq/11-coupled receptors.

Conclusions: Our study indicates prostanoids provide inotropic support in normal myocardium by increasing MLC-2 phosphorylation, likely enhancing myofilament calcium sensitivity. This enhancement of myofilament calcium sensitivity, representing a less energy-demanding mechanism of inotropic support may be advantageous in HF, compared to cAMP-mediated mechanisms. As such, the significant reduction of prostanoid inotropic support observed in both human and rat HF ventricle may be detrimental. Understanding the cardiac role of prostanoids is necessary for the development of therapy for HF patients that can utilize the beneficial hemodynamic effects of prostanoids while mitigating potentially deleterious effects upon contractility.

1558 Isolated factors identified in the effluent of preconditioned hearts offers cardioprotection

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One or several short episodes of non-lethal ischemic episodes administered to a heart prior (Ischemich Preconditioning, IPC) or directly after (Ischemic Postconditioning, IschPost) a major ischemic episode facilitates myocardic survival during the reperfusion phase.

There is strong evidence that this protection is due to a factor released from the heart during the conditioning phase. The perfusate containing protects the heart when administered 10 minutes prior and after ischemia (25% ± 3% and 30% ± 3% vs 55%± 3% for control, $p < 0.05$). The factor is so potent that when administered only in 3 x 30" bursts after ischemia it is enough to significantly lower infarct sizes (40% ± 5% vs control 56%± 4%, $p<0.05$.

We have attempted to characterise and isolate this factor from effluate released during preconditioning of isolated rat hearts in a Langendorff setup. We fractionated the proteins in the effluent based on both size and hydrophobicity and the fractions were administered to unconditioned rat hearts prior to a 30 min ischemic event and the infarct/risk ratio was calculated after 120 min reperfusion. Hearts perfused with a hydrophobic fraction over 10kDa had ischemic injuries at the level of an IPC heart (25% ± 6% vs 22% ± 3%, $p < 0.05$) and over twotimes lesser injuries than the hearts that were perfused with a hydrophilic fraction and the hearts that were perfused solely with Krebbs Heinsleitt Buffer (KHB) (25% ± 3% vs 55% ± 3% and 50% ± 12%, $p < 0.05$). We analysed the different fractions by LC-MSMS and identified a total of 88 proteins released during preconditioning and of several of them were only identified in fractions which were protective.

1563 Activation of Notch signalling in cardiomyocytes during post-infarction myocardial remodelling

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Purpose: Notch signalling is crucial for cell-to-cell interaction during cardiovascular development and may influence differentiation, proliferation, apoptotic events, and mediate inflammatory responses. Thus, the Notch pathway may be a signalling system with an important role also during remodelling of adult myocardium in heart failure (HF) development. The purpose of the present study was to investigate whether Notch receptors (Notch1-Notch4) and ligands (Jagged 1, Jagged2, Delta-like (Dll)-1, and Dll4) are expressed and regulated in myocardial tissue and in isolated cardiomyocytes in experimental and clinical HF.

Methods: Myocardial infarction (MI) and HF were induced in rats by coronary ligation. Gene expression analysis was performed in myocardial tissue and in isolated cardiomyocytes in the acute (7 days) and in the more chronic phase (28-56 days) after MI and in sham-operated control rats as well as in myocardial tissue from patients with severe chronic HF and in healthy controls. Expression levels of Notch intracellular domain (NICD), which indicates Notch signalling, in cardiomyocytes were investigated by Western blotting. The localisation of the different Notch signalling components was examined by immunohistochemistry.

Results: Gene expression of all Notch receptors and ligands investigated were found in rat and human myocardial tissue. Notch2 and Dll4 mRNA levels were significantly increased in non-infarcted

left ventricle (LV) of rats with chronic HF and in failing human LV. Notch2, Notch3, Notch4, and Jagged1 mRNA levels were higher and Jagged2 mRNA levels lower in the ischemic region of rats with MI compared to non-ischemic tissue and sham myocardium ($P<0.05$). The Notch receptors and ligands as well as NICD were localised to cardiomyocytes and vessels. In isolated rat cardiomyocytes, Notch3 and Notch4 appeared to be the predominant Notch receptors, and mRNA levels of these receptors in cardiomyocytes were upregulated in chronic HF together with mRNA levels of the Notch ligands Jagged1 and Dll4 ($P<0.05$). A parallel increase in NICD-3 and -4 was found in cardiomyocytes ($P<0.05$), indicating increased Notch3 and Notch4 signalling.

Conclusions: The present study demonstrates presence of Notch receptors and ligands as well as NICD in adult rat and human myocardial tissue and in isolated cardiomyocytes with differences in their relative expression levels and altered expression levels in failing vs. non-failing myocardium. Thus, our data indicate a role of Notch signalling during myocardial remodelling in HF.

1590 Increased production of CXCL16 in experimental and clinical heart failure; a possible role in extracellular matrix remodeling

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Background: Although both experimental and clinical studies indicate a role for inflammation in the development of myocardial failure, knowledge about the production and functional role of the different inflammatory actors in heart failure (HF) remains incomplete. Based on its combined role in inflammation and vascular remodeling, we hypothesized a role for CXCL16 in the pathogenesis of HF.

Methods and Results: Our main findings were: (i) Patients with chronic HF (n=188) had significantly raised plasma levels of CXCL16 as compared with healthy controls (n=20), that significantly correlated with the degree of disease severity. (ii) Left ventricular (LV) tissue from patients with severe HF (n=8) showed enhanced production of CXCL16 compared to non-failing LV (n=6) as assessed by Western blotting. (iii) In mice exposed to pressure overload we found enhanced CXCL16 mRNA levels in the LV, with particularly high levels in those with decompensated hypertrophy. In mice with post-myocardial infarction (post-MI) HF, expression of CXCL16 was increased both in the infarcted and the non-infarcted areas of LV 3 and 7 days after coronary ligation, indicating early onset of increased CXCL16 production. The increase in CXCL16 in the tissue at 7 days post-MI was associated with increased CXCL16 levels both in cardiomyocytes and in non-cardiomyocytes (i.e., endothelial cells and fibroblasts). (iv) In vitro experiments showed that CXCL16 induces enhanced protein synthesis in neonatal rat cardiomyocytes, and promotes proliferation and matrix metalloproteinase (MMP) activity in myocardial fibroblasts accompanied by a significant increase in gelatinolytic activity. Furthermore, CXCL16 induced increased MMP activity in cardiomyocytes, primarily reflecting increased MMP-2 levels. (v) Using specific inhibitors in cell experiments, we showed that the effect of CXCL16 on fibroblasts involved activation of the c-Jun N-terminal kinases.

Conclusion: We demonstrate enhanced myocardial expression of CXCL16 in both experimental and clinical HF. The combined effect of CXCL16 on cardiomyocytes and myocardial fibroblasts suggest a role for CXCL16 in extracellular matrix remodeling and ultimately also in the development of HF.

1591 Signalling and gene expression by interleukin-1beta (IL-1beta) and IL-33 in cardiomyocytes

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Purpose: IL-1β and IL-33 are related cytokines implicated in cardiomyocyte pathology. The hypertrophic agonist endothelin-1 (ET-1) modulates expression of IL-33 receptors (ST2) and may influence IL-33 signalling. We compare the effects of IL-1β and IL-33 on cardiomyocyte signalling and gene expression, and examine the effects of ET-1 pretreatment.

Methods: Neonatal rat ventricular myocytes were exposed to IL-1β/33 ± ET-1 (5 h). Activation of mitogen-activated protein kinases (MAPKs) was assessed by immunoblotting and protein kinase assays. Significant changes in RNA expression

(0.5, 1, 2 h) were determined using microarrays (FDR<0.05;>1.5-fold) and quantitative RT-PCR.

Results: IL-33 activated ERK1/2, JNKs and p38-MAPK (maximal at 15 min), but the degree of activation was substantially less than that induced by IL-1β. Consistent with relative MAPK activation, IL-1β had a greater effect on cardiomyocyte RNA profiles (373 RNAs up- and 271 down-regulated) than IL-33 (127 RNAs up- and 25 down-regulated). Only 17 RNAs were modulated by IL-33 alone including IL-1α and IL-1β, and IL-33 was generally effective in upregulating mRNAs encoding chemokines/cytokines. Using qPCR, we confirmed that IL-33 rapidly increased IL-1α/β mRNAs. The response to IL-1β was less than that of IL-33 and was delayed. In contrast, IL-1β more potently induced mRNAs for IL-6, TNFα, and colony stimulating factors. ET-1 increased expression of soluble ST2 mRNA and protein which may prevent IL-33 activation of membrane bound receptors. However, ET-1 pretreatment inhibited activation of p38-MAPK, not ERK1/2 or JNKs, by IL-33 or other other stimuli (IL-1β, H2O2, TNFα). Thus, inhibition of p38-MAPK by ET-1 reflects modulation of the signalling cascade rather than any effect on IL-33 receptor expression. The degree of phosphorylation of upstream kinases for p38-MAPKs in response to IL-1β was similar with/without ET-1 pretreatment suggesting an effect at the MAPK level. Apart from influencing p38-MAPK activation, ET-1 also modulated changes in mRNA expression (e.g. IL-6, TNFα) induced by IL-1β.

Conclusion: IL-1β activates intracellular signalling and gene expression more potently than IL-33 in cardiomyocytes. ET-1 pretreatment modulates these responses providing evidence of signal integration. We conclude that hypertrophic stimuli may attenuate the cardiomyocyte inflammatory response.