1006-16 Apoptosis in Human Abdominal Aortic Aneurysms Is Associated With Increased Expression of p53

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Abdominal aortic aneurysms (AAA) are characterized by degeneration of the cellular and matrix components of the aortic wall. We speculate that loss of vascular smooth muscle cells (VSMC) might contribute to medial degeneration and that this might occur through programmed cell death or apoptosis. To determine if apoptosis might play a role in the pathogenesis of AAA, aortic specimens from 5 normal transplant donors and 5 patients undergoing surgical repair of AAA were examined. Using morphometric analysis of α-actin stained tissue sections; the mean density of medial VSMC was reduced by 74% in AAA as compared with normal aorta. Furthermore, ultrastructural analysis of the remaining VSMC demonstrated loss of cell volume and significant alterations in chromatin. *In situ* labeling of fragmented DNA demonstrated extensive nuclear staining, clearly indicative of apoptosis, throughout the medial layers of each aneurysm specimen. No significant areas of positive staining were observed in normal aortas. Because p53 is often associated with the induction of apoptosis, RT-PCR was employed to detect p53 mRNA. A 4-fold increase in p53 expression was observed in AAA specimens as compared to normal aorta. A similar increase in the amount of immunoreactive p53 protein was demonstrated by Western blot and immunohistochemistry. These results indicate that the loss of VSMC that occur with aortic medial degeneration might be due to alterations in p53-mediated cell cycle regulation with the induction of apoptosis and might be responsible in part for the development of aortic aneurysmal disease.

1006-17 Brief Myocardial Ischemic Insult Results in Activation of the Stress Activated Protein Kinase

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Acute, severe and prolonged ischemic myocardial insult results in myocardial necrosis. Brief episodes of ischemia do not cause any immediate structural abnormality but their recurrences may subsequently result in cardiomyopathic state and congestive heart failure. Variable amount of myocardial hypertrophy is an obligatory component of cardiomyopathy which results from activation of AP1-binding proteins. We investigated the effect of hypoxia on stress activated protein kinase (SAPK) which is a transactivator of AP1 proteins.

Rat embryonic cardiocytes (H9C2) were subjected to hypoxia for 5, 15, 30, 60, 180, and 360 min. The cells were harvested, lysed and immunoprecipitated with anti-SAPK antibody. In vitro immune complex kinase assays in the protein precipitates using GST-Jun (2–100) fusion protein as a substrate demonstrated an early activation of SAPK activity. There was 10 to 15-fold increase in the SAPK activity at 15–30 min as compared to control cells. The intensity decreased at 60 min and reduced to nearly basal levels by 3 hours.

To investigate pathophysiological significance of SAPK, LAD coronary artery in 5 rats was occluded for 5, 10, 15, 30 and 60 min. The SAPK in the ischemic anterior wall increased to the peak level by 15 min and then reduced significantly to basal levels by 1 H.

Transient transfections of H9C2 cells with wild-type SEK1 and dominant-negative SEK1 vectors (SEK1 K-R) demonstrated SEK1 involvement in SAPK induction. The studies with transient tarnsfections with c-abl and Rac-1/Rho are in progress to elucidate the upstraem cascade activating SEK1.

The immediate activation of SAPK in myocardial cells may explain long-term deleterious effects of brief episodes of hypoxia in evolution of ischemic cardiomyopathy.

1006-18 Can the Geometry of a Coronary Stenosis Predict Occlusion and Myocardial Infarction in the Following Year

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The risk of myocardial infarction (MI) is known to be poorly related to the severity of pre-existent coronary stenoses. In order to test whether their shape could be a stronger predictive marker, we have studied 38 patients (pts) who underwent a coronary angiogram (angio) less than 12 months (m) before a myocardial infarction (MI) documented by a second angio which allowed for the identification of the culprit lesion and who did not need revascularisation in the interval. The culprit and the other stenoses (control) of the first angio were quantitatively analysed and the following geometric characteristics were measured: percent stenosis, symmetry index (from 0 — totally eccentric — to 1 — perfectly concentric), length, maximal as well as average inflow and outflow angles. The comparison between the 38 culprit and the 130 control stenoses in the same pts gave the following results:

	Culprit	Control	p
Percent stenosis (%)	50.2 ± 13.9	40.1 ± 13.4%	<0.0001
Symmetry index	0.69 ± 0.26	0.49 ± 0.28	<0.0001
Length (mm)	10.3 ± 4.9	8.7 ± 4.8	0.006
Maximal outflow angle (°)	30.3 ± 10.7	23.6 ± 8	<0.0001

The minimal lumen diameter and the other angles were not significant.

In conclusion, the shape of coronary stenoses as defined by the length, the symmetry index and the maximum outflow angle is significantly associated with the subsequent occurrence of a MI, at least in the first year of follow-up.

1006-19 Platelets Are Not Degranulated by a Nonionic Dimeric Contrast Agent During Diagnostic Coronary Angiography and Coronary Interventions

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Flow cytometric studies have shown that contrast media (CM) (nonionic iohexol and ionic diatrizoate) degranulate platelets in vitro. To further address this matter, blood from 30 adult patients (pts) undergoing diagnostic cardiac catheterization was added in vitro to equal volumes of ioxaglate (ionic dimer), iodixanol (nonioinic dimer), buffer, or buffer with 10 μM U46619, a thrombin-independent platelet agonist. All pts received IV heparin, had taken ASA 325 mg/day for ≥ 1 day, and were also randomized to receive either ioxaglate or iodixanol. Blood was sampled sequentially from the coronary artery and the right atrium, before and after the administration of CM during diagnostic angiography, and, for a subset of 12 pts, before and during coronary intervention (PTCA and/or stent placement). For the latter pts, heparin was given to assure an ACT of ≥ 275 sec. In vitro, as assayed by whole blood flow cytometry, a small degree of degranulation (as determined by % platelets positive for the α-granule protein P-selectin, mean ± SE) was observed with ioxaglate, but none was seen with iodixanol (Table). In vivo, no degranulation was seen for either CM for any pt probably due to rapid hemodilution of CM with coronary blood flow. Platelet adhesion/aggregation (platelets/cm^2 × 10^6, mean ± SE) to a collagen substrate in whole blood at 270 sec^{-1} was reduced by ioxaglate in vitro, but unaffected by iodixanol in vitro (Table) and by both CM in vivo. No pt suffered a thromboembolic event. In summary, both in vitro and in vivo, the nonionic dimer iodixanol results in neither enhanced platelet adhesion/aggregation under arterial-like rheologic conditions nor platelet degranulation.

	U46619	Buffer	Ioxaglate	Iodixanol
P-selectin	70.5 ± 4.2	3.1 ± 0.3	6.0 ± 0.8	3.0 ± 0.3
Aggregation	N/A	1.7 ± 0.2	0.2 ± 0.04	1.8 ± 0.2

1006-20 The Role of Proteoglycan (Versican) Cleavage by the Metalloenzyme Matrilysin in Unstable Atherosclerotic Lesions in Patients

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Recent descriptions of the expression of matrix metalloproteinases, such as collagenase and gelatinase, in atherosclerotic lesions indicate a potential role in plaque rupture by degrading matrix proteins, which compromises the structural integrity of the lesion. Because components of atherosclerotic lesions also comprise proteoglycans and elastin, we sought to define the sites and cell source of metalloenzymes that could specifically cleave these substances: namely, matrilysin (ML) and macrophage metalloelastase (ME). Samples of lesions from patients undergoing carotid endarterectomy for clinical indications (n = 18) were analyzed by Northern hybridization. Both ML and ME were expressed in atherosclerotic lesions, but not in normal arteries (n = 2). In situ hybridization and immunohistochemistry revealed prominent expression of ML by cells confined strictly to the border between acellular lipid cores and overlying fibrous cap regions. ME was expressed in these same border areas. Staining with CD-68 antibody demonstrated that ML was produced by lipid-laden macrophages, and organ cultures exhibited release of ML from endarterectomy tissue. Immunohistochemical staining for versican demonstrated that this vascular proteoglycan was present at sites of ML expression. Biochemical studies showed that ML degraded versican much more efficiently than did other metalloproteinases present in atherosclerotic lesions. Our findings suggest that the site-specific expression of ML

in atherosclerotic lesions may cleave structural proteoglycans, which could induce potential separation of caps and shoulders from lipid cores. Because ML exhibits limited inhibition by tissue inhibitors of metalloproteinases as compared with collagenase or gelatinase, it is a likely candidate for lesion destabilization.

1006-21 Cytokine Profiles of T Cell Clones From Human Atherosclerotic Plaques

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T cells can be divided into three subpopulations: T_H1 cells, which produce IL-2 and IFN-γ, T_H2 cells which produce IL-4, IL-5 and IL-10, and T_H0 cells which produce both IFN-γ and IL-4. Atherosclerotic lesions are characterized by the presence of an inflammatory infiltrate. Besides activated (HLA-DR$^+$) macrophages, activated memory T lymphocytes are also present in such lesions. The role of these T lymphocytes in relation to the pathogenesis of atherosclerosis is unknown. The purpose of the present investigation is to analyse the cytokine profiles of T cells in atherosclerotic plaques.

Aortas were collected at autopsy within 5 hours postmortem. The lesional intima was removed, T lymphocytes were isolated, and polyclonally expanded using PHA. After approximately one week the obtained cell lines were cloned using limiting dilution. Cytokine profiles of the obtained T cell clones were analyzed. Therefore, supernatants were collected after 24 hours of culture with coated OKT-3 and PMA, and the production of IL-4, IFN-γ and IL-2 was analyzed using a sandwich ELISA.

From four donors, a total of 81 T cell clones were generated, 72 of them were CD4$^+$, and 9 were CD8$^+$. Most CD4$^+$ clones (± 90%) produced both IFN-γ and IL-4 (T_H0 type). However, 10% of the CD4$^+$ clones showed a T_H1-like cytokine profile.

Our results indicate that the T cell population in plaques is heterogeneous and both IL-4 and IFN-γ may participate in regulating local inflammatory responses.

1007 Hypertension

Wednesday, March 27, 1996, 9:00 a.m.–11:00 a.m.
Orange County Convention Center, Hall E
Presentation Hour: 9:00 a.m.–10:00 a.m.

1007-85 Recent Trends in Antihypertension Therapy

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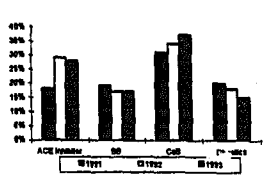
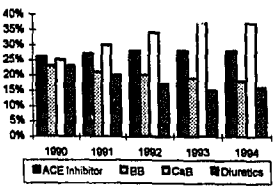
Administrative data were used to evaluate the effects of JNC V on treatment patterns of hypertension (HTN) in a 2 million member independent practice HMO with an open formulary. Newly diagnosed members with HTN were defined on the date of their first claim/encounter with HTN-specific ICD-9 criteria, preceded by at least a 12 month interval without HTN criteria, and corroborated by a second criterion (drug claim within ± 6 months or a second ICD-9 encounter/claim within + 12 months). As shown below, calcium blockers (CaB) were preferentially chosen for new starts 1991 to 1993 with a concomitant decline in diuretics and beta blockers (BBs). This correlated with an increasing penetration of CaB in point prevalence of treatment patterns for all members with HTN (new and previously diagnosed) at year end 1990 to 1994

First Drug Used for New HTN



Drugs for HTN by Year



Thus, despite JNC V's emphasis on BBs and diuretics as preferred first-line agents, CaBs continue to show increased penetration for both new starts and overall treatment of HTN.

1007-86 Significantly Increased Left Ventricular Mass Is Detected by 3D Echo in Clinically Normotensive Patients With Exercise Induced Hypertension

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One-third of clinically normotensive patients with exercise induced hypertension (ExHBP) will develop overt hypertension within 5 years. The presence of increased LV mass in these patients remains controversial. We hypothesize that previous failures to detect increased mass by 1D and 2D echo have been due to their large measurement variability which has masked a relatively small difference of LV mass compared to normal subjects. The purpose of this study was to compare the 3D, 2D and 1D echo methods in a group of 20 patients with atypical chest pain, negative treadmill test and ExHBP (Increase of SBP > 85 mmHg in males, increase of SBP > 60 mmHg in females) to 20 age and sex matched normal subjects. LV mass was calculated for 3D echo by ventricular surface reconstruction using a real-time scanner to acquire 8–10 short axis cross-sections, an acoustic spatial locater, a personal computer and the line of intersection display for operator guidance. LV mass was calculated by 2D echo (ASE truncated ellipsoid method) and for 1D echo (Penn method). Mass was indexed to height^2. Data were analyzed by the unpaired T test.

	Mass Index Mean ± SD	T	p
3D-ExHBP	54.6 ± 7.9 g/m^2	4.44	0.0001*
3D-Normal	43.7 ± 6.0 g/m^2		
2D-ExHBP	51.5 ± 14.5 g/m^2	0.13	0.90
2D-Normal	50.8 ± 20.2 g/m^2		
1D-ExHBP	69.5 ± 33.5 g/m^2	0.41	0.68
1D-Normal	65.7 ± 23.3 g/m^2		

Conclusion: LV mass in clinically normotensive patients with exercise induced hypertension is significantly increased compared to normal subjects. 3D echo but not 2D or 1D echo detects this change due to its smaller measurement variability (standard deviation).

1007-87 Reliability in Patient-Reported Ambulatory Blood Pressure Monitoring

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Blood pressure (BP) recordings taken by patients are used with increasing frequency to establish the diagnosis of hypertension (HTN) and judge the adequacy of treatment. It is important for physicians to be confident of the reliability of these recordings. Thirty-eight subjects (14 with high BP, 12 borderline high BP and 12 normotensives) wore a 24-hour ambulatory BP monitor with a memory chip capable of storing all measurements made during the testing period. Subjects were instructed to record their BP on diary cards when it was displayed by the monitor. They were not told of the memory chip until after the testing period. Of all valid BP measurements made on 14 hypertensive subjects, 21.5% involved significant errors: the subject neglected to record any BP value or the recorded value was incorrect. The normotensives' error rate was 18.6% (12 subjects) and borderline hypertensives' error rate 27.4% (12 subjects). Subject reported mean arterial pressure differed significantly from machine reported mean arterial pressure ($t = 2.16$, $df = 37$, $p = 0.38$). Hypertensives made a disproportionately larger number of misreporting errors which were above the true BP ($f = 6.54$, $df = 1$, $p = 0.18$). This pilot study suggests that home BP measurements reported by patients may not be as reliable as is generally thought.

1007-88 DD Genotype of the Angiotensin-Converting Enzyme (ACE) Gene Is Associated With Abnormal Diastolic Function in Essential Hypertension

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An insertion/deletion polymorphism in the ACE gene accounts for 50% of the variation in serum ACE activity. ACE is responsible for the generation of angiotensin II which has not only pressor and mitogenic activity but also impairs active myocardial relaxation. We investigated the contribution of genetic polymorphisms a the ACE gene to the development of diastolic functional abnormalities in 100 patients with essential hypertension. All patients underwent echocardiographic assessment of left ventricular mass index (LVMI) and diastolic function ratios of peak and integrals of early to late filling (E/Ap and E/A_I respectively), and determination of ACE genotype from leukocyte DNA. There was no significant difference in age, sex blood pressure or LVMI among genotypic groups. Analysis of covariance (ANOVA) modelled for indices of diastolic function, adjusting for age, sex, heart rate and LVMI demonstrated the E/Ap interacted with age ($p < 0.0001$), heart rate ($p <$