CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
RESPIRATORY AND CARDIOVASCULAR SYSTEM

ORGANIZED BY

SOCIETY OF TOXICOLOGIC PATHOLOGY - INDIA (STP-I)

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Induced lesions of the vascular system
The International Federation of Societies of Toxicologic Pathologists is pleased to sponsor the lectures given by:

Kevin ISAACS

during the 4th STPI conference
1-3 November 2012, in Bangalore

http://www.ifstp.com
Introduction

Basic points
- No time for ‘Vascular system 101’
- Vessels are *almost* ubiquitous
- Not just conduits

Functions of vessels
- Carrying blood & lymph with contents
  - Gases
  - Cellular factors
  - Humoral factors
  - Membrane transport
- Permeability
  - Endocytosis
  - Transcytosis
  - Fenestrae
  - Ion channels
- Maintain gradients
  - Osmotic
  - Hydrostatic
  - Concentration
  - Ionic

Not all the same
- Specialised function
  - Splanchnic bed
  - Coronary arteries
  - BBB
  - Testes
- Specialised structures
  - Glomeruli
  - JG apparatus
  - Plexi (pampiniform, ocular)
Animal models - not covered here

Animal models used extensively

- Attempt to mimic human disease
  - Atherosclerosis
  - Diabetes
  - Hypertension
  - Immune-mediated diseases

Drug candidate identification

- Pre-development studies
- Proof of concept

Mechanistic research

- Problem solving novel findings
Vessel structure 1

Arteries
- Elastic
- Muscular
- Large to small
- Specialisations
- Anastomoses
- Carotid and aortic bodies
- Baroreceptors
- Chemoreceptors
- Vasa vasorum
- Species differences

Arterioles
- 10 - 100µm, smooth muscle
- Metarterioles
- Arteriovenous anastomoses
- Pre-capillary sphincters
- Peripheral resistance
- Tissue specialisation
- JG apparatus

Capillaries
- 5 - 10µm, no smooth muscle or adventitia
- Specialisations
  - Blood brain barrier
  - Blood-CSF barrier
  - Continuous BM
  - Fenestrated BM
- Sinusoidal
  - Continuous basal lamina
  - Endocrine glands, gut, pancreas, kidney
  - Discontinuous basal lamina
  - Liver, spleen

Retia mirabilia
- Arteriovenous complexes
- Counter-current exchange
- Solutes
- Kidney medulla/papilla
- Temperature
- Testes
- Eye

Anatomy has a profound influence on disease processes
Venules
- 8 - 100µm, thinner wall than arterioles
- Specialisations
  - Anastomoses
  - High endothelial venules

Veins
- Valves
- Tissue specialisations
  - Lungs
  - Oxygenated blood
  - Rodent cardiac muscle
  - Hepatic portal vein
  - Valveless in dura mater
  - Plexi
  - Vasa vasorum
  - Large veins

Lymphatics
- Carry lymph
- Not in CNS
- Valves
- Discontinuous BM

Lymphatic capillaries
- Blind ending
- No smooth muscle or adventitia
# Cell types

<table>
<thead>
<tr>
<th>Endothelial cells</th>
<th>Smooth muscle cells</th>
<th>Pericytes/veil cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Permeability barrier</td>
<td>• Contractile</td>
<td>• Support small vessels</td>
</tr>
<tr>
<td>• Fluid filtration</td>
<td>• Adrenergic innervation</td>
<td>• Pluripotential</td>
</tr>
<tr>
<td>• Biologically active</td>
<td>• α₁, α₂, β₂</td>
<td></td>
</tr>
<tr>
<td>• Haemostasis</td>
<td>• Proliferative potential</td>
<td></td>
</tr>
<tr>
<td>• Vascular tone</td>
<td>• Atheroma</td>
<td></td>
</tr>
<tr>
<td>• Inflammation</td>
<td>• Inflammation</td>
<td></td>
</tr>
<tr>
<td>• Proliferative potential</td>
<td>• Elastin synthesis</td>
<td></td>
</tr>
<tr>
<td>• Angiogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Wound healing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Tumours</td>
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</tr>
</tbody>
</table>
### Endogenous agents altering peripheral resistance

**Vasoconstrictors**
- Catecholamines
  - Epinephrine
  - Norepinephrine
  - Dopamine
- Endothelin
- Serotonin
- Angiotensin II
- Vasopressin

**Vasodilators**
- Histamine
- Adenosine
- Nitric Oxide (NO)
- Carbon Dioxide
- Potassium
- Hydrogen Ion
- Prostaglandins
- Acetylcholine
- Bradykinin

Many of these are targets for pharmacologically active agents.
Local factors altering blood flow

Hypoxia
- Reactive hyperaemia

Tissue metabolites and ions
- Adenosine
- Potassium ions
- Carbon dioxide
- Hydrogen ion
- Lactic acid
- Inorganic phosphate

Myogenic autoregulation
- Vascular smooth muscle reacts to restore vessel diameter and resistance
- Vascular smooth muscle cells depolarize when stretched
  - Activation of membrane calcium channels

Endothelial factors
- Vasoactive substances released from endothelium:
  - Nitric Oxide (NO)
  - Endothelium-derived relaxing factor
  - Prostacyclin
  - Endothelin
  - Endothelial-derived hyperpolarizing factor (EDHF)
Factors affecting blood vessels

Physical interventions
- Transmural pressure
- Needles/catheters
- Stents

Systemic
- Altered vascular tone
  - Humoral
  - Nervous
- Clotting factors
- Inflammation & immune system
- Altered lipid metabolism
- Vitamins/mineral levels
  - Vit D and Ca++
- Direct toxicity
  - Smooth muscle
  - Endothelium

Local specialisation
- E.g. Kidney
- Glomerular
- JG apparatus
- Papilla
Morphological reaction of vessel walls to injury is limited

- Few unique histological lesions
- May see a variety of appearances
  - Differences in distribution
  - Differences in combination of changes

Spontaneous disease

- Major confounding factor
- Experience helps
- Methodical approach is necessary
Lesions
Target sites of the vessel

Tunica intima
- Mainly endothelium with some connective tissue
- Permeability & physical barrier
- Metabolic activity

Tunica media
- Smooth muscle

Tunica adventitia
- Pericytes/veil cells
- Vasa vasorum

Nerves
- Sympathetic
- Parasympathetic

Connective tissue
- Fibroblasts
  - Collagen
  - Elastic fibres
  - GAGs

Inflammatory cells
- PMNS, macrophages, lymphocytes
## Limited set of reactions

<table>
<thead>
<tr>
<th>Inflammation</th>
<th>Thrombosis</th>
<th>Haemorrhage</th>
<th>Necrosis</th>
<th>Mineralisation</th>
<th>Immune-mediated</th>
</tr>
</thead>
</table>
| • Vessel wall  
  • Perivascular  
  • Intima  
  • Atheroma | • Aseptic  
  • Septic | • Clotting defects  
  • Platelets  
  • Clotting factors  
  • Wall damage  
  • Arteriopathy  
  • Lathyris | • Smooth muscle  
  • Arteriopathy | • Dystrophic  
  • Generalised | • Ag-Ab complexes  
  • Vasculitis  
  • Systemic  
  • Local |

<table>
<thead>
<tr>
<th>Intimal proliferation</th>
<th>Pigment deposition</th>
<th>Vacuolation</th>
<th>Atheroma</th>
<th>Neovascularisation</th>
<th>Neoplasia</th>
</tr>
</thead>
</table>
| • Shear forces  
  • IEL rupture  
  • Endothelial hyperplasia  
  • Smooth muscle hyperplasia | • Haemosiderin | • Endothelial  
  • Smooth muscle | | • Angiogenesis  
  • Vasa vasorum | • Multiple sites |
Biomarkers
## Vascular inflammatory disease – some potential biomarkers

<table>
<thead>
<tr>
<th>Inflammatory</th>
<th>Endothelial</th>
<th>Angiogenesis</th>
<th>Oxidative stress</th>
</tr>
</thead>
</table>
| - High-sensitivity C-reactive protein  
- IL-6  
- IL-8  
- Myeloperoxidase  
- MCP-1  
- Lipocalin-2  
- TNF receptor 1  
- TIMP-1 | - Circulating endothelial cells  
- EPCs (progenitor cells)  
- ADMA  
- vWF  
- Angiotensin 1  
- Endothelin 1  
- Caveolin 1  
- vCAMs  
- Selectins | - vEGF | - Isoprostanes  
- Homocysteine |
| Coagulation factors | Ischaemia | Smooth muscle | Matrix factors |
| - Thrombomodulin  
- Thrombospondin  
- Fibrinogen  
- TPA | - B2 microglobulin | - H1-Calponin  
- SM actin | - MMP-9  
- Adiponectin  
- ICAM-1  
- Osteoprotegrin |
PDE3 inhibitors

A case study illustrating numerous aspects of induced vascular disease
What are PDE inhibitors?

Large class of compounds
- Methylxanthines

11 families of PDE
- Non-selective inhibitors
  - Caffeine, aminophylline, theophylline

PDE3 inhibitors
- Inotropic/vasodilating compounds
  - Intended as alternative to cardiac glycosides
    - Acute heart failure
    - Now making a comeback?
      - Milrinone, inamrinone, ciostazol

PDE4 inhibitors
- Asthma, COPD

PDE5 inhibitors
- Erectile dysfunction
Reported as ‘coronary arteritis’

- Dose-related
- Previously unreported effect
- No other organs affected

Other cardiovascular findings

- ‘Jet’ lesions
- Papillary necrosis
- Atrial haemorrhage
- Decreased BP
- Tachycardia
Establishing the facts

- Are you sure this is real?
- Is this a class effect?
- Is it species specific?
- Is it a risk to man?
- Is there a safety margin?
### Features of PDE3-treated lesions in dogs

<table>
<thead>
<tr>
<th>Category</th>
<th>Details</th>
</tr>
</thead>
</table>
| **No consequential damage**                  | - No thrombi  
- No occlusion  
- No infarcts                                                                                                                     |
| **Inflammation relatively minor and sporadic** | - Two cases with widespread inflammation                                                                                             |
| **Prominent changes in all layers (sub-chronic)** | - Intimal thickening  
- Rupture of IEL  
- Adventitial fibrosis  
- Neovascularisation                                                                                                               |
| **Acute changes**                             | - Medial haemorrhage & necrosis  
- Adventitial oedema & inflammation                                                                                                    |
| **Distribution of lesions**                  | - Multiple sites in coronary tree                                                                                                      |
Inflammation, circumflex vessels (Hartman)

- Non-suppurative inflammation
  - Low grade
- Focal lesions
  - Segmental
- Sporadic, low incidence
  - Numerous breeds
- No clinical signs
  - Undetectable ante mortem

INAS (IJAS, Beagle Pain Syndrome)

- Necrotising arteritis
  - Usually severe
  - Fibrinoid change
- Sporadic
  - Usually seen in treated animals
- Clinical signs
  - Pyrexia
  - Pain
  - Neutrophilia

Other minor changes

- Replication of IEL
  - Atrial branches
- Hypertrophy, media
  - Papillary muscle branches
Questions arising

Is it spontaneous?
- Exacerbated background lesions?

What are early treatment-related changes?
- Lesions seen so far are sub-chronic
  - 28 day study
  - Do they give clues to pathogenesis?

Did it occur with similar compounds?
- Not reported

Is there anything in the literature?
- No
Other vascular and treatment-related changes

Atrial haemorrhage
- Mainly right side
- Not specific for PDE inhibitors
- Related to endothelin receptors
  - Not seen in man (Minoxidil)

Hypertrophy of media in papillary arteries
- Related to motility
  - Pacing studies (unpublished)

Left papillary muscle necrosis
- Ischaemia due to limited vascular supply
- Increased oxygen demand & consumption
- Tachycardia
- Sudden drop in BP
- Not specific for PDE3 antagonists
Changes are consistent with increased transmural pressure

- Looks like some hypertensive changes in man
- Increased coronary flow confirmed
  - Pressure not measured
- Similar changes in rats confirmed
  - Antagonism studies

Confirmation not followed through in dog

- Compounds dropped
  - Clinical data not good
  - Never followed up fully
What conclusions were reached?

- Is it real?
  - Yes, it is reproducible and dose-related

- Is it species specific?
  - Not really

- Is this a class effect?
  - Yes

- Is there a reasonable safety margin?
  - Not with the compounds we used

- Is it a risk to man?
  - I would say yes
Species specificity

- Occurs in dog, rat, rabbit, (NHP?)
  - Not in pig as far as I know

- Leads to increased mortality in man

- Not possible to monitor in life or in man
  - No reliable clinical biomarkers
    - Not monitored in detail at human necropsy
    - Coronary arterial disease is widespread

- Was generally regarded as dog-specific
  - Erroneous in my view
Other findings with PDE inhibitors

<table>
<thead>
<tr>
<th></th>
<th>Dogs</th>
<th>Rats</th>
<th>Pigs</th>
<th>Primates</th>
<th>Rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Combined PDE3 inhibitor and immunomodulator</td>
<td>Mesenteric 1° branches</td>
<td>Nothing with PDE3 inhibitors</td>
<td>Nothing reported initially</td>
<td>Coronary arteriopathy</td>
</tr>
<tr>
<td></td>
<td>Dose related INAS-type lesions</td>
<td>Related to increased transmural pressure</td>
<td>Vascular lesions with a PDE4 inhibitor</td>
<td>A different lesion with one PDE3 inhibitor</td>
<td>Far worse than dogs – killed animals</td>
</tr>
<tr>
<td></td>
<td>Not published</td>
<td>Hindlimb arteries</td>
<td>SCH 351591 causes vascular lesions</td>
<td>PDE4 inhibitor</td>
<td>Not published</td>
</tr>
</tbody>
</table>
Neoplasia

Endothelial
- Haemangioma
- Haemangiosarcoma
- Lymphangioma
- Lymphangiosarcoma

Smooth muscle
- Leiomyoma
- Leiomyosarcoma

Pericyte
- Pericytoma
- Fibroma
- Fibrosarcoma

Neural
- Benign schwannoma
- Malignant schwannoma
NTP data for spontaneous vascular neoplasms

**B6C3F1 mice spontaneous rates**

- Haemangioma
  - 1% male; 2% female (range 1-15%)
  - Liver, skin, uterus, ovary
- Haemangiosarcoma
  - 5% male; 5% female (range 0-5%)
  - Liver, skin, spleen, bone marrow, heart, uterus

**F344 rats**

- Haemangioma
  - 0.3% male; 0.25% female
  - Skin, uterus
- Haemangiosarcoma
  - 0.5% male; 0.4% female
  - Skin, spleen, heart, uterus
Vascular neoplasia

**Rare in humans**
- More common in mice
- Less common in rats

**DNA-reactive agents**
- Vinyl chloride
- Thorotrast
- Urethane

**Non-genotoxic inducers**
- PPAR-γ agonists
- RAR agonists (retinoic acid receptor agonists)
- PDE-5 inhibitors
- DPP-4 inhibitors
- Gonadotropin antagonists
- NO releasers
- vEGF inducers
NTP data for induced vascular neoplasms

25/290 carcinogens (550 studies)
- 2/25 had vascular neoplasia only
- 19/25 studies in mice only
  - 10 in both sexes
- 3/25 studies in rats only
- 3/25 studies in rats and mice
- Induced incidences in mice: 22%
  - Range: 8 – 100%

Highest incidences (>75%)
- Riddeline (pyrolozidine alkaloid)
- 2-methyl-1-nitroanthroquinone
- Cupferron
- Tetrafluoroethylene
- o-nitrotoluene

Suggested potential mechanisms
- Haemolysis/Fe overload/haemosiderosis
- Hormonal perturbations
- Reduced antioxidant defence mechanisms
- Genotoxic events
- Increased cell proliferation/apoptosis
- Dysregulation of cytokines/growth factors
Mainly haemangiosarcomas induced

- Liver
- Spleen
- Heart
- Adipose tissue
  - PPAR agonists
  - Late occurrence >18 months

Haemangioma less frequently induced

- Many carcinogens have no effect on their incidence
- May be relevant
  - Difficult to ignore
Precursor lesions?

- Not well documented
  - Angiomatous hyperplasia is recorded
  - Not illustrated!
- All vascular lesions should be recorded accurately

Dysregulation of angiogenesis and/or erythropoiesis

- Haemolysis
- Local tissue hypoxia
- May be common convergence
- Release of endothelial growth factors & cytokines

Significance for man

- Species differences in responses to chemicals
- Different responses in different tissues
- Significance still uncertain
Secondary vascular lesions
Exaggerated pharmacology
Angiotensin II antagonist

Expected pharmacology

Well established mechanism

Changes can be marked
- High doses
- Prolonged administration

Changes still need to be recorded
- Proves absorption
- Expected by reviewers
Intravenous injection
Intravenous injection

A common and simple means of administration
- Dog, rat, NHP, minipig
- Dosage route in man for some compounds

Formulation
- Components
- Irritancy
- pH
- Solubility

Technical aspects
- Trained staff
- Species
  - Bigger animals are easier
- Site
  - Can be problems with jugular, hind limbs, tail
- Number of injections
  - More than one site
  - Clear marking for sampling at necropsy
- Rate of administration
# Intravenous injection lesions

## Procedural - needle insertion

- **Number of injections is important**
  - Haemorrhage
  - Necrosis and repair of vein wall
  - Necrosis and repair of perivenous tissue
  - Changes in overlying skin

## Treatment related changes

- **Increased incidence and/or severity in comparison with controls**
  - **Vein wall**
    - Necrosis
    - Inflammation
    - Fibroplasia
    - Thrombosis
    - Endothelial hyperplasia
    - Recanalisation
  - **Perivenous tissue**
    - Haemorrhage
    - Inflammation
    - Acute
    - Chronic
    - Granulomatous
    - Fibrin
    - Deposition of injected material
    - Fibroplasia
  - **Overlying skin**
    - Ulceration
    - Scab formation
    - Dermal inflammation
    - Dermal fibrosis
    - Epidermal hyperplasia
Intravenous infusion
Administration of biopharmaceutical compounds

- Short half-life
- Maintain reasonable blood levels
- Few weeks to 6 months
- Dogs and rats, usually

Technically demanding

- Surgical preparation
  - Catheter implantation
- Animals must be trained
- Possibility of infection
- Possibility of catheter blockage
- Wide range of procedure-related effects
Intravenous catheter procedural lesions

- Duration of treatment
- Infection tracking from skin wound
- Changes in vein wall
  - Inflammation & fibrosis
  - Endothelial hyperplasia
  - Thrombi
    - Aseptic
    - Septic
- Downstream changes
  - Emboli
  - Thrombi
    - Aseptic
    - Septic
Increased incidence and/or severity in comparison with controls

Novel changes

Complex lesions
- Acute on top of chronic

Changes in vein
- Endothelial hyperplasia
- Thrombi
  - Aseptic
  - Septic
- Inflammation & fibrosis
  - Acute
  - Chronic
- Precipitation of dosing material

Downstream changes
- Vena cava
- Heart
- Lungs
- Emboli
  - Aseptic
  - Septic
  - Mineralised
- Thrombi
  - Aseptic
  - Septic