CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
RESPIRATORY AND CARDIOVASCULAR SYSTEM

Fourth Conference

ORGANIZED BY

SOCIETY OF TOXICOLOGIC PATHOLOGY - INDIA (STP-I)

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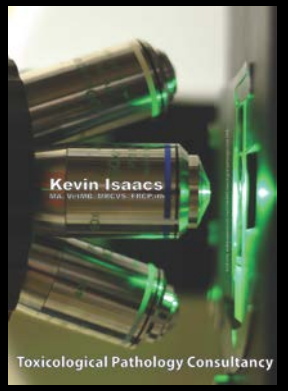

Induced lesions of the vascular system

International Federation of Societies of Toxicologic Pathologists

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 anastamoses
- 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

Vessel structure 2

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

Endothelial cells

- Permeability barrier
  - Fluid filtration
- Biologically active
  - Haemostasis
  - Vascular tone
  - Inflammation
- Proliferative potential
  - Angiogenesis
    - Wound healing
    - Tumours

Smooth muscle cells

- Contractile
  - Adrenergic innervation
    - $\alpha 1$, α 2, $\beta 2$
- Proliferative potential
  - Atheroma
  - Inflammation
  - Elastin synthesis

Pericytes/veil cells

- Support small vessels
- Pluripotential

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

Pathological reactions to injury

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

Inflammation
- Vessel wall
- Perivascular
- Intima
  - Atheroma

Thrombosis
- Aseptic
- Septic

Haemorrhage
- Clotting defects
  - Platelets
  - Clotting factors
- Wall damage
  - Arteriopathy
  - Lathyrism

Necrosis
- Smooth muscle
  - Arteriopathy

Mineralisation
- Dystrophic
- Generalised

Immune-mediated
- Ag-Ab complexes
- Vasculitis
  - Systemic
  - Local

Intimal proliferation
- Shear forces
  - IEL rupture
- Endothelial hyperplasia
- Smooth muscle hyperplasia

Pigment deposition
- Haemosiderin

Vacuolation
- Endothelial
- Smooth muscle

Atheroma

Neovascularisation
- Angiogenesis
  - Vasa vasorum

Neoplasia
- Multiple sites

Biomarkers

Vascular inflammatory disease – some potential biomarkers

Inflammatory

- High-sensitivity C-reactive protein
- IL-6
- IL-8
- Myeloperoxidase
- MCP-1
- Lipocalin-2
- TNF receptor 1
- TIMP-1

Endothelial

- Circulating endothelial cells
- EPCs (progenitor cells)
- ADMA
- vWF
- Angiotensin I
- Endothelin 1
- Caveolin 1
- vCAMs
- Selectins

Angiogenesis

- vEGF

Oxidative stress

- Isoprostanes
- Homocysteine

Coagulation factors

- Thrombomodulin
- Thrombospondin
- Fibrinogen
- TPA

Ischaemia

- B2 microglobulin

Smooth muscle

- H1-Calponin
- SM actin

Matrix factors

- 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

28-day study in dogs

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

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

Spontaneous disease

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 PDE_3 antagonists

Potential mechanism

Changes are consistent with increased transumural 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

Dogs	Rats	Pigs	Primates	Rabbits

• Combined PDE3 inhibitor and immunomodulator
  • Dose related INAS-type lesions
  • Not published
	• Mesenteric 1° branches
	  • Related to increased transmural pressure
	• Hindlimb arteries
		• Nothing with PDE3 inhibitors
		• Vascular lesions with a PDE4 inhibitor
			• Nothing reported initially
			  • A different lesion with one PDE3 inhibitor
			• PDE4 inhibitor SCH 351591 causes vascular lesions
				• Coronary arteriopathy
				  • Far worse than dogs – killed animals
				  • Not published

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 (pyrrozolidine 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

Haemangioma & haemangiosarcoma

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

Haemangioma & haemangiosarcoma

Precursor lesions?

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

Dysregulation of angiogenesis and/or erythropoesis

- 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

Basic considerations

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

Procedural result of indwelling catheter

- 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

Treatment related changes

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