TOPICALLY APPLIED CORE MULTISHELL NANOCARRIERS REMAIN IN THE STRATUM CORNEUM, BUT THEIR CARGO, TACROLIMUS, REACHES THE VIABLE SKIN IN A MURINE MODEL OF ATOPIC DERMATITIS

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Introduction: Core multishell nanocarriers (CMS) are biocompatible drug transporters designed for topical therapy of skin inflammation. Here, the penetration of the carrier, its cargo and effects on the skin were analysed in a murine atopic dermatitis (AD) model after topical application.

Materials and Methods: Fluorescently-labelled, tacrolimus-loaded or unloaded CMS, tacrolimus standard ointment or the solvent were applied topically to the inflamed skin. Carriers were localized by fluorescence microscopy. Tacrolimus was quantified in different skin layers and liver via liquid chromatography tandem–mass spectrometry. Effects of CMS on the skin were analysed via Raman spectroscopy. Epidermal thickness and immunohistochemically CD3-positive T cells were quantified.

Results: CMS were exclusively localized in the stratum corneum; however, their cargo was found in deeper skin layers and the liver. The amount of tacrolimus in these tissues was lower compared with the standard. However, tacrolimus-loaded CMS reduced the epidermal thickness and T-cell infiltration similarly to the standard tacrolimus ointment. The lipid layer of the stratum corneum was disorganized after application of CMS.

Conclusions: CMS did not 'carry' its cargo into deeper skin layers by translocation of the loaded carrier with subsequent release. However, its topical application led to a disorganization of the lipid layers, which may have facilitated the entry of the cargo into viable skin layers after release from its depot in the stratum corneum. Tacrolimus-loaded CMS ameliorated AD as effectively as the standard tacrolimus ointment. Advantages in other aspects make CMS promising topical carrier candidates (e.g. to reduce systemic side-effects).

REDISTRIBUTION OF SIALIC ACID RECEPTORS IN THE CHICKEN TRACHEA HAMPERS SUPERINFECTION WITH INFECTIOUS BRONCHITIS VIRUS, BUT NOT WITH LOW PATHOGENIC AVIAN INFLUENZA VIRUS

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Introduction: Avian infectious bronchitis virus (IBV) is known to replicate in the chicken trachea. To establish an infection, IBV uses, like avian influenza viruses (AIVs), α2, 3-linked sialic acids as receptors. Previously, it has been shown that IBV infection changes sialic acid expression in chicken bronchial explants *ex vivo*. The aim of this study was to investigate the consequences of primary IBV infections on tracheal morphology and sialic acid expression in relation to interference with secondary IBV and AI infections.

Materials and Methods: In-vivo IBV-infected chicken tracheas (approved by the Dutch 'DierEthische Commissie', GD Deventer number 278) were compared regarding histopathological changes and binding patterns of recombinantly-produced viral proteins (IBV spike, AIV haemagglutinin). A tracheal organ culture (TOC) model was validated to subsequently study IBV and low pathogenic AI growth kinetics in sequential infections, using qPCR to quantify viral RNA production and immunohistochemistry to visualize viral protein expression.

Results: IBV-induced tracheal morphological changes were comparable between IBV strains. IBV-induced changes markedly hampered IBV spike binding on tissue slides, while they still enabled AI haemagglutinin binding. In chicken TOCs, a primary IBV infection suppressed RNA production of sequentially-inoculated IBV. In contrast, LPAI RNA production was only mildly hampered by a preceding IBV infection, with delay early in infection, but no difference with control tracheas (LPAI only) later in infection.

Conclusions: IBV infection markedly reduces tracheal susceptibility to secondary IBV infection, but barely changes AI replication. Based on viral protein binding studies, this difference might originate from virus-specific binding to sialic acids on the host cell surface.

RETINAL FEATURES IN IODOACETIC ACID-TREATED BIOMEDICAL PIGS AS A MODEL FOR PHOTORECEPTOR DISORDERS

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Introduction: Swine are a well-recognized model for human ocular disease, considering the similar size and the presence of a cone-dominant visual streak analogous to the human macula. The aim of this study was to detect the ocular morphological features of iodoacetic acid (IAA)-treated pigs.

Materials and Methods: Three commercial hybrid pigs treated with 12 mg/kg IAA and three controls were examined at necropsy. The ocular globes were formalin fixed and stained with HE. The retinal thickness was measured using ImageJ. IHC using antibodies to Iba1, GFAP, Olig-2 and Caspase-3 was performed.

Results: Bilaterally, all of the IAA-treated pigs showed a selective loss of rods with sparing of cones (early retinal atrophy) in the central visual streak, combined with areas of complete loss of the outer retina (end stage retinal atrophy) in the peripheral zones. The atrophy was selective to the outer retina (OR) (138 μm; 124.9–330.7 μm, median; range) rather than the inner retina (IR) (588.2 μm; 367.4–867.7 μm) in the treated animals compared with the controls (OR 425.8 μm; 272.3–529.3, IR 460.2; 225.7–685.8 μm) ($P = 0.008$; Mann Whitney U test). Numerous activated microglial cells, forming glial nodules, were present in areas of selective atrophy of rods, compared with the control retinae that had only rare ameboid microglia. The end stage retinal atrophy had numerous, haphazardly arranged Müller cells (glial scar), with loss of the columnar alignment. Apoptotic rods were highlighted in only one animal.

Conclusions: IAA causes photoreceptor atrophy, which activates a microglial and marked Müller cell response. Apoptosis of rods is a rare event.

NEOPLASIA IN SYNGNATHIDAE – HISTOLOGICAL AND IMMUNOHISTOLOGICAL CHARACTERIZATION OF 18 CASES

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Introduction: Syngnathidae (seahorses, pipefish and sea dragons) are common in commercial aquaria, but published literature on syngnathid diseases is limited and immunohistochemical techniques not routinely employed.

Materials and Methods: A retrospective review was undertaken of 2,541 syngnathid submissions received between March 2003 and October 2016, identifying 18 neoplasms further classified based on morphology. Nine commercial antibodies were trialled for immunohistochemical characterization of neoplastic tissue using appropriate syngnathid and non-syngnathid positive controls.

Results: Most commonly submitted were big bellied ($n = 495$), slender ($n = 318$), lined ($n = 302$) and yellow ($n = 213$) seahorses, broadnose pipefish ($n = 142$) and weedy sea dragons ($n = 114$). Eighteen cases of neoplasia were identified: ovarian and testicular germ cell tumours ($n = 4$), exocrine pancreatic and intestinal carcinoma (each $n = 3$), chromatophoroma ($n = 2$), lymphoma, seminoma, thyroid and renal carcinoma, swim bladder and pituitary adenoma (all $n = 1$). Big bellied seahorses comprised 19% of submissions, but 50% of neoplasms. Neoplasia in pipefish was rare ($n = 3$) and no cases occurred in sea dragons. Carcinomas labelled positive with pan-cytokeratin, while vimentin, PNL-2, melan-A, S100, CD20, CD3, IBA-1 and lysozyme were not cross reactive. Neoplasms were the primary cause of death in seven cases (38%), branchitis in a further seven (38%) and the cause of death was unclear in four cases (22%).

Conclusions: In contrast to a previous review (172 sygnathids, neoplasia prevalence 4.1%), the prevalence of neoplasms in our study of captive sygnathids was low (0.7%). Germ cell tumours not identified previously in sygnathids were most common in the study population (4/18, 22%). Many commercially available immunohistology markers are not cross reactive in sygnathids.