J. Comp. Path. 2009, Vol. 140, 217–224

ELSEVIER
www.elsevier.com/locate/jcpa

Histopathological Examination of the Pancreas of the Koala (*Phascolarctos cinereus*)

D. P. Higgins and P. J. Canfield

Koala Infectious Diseases Research Group, Faculty of Veterinary Science, B14, University of Sydney, NSW 2006, Australia

Summary

This retrospective study, which was based on koala pancreatic specimens taken 2, 24, 48 and 72 h after death, showed that the degree of autolysis did not necessarily exclude histopathological examination. Disorders not previously reported in the pancreas of koalas included the following: inflammation and necrosis; atrophy and fibrosis of exocrine pancreatic tissue; lymphosarcoma; pancreatic heterotopy; and ductal adenocarcinoma.

Keywords: autolysis; koala; pancreas; *Phascolarctos cinereus*

Introduction

Many diseases of koalas (*Phascolarctos cinereus*) have been described in the literature, but there is only one report of pancreatic disease, namely a case of diabetes mellitus (Hemsley *et al.*, 1998). However, because rapid post-mortem autolysis occurs in the pancreas (Shimizu *et al.*, 1990) and because many reported koala necropsies were not carried out until 24–72 h after death (Canfield, 1990, 1991; Stalder, 2003), pancreatic abnormalities in this species may have been overlooked in the past. The aims of this retrospective study were (1) to assess the effect of different degrees of autolysis on the suitability of koala pancreatic specimens for histopathological examination, and (2) to describe pancreatic abnormalities detected in free-ranging koalas.

Materials and Methods

Cases and Specimens

A database of necropsy results for approximately 1200 koalas from the Port Macquarie district between 1990 and 2003, and necropsy records of 90 koalas from southeast Queensland between 2000 and 2003, were searched for comments indicative of cases with pancreatic abnormalities. Histological sections of these cases were reviewed and collated with any available clinical and gross pathology data. Necropsy had been performed on koalas from southeast Queensland within 2 h of euthanasia and pancreatic tissue collected from all animals. Koalas recorded in the database, however, had been transported chilled for 24–72 h before necropsy and pancreatic tissue collected from only a minority.

Duplicate sections of normal pancreata, collected from chilled koala carcases at 2, 24, 48 and 72 h *post mortem*, were examined for histological signs of autolysis.

Thirteen cases, numbered 1–13, with pancreatic abnormalities were identified; of these, eight originated from southeast Queensland koalas and five from database koalas sampled 24 h after death.

Tissues collected at necropsy were fixed in 10% buffered formalin and processed by standard methods, and sections (5 μm) were stained with haematoxylin and eosin (HE). In addition, selected sections were stained with Van Gieson, Congo red, and Gram–Twort stains for collagen, amyloid and bacteria, respectively.

Immunohistochemistry (IHC)

Beta islet cells were labelled on some sections to confirm their identity. As described by Hemsley *et al.* (1998), sections (4 μm) were incubated with guinea-pig anti-porcine insulin (Dakopatts, Glostrup, Denmark; A0564), followed by biotinylated

Correspondence to: D.P. Higgins (e-mail: damienh@vetp.usyd.edu.au).

0021-9975/$ - see front matter
doi:10.1016/j.jcpa.2008.07.009

anti-mouse/rabbit Ig and streptavidin–biotin–horseradish peroxidase (ABC Duet K0492; Dako, Australia) and 3,3′-diaminobenzidine (DAB; K3466; Dako, Australia); the sections were counterstained with haematoxylin. Sections of normal koala pancreas were used as positive and negative controls; primary antibody was omitted as a negative control. Cases of lymphosarcoma were typed by a similar immunoperoxidase method, with cross-reactive anti-human CD3 (T-cell) and anti-human CD79b (B-cell) antibodies (MCA1477 and MCA2209; Serotec, Oxford, UK), applied after heat treatment in antigen retrieval solution (S1699; Dako, Australia).

Results

Post-mortem Autolysis in Normal Pancreata

Pancreatic tissues collected within 2 h of death displayed minimal histological features of autolysis (Fig. 1a). At 24 h, there was an expansion and disaggregation of connective tissue around lobes and separating lobules. Islets were still distinct, but islet cells were often separated due to contraction and they showed increased cytoplasmic eosinophilia and early pyknosis. There was loss of polarity of acinar cells, with contraction and detachment from adjacent cells and stroma. Nuclear changes varied from early pyknosis to karyolysis (Fig. 1b). Bacteria were not detected. In tissue collected 48 h *post mortem*, acinar structure was still discernible, but advanced karyolysis was widespread and acinar cell cytoplasm was eosinophilic and fragmented (Fig. 1c). In tissue collected at 72 h, karyolysis of acinar and islet cells was near completion, islets were not discernible, and acinar cells remained only as fragments of cytoplasmic eosinophilic material. However, connective tissue structure was similar to that seen 24 h *post mortem*. Incidental lymphoid and phagocytic cells scattered between lobules showed mild morphological changes but remained clearly identifiable (Fig. 1d).

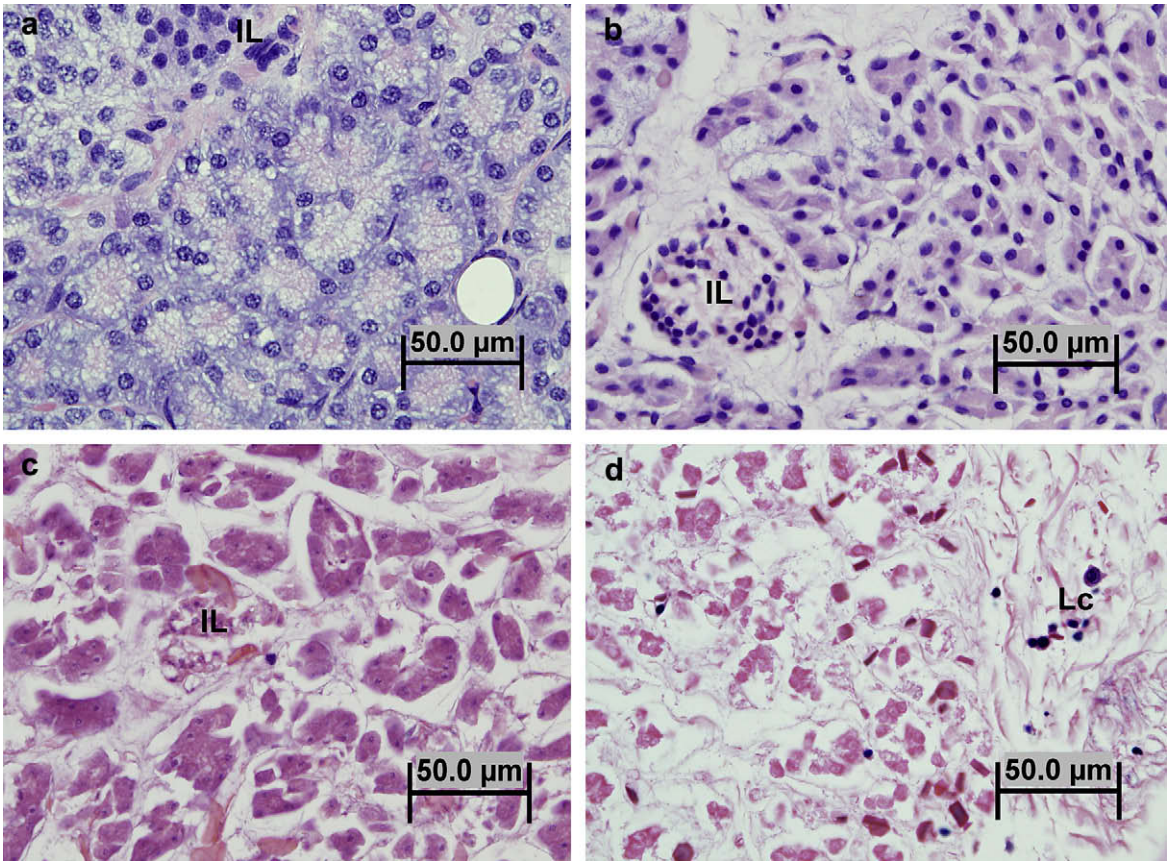

Fig. 1. a–d. Histological sections of koala pancreas fixed in 10% buffered formalin, at (a) 2 h, (b) 24 h, (c) 48 h and (d) 72 h *post mortem*. In (b) most of the pancreatic cells have pyknotic nuclei and condensed cytoplasm. Acinar cells have separated from one another. In (c) there is advanced karyolysis and increased eosinophilia of cytoplasm. In (d) acinar cells are unrecognizable and are left as eosinophilic debris. IL, islet of Langerhans; Lc, lymphocytic and phagocytic cells. HE. Bars, 50 µm.