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Experimental Gerontology 38 (2003) 1101–1107
Experimental Gerontology
www.elsevier.com/locate/expgero

Hepatic sinusoidal pseudocapillarization with aging in the non-human primate

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Received 7 April 2003; received in revised form 30 July 2003; accepted 30 July 2003

Abstract

Background/Aims: Age-related changes in the hepatic sinusoid termed pseudocapillarization have been reported in the rat and human and have implications for disease susceptibility in old age. In this study, we investigated whether similar changes occur in the livers of old baboons and thus represent a widespread aging change. *Methods*: Liver tissue from five young baboons (5.4 ± 0.5 yrs) and five old baboons (21.8 ± 0.7 yrs) was compared by transmission electron microscopy, scanning electron microscopy and immunohistochemistry. *Results*: The thickness of the sinusoidal endothelium was increased in old baboons (130 ± 8 nm versus 186 ± 9 nm, $P < 0.001$) and the frequency of endothelial fenestrae decreased, with the porosity declining from 4.2 ± 0.5% to 2.4 ± 0.4% ($P = 0.006$). The expression of laminin and von Willebrands factor was more extensive in old baboons. Novel perisinusoidal ring-shaped cells, probably fat-engorged stellate cells, were prominent in the old baboons. *Conclusions*: Pseudocapillarization is a significant age-related change in the baboon liver. Aging in baboons is associated with a novel aging change in the stellate cell not reported in other species. Hepatic pseudocapillarization is a widespread aging liver change found in several species including humans and other non-human primates.

Keywords: Aging; Baboons; Defenestration; Pseudocapillarization

1. Introduction

The liver has often been considered to be relatively unaffected by the primary aging process and spared the effects of age-related diseases (Jansen, 2002; Popper, 1986; Schmucker, 1998; Vestal, 1989). At the light microscopic level, aging is associated with lipofuscin accumulation and an increase in multinucleate hepatocytes (Popper, 1986). Functional changes, particularly related to impaired drug metabolism, have been attributed to age-related reduction in blood flow and liver mass (Vestal, 1989).

Recently, we have shown that aging is associated with marked ultrastructural alterations in the liver sinusoidal endothelium and space of Disse in the rat (Le Couteur et al., 2001) and in surgical and post mortem specimens from human livers (McLean et al., 2003). These changes, termed 'pseudocapillarization', are characterized by defenestration, thickening of the endothelium, and deposition of basal lamina and extracellular matrix in the space of Disse. Normal fenestrated endothelial cells of the liver sinusoids act as a dynamic filter that permits exchange of fluid, solutes and particles between the sinusoidal lumen and space of Disse (Fraser et al., 1995; Wisse et al., 1996). Age-related changes in the liver sinusoids and space of Disse are implicated in the association between aging and impaired clearance of drugs (Le Couteur and McLean, 1998), chylomicron remnants (Cassader et al., 1996) and neurotoxins (Yang et al., 2002) and may provide a mechanistic link between primary aging processes and age-related disease (Le Couteur et al., 2002).

In this study, we examined the livers from young and old non-human primates (*Papio hamadryas*) in order to determine whether pseudocapillarization is a widespread

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doi:10.1016/j.exger.2003.07.002

aging process, regardless of average life expectancy and species.

2. Methods

The study was approved by the Central Sydney Area Health Service Animal Welfare Committee. The baboons were recruited from the National Baboon Colony, Sydney, Australia. The animals are members of a captive breeding colony of *Papio hamadryas* that was established in 1981. All animals are housed in family groups, fed a standard diet and are under the supervision of an experienced primate veterinarian. This colony has been reported previously (Birrell et al., 1996; Harewood et al., 1999).

Liver specimens were obtained by needle biopsy ($n = 8$), open surgical biopsy ($n = 2$) and as part of euthanasia of two older animals ($n = 2$). For the biopsy samples, animals were anaesthetized with ketamine hydrochloride (6 mg/kg) and samples were obtained using Gallini 16 gauge tru-cut percutaneous biopsy gun or by open biopsy via a 5 cm right upper quadrant laparotomy incision. Metoclopramide, atropine and buprenorphine were administered during the perioperative period. All animals recovered well from the procedure. Fresh post mortem tissue was obtained from animals that were euthanased because of old age and loss of condition ($n = 2$). Animals were sacrificed by injection of pentobarbitone (80 mg/kg) following initial sedation with ketamine hydrochloride.

Liver specimens were fixed for light microscopy in 4% buffered paraformaldehyde and for electron microscopy with 2% glutaraldehyde/3% paraformaldehyde in 0.1 M sodium cacodylate buffer (0.1 M sucrose, 2 mM $CaCl_2$). The surgical and post mortem specimens also were perfused with fixative for scanning electron microscopy analysis by injecting an aliquot of glutaraldehyde fixative into the specimen with a 25G needle until the tissue hardened. Tissue was post-fixed for 2 h.

All tissue was examined by a hepatopathologist (RF) for evidence of underlying disease such as infection, tumor, fibrosis or cirrhosis.

For light microscopy and immunohistochemistry liver specimens were embedded in paraffin blocks. Sections from each animal were stained with Hematoxylin and Eosin, Masson's trichrome, Wilder's reticulin and Sirius red. Immunohistochemistry was used to detect the expression of collagen IV, laminin, von Willebrands factor (vWF), fibronectin, synaptophysin, α-smooth muscle actin (α-SMA), desmin, glial fibrillary protein (GFAP) and vimentin modified from previous descriptions (Le Couteur et al., 2001; McLean et al., 2003). Sections were pre-treated with proteinase K (collagen IV, laminin, vWF and GFAP) or microwave antigen retrieval (fibronectin, vimentin, desmin and synaptophysin) after deparaffinization. To prevent endogenous peroxidase activity and biotin binding, sections were pre-treated with 0.3% H_2O_2 in PBS and avidin–biotin blocking solutions (Miller and Kubier, 1997). Sections were then incubated with the primary antibodies: vWf (1:800 rabbit, Sigma, St Louis, MO), collagen IV (1:500 mouse, Sigma), laminin (1:30 rabbit, Sigma), fibronectin (1:50 mouse, Sigma), desmin (1:100 mouse, Dako), α-SMA (1:800 mouse, Sigma), GFAP (1:500 rabbit, Dako), vimentin (1:300 mouse, Dako) and synaptophysin (1:100 rabbit, Dako, Carpenteria CA). Secondary biotinylated antibodies (goat anti-mouse 1:400 and anti-rabbit 1:800, Sigma) were incubated for 45 min. Sections were incubated with extravidin peroxidase (30 min, 1:100, Sigma) and peroxidase activity was revealed using 3,3′-diaminobenzidine. All slides were graded according to intensity of staining (0, +, ++, +++) and differentiation between periportal and pericentral staining was undertaken.

For transmission electron microscopy, fixed tissue was processed and embedded in Spurr's resin. Blocks were sampled at random for light microscopic assessment. Two blocks per liver were finally studied, selected randomly from those satisfying requirements for quality of fixation and tissue integrity (Cogger et al., 2001; Le Couteur et al., 2001; McLean et al., 2003).

Ultrathin (70–90 nm) sections were taken from each block for initial scanning (magnification × 8000) using a Philips CM 120 Transmission Microscope. A pool of suitable sections resulted from the low power scanning process, and 10 sections were chosen at random for ultrastructural measurement from each liver. In each of the ten sections representative fields were chosen by an operator blinded to tissue category.

Transmission electron micrographic measurements (magnification × 17000) of the thickness of the sinusoidal endothelial cells were made using Mitutoyo calipers and the number of fenestrations was counted. The presence of extracellular matrix deposition and basal lamina formation was noted.

For scanning electron microscopy, fixed tissue was osmicated (1% OsO_4/0.1 M sodium cacodylate buffer), dehydrated in an ethanol gradient to 100% and critical point dried using a Bal-Tec CPD 030. Tissue was then mounted on stubs, splutter-coated with gold and examined using a Philips XL30 Scanning Microscope. Ten images were taken from each animal for analysis of fenestrae diameter and endothelial porosity using the Zeiss KS Image Analysis program.

The results are expressed as mean ± sem. Comparison of the electron microscopy data for the two age groups was performed using the Student t-test or the z-test. Differences were considered significant when $P < 0.05$.

3. Results

Two young baboons were omitted from this analysis (males, aged 7 and 10) on the basis of extensive hepatic fibrosis and inflammation. These two baboons were

Table 1
Age, gender and liver function tests of baboons

	Young baboons ($n = 5$)	Old baboons ($n = 5$)
Age (yrs)	5.4 ± 0.5	21.8 ± 0.7
Male: female	1:4	3:2
AST (U/l)	40.8 ± 10.5	34.4 ± 5.2
GGT (U/l)	34.8 ± 4.4	26.6 ± 2.6
Albumin (g/l)	33.6 ± 0.9	31 ± 1.9
Bilirubin (μmol/l)	2.2 ± 0.5	3.4 ± 0.7
Cholesterol (mmol/l)	2.8 ± 0.2	2.4 ± 0.1
Triglycerides (mmol/l)	0.54 ± 0.08	1.04 ± 0.29
Uric acid (mmol/l)	0 ± 0	0 ± 0

subsequently euthanased because of poor health and autopsy revealed widespread lymphoma. All other animals included in the analysis had normal liver morphology on light microscopy. Liver function tests from these animals are shown in Table 1.

3.1. Light microscopy

Lipofuscin deposits and multinucleate hepatocytes were noted in the older animals. On light microscopy no other age-related differences in Hematoxylin and Eosin, Masson's trichrome, reticulin or Sirius red stains were seen. All animals had numerous granulocytes and prominent Kupffer cells in the sinusoids.

Unusual, large, round cells were noted mostly in the livers of older animals (Fig. 1). They were present with high frequency in four of the five aged baboon livers and in small numbers in two of the young baboons. The nucleus of these cells was peripherally located giving the appearance of a signet ring. These age-related ring-shaped cells were perisinusoidal.

3.2. Immunohistochemistry

Extensive and diffuse perisinusoidal staining for laminin and vWF was seen in the livers of old baboons, but was less intense and focally distributed in livers of young baboons (Fig. 1). Diffuse perisinusoidal staining for collagen IV and fibronectin was seen in both young and old baboons and there were no age-related differences in the staining of these two antigens.

Staining of the age-related ring-shaped cells for synaptophysin, desmin, α-SMA, GFAP and vimentin was

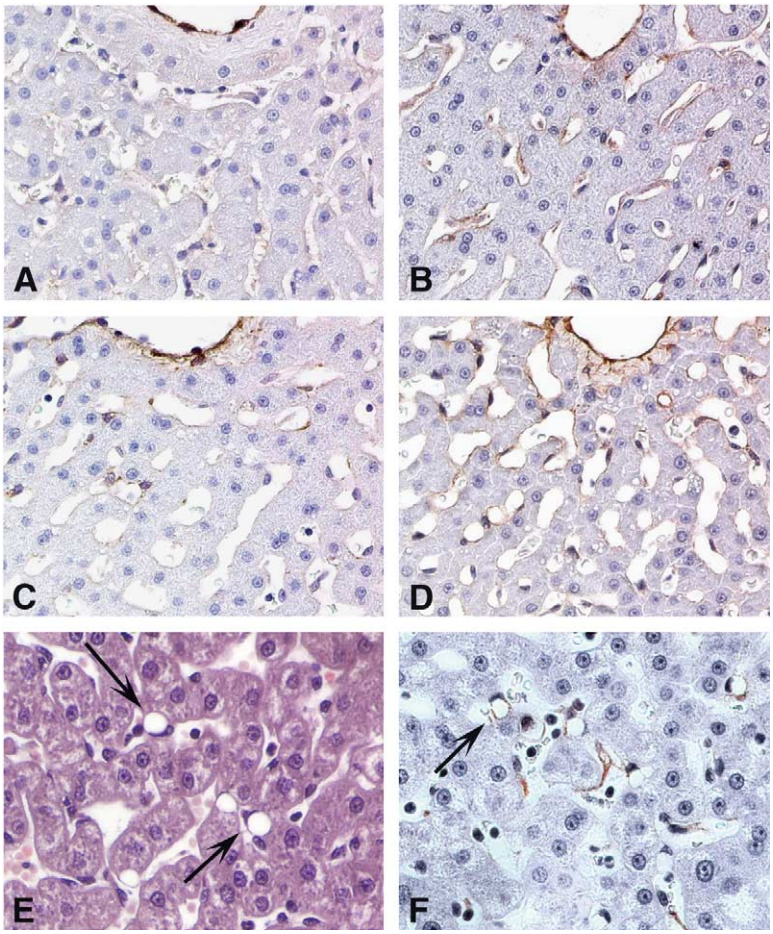

Fig. 1. Immunohistochemistry of the liver for vWf ((A) young baboon, (B) old baboon), laminin ((C) young baboon, (D) old baboon). The perisinusoidal expression is increased in the old baboons. A representative haematoxylin and eosin section of an old liver showing numerous ring-shaped stellate cells is shown in (E). These cells are express vimentin (F).

performed to further characterize these cells. All ring-shaped cells were positive for vimentin (Fig. 1), some for synaptophysin and no staining was detected for the other antibodies.

3.3. Transmission electron microscopy

In all specimens the morphology of the hepatocytes was well preserved with no evidence of autolytic changes or fixation artifacts. In particular, there were no signs of organelle swelling and the structure of the mitochondria and microvilli was intact. Cellular glycogen reserves were abundant. With the exception of the increased frequency of multinucleate cells in the older group, no difference between young and old animals could be detected on the basis of hepatocyte morphology.

Sinusoidal endothelium thickness was significantly increased in the old animals (Fig. 2, Table 2). The number of endothelial fenestrations was significantly decreased in the old animals (Table 2). All of the older animals had extracellular matrix deposition in the space of Disse whereas this was observed in only one of the younger animals. Two of the old animals had extensive basal lamina formation (Fig. 2).

The age-related ring-shaped cells were located in the space of Disse. These cells contained a single large osmium dense lipid droplet, surrounded by a thin layer of cytoplasm (Fig. 2).

3.4. Scanning electron microscopy

Tissue from two old baboons and two young animals were perfusion-fixed for scanning electron microscopy analysis. There was defenestration and loss of sieve plates in the sinusoidal endothelium of the older animals (Table 2, Fig. 2). Average fenestration diameter was increased slightly with age.

4. Discussion

The sinusoidal region of the baboon liver undergoes significant age-related ultrastructural changes. First, we noted a 40% increase in thickness in the sinusoidal endothelium from 130 ± 8 nm to 186 ± 9 nm. In rats we have previously noted that the endothelium increased in thickness by approximately 40% from 230 ± 50 nm to 320 ± 80 nm (Le Couteur et al., 2001) and in humans by 75% from 165 ± 17 nm to 289 ± 9 nm (McLean et al., 2003). In addition, we noted a reduction in the number of fenestrations in the sinusoidal endothelium (‘defenestration’). In the aged baboon, the porosity of the endothelium determined on scanning electron microscopy was reduced from 4.2 ± 0.5% to 2.4 ± 0.4%. This is very similar to the reduction of porosity in old age in the rat from 4.1 ± 2.3% to 2.5 ± 1.2% (Le Couteur et al., 2001). In human tissue, we were unable to perform scanning electron microscopic analysis, however, the fenestration count determined on transmission electron microscopy was reduced by approximately 80% (McLean et al., 2003). This age-related change in the ultrastructure of the sinusoidal endothelium is associated with an increased expression of vWF in baboons, humans and rats. Endothelial cells in tissue beds outside the liver express vWF, a glycoprotein mediating attachment of platelets after endothelial injury, but it is not expressed in sinusoidal cells of normal young liver. The upregulation of vWF in old age confirms that significant biological changes are occurring in the endothelium and is consistent with the electron microscopic analysis.

The sinusoidal endothelium has a pivotal functional role in hepatic metabolism. The endothelium is very thin and perforated with fenestrations that allow graded transfer of fluid, substrates and large particles such as lipoproteins between blood, the space of Disse and the hepatocytes (Wisse et al., 1985). Clearly this structural design facilitates the role of the liver in the regulation of systemic metabolism and detoxification of xenobiotics (Braet et al., 1995). Therefore, the age-related changes in the sinusoidal endothelium consisting of defenestration and thickening will interfere with the flow and/or diffusion of substrates from blood into the hepatocytes. Pseudocapillarization may provide a mechanism linking old age with impaired chylomicron remnant clearance and hence atherosclerosis (Le Couteur et al., 2002), impaired oxygen and drug uptake and hence adverse drug reactions (Le Couteur and McLean, 1998), and finally the clearance of neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Yang et al., 2002).

Despite the homologies, there were some differences between baboons, rats and humans in terms of extracellular matrix changes. In baboons we noted that there was age-related upregulation of laminin, but not of collagen, and, likewise, transmission electron microscopy did not reveal any major changes in collagen deposition in the space of Disse. Two of the old baboons demonstrated basal lamina formation. In contrast, there was substantive deposition of collagen and basal lamina formation in the livers from older humans and this was associated with increased perisinusoidal staining of Masson’s trichrome and collagen (Le Couteur et al., 2001). Rats had changes intermediate between those we saw in humans and baboons (McLean et al., 2003). These species differences in extracellular matrix changes may indicate differences in co-existent diseases or ‘rates of aging’ between species. Age-related changes in extracellular matrix will also influence the transfer of substrates between blood and hepatocyte through changes in the permeability barrier.

Major age-related changes in the liver appear to be confined to the perisinusoidal regions, in particular the endothelium. In the baboon, as in most other animals, old age was found to be associated with some deposition of lipofuscin and multinucleate cells in the hepatic

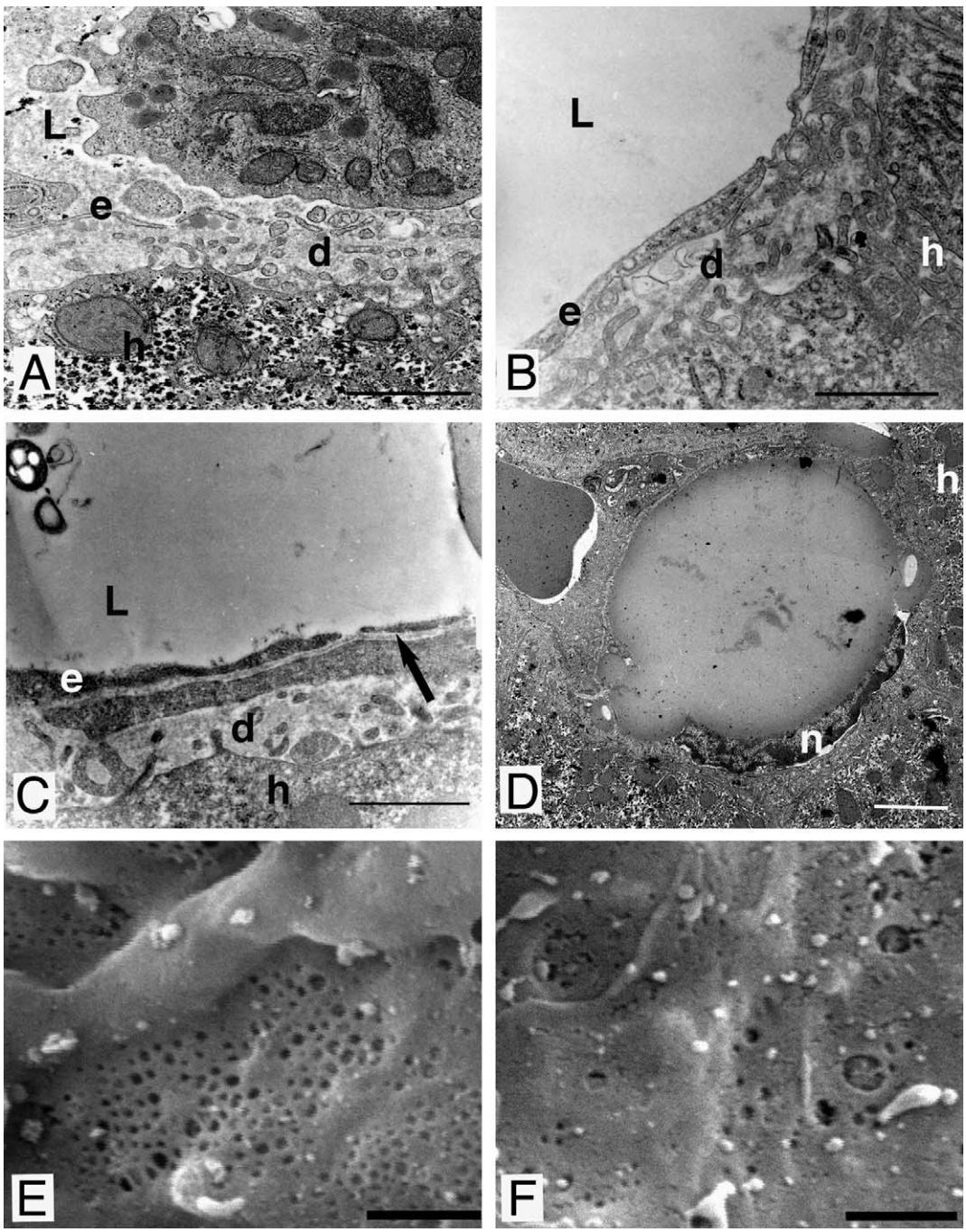

Fig. 2. Electron microscopy of the baboon liver. Transmission electron micrographs of the young (A) and old baboon liver (B–D). The endothelial cells (e) are thickened and defenestrated with old age. Increased extracellular matrix is found in the space of Disse (d) in the old livers. There is basal lamina deposition beneath the endothelial cells in some old baboons (→ (C)). A lipid laden ring-shaped cell is shown in (D). The nucleus (n) is located in the rim of cytoplasm that surrounds the lipid droplet. Scanning electron micrographs of the young (E) and old (F) liver. There is defenestration of the endothelium in the old baboons. (Abbreviations: h, hepatocyte; e, endothelial cell; L, sinusoidal lumen; n, nucleus; d, space of Disse. Scale bars: (A–C, E and F) = 1 μm, (D) = 5 μm).

parenchyma, however, no other changes were observed on light microscopy or electron microscopy. However, we did note a novel, presumptively age-related cell in the baboon. These cells had a ring-like appearance on light microscopy and were in the perisinusoidal regions. Electron microscopy and immunohistochemistry indicated that these cells are most likely to be abnormal stellate cells that are engorged with a single fat globule. The significance of these ring-shaped stellate cells for aging is not clear but presumably they indicate that a process of hepatic stellate cell modification is occurring in old baboons. Similar swollen stellate cells have been found in polar bears, where the area of lipid droplets was found to be up to 43 times as large as those seen in rats (Higashi et al., 2002).

Table 2
The effects of old age on the electron microscopy of the liver sinusoidal endothelial cell and space of Disse

	Young baboons ($n = 5$)	Old baboons ($n = 5$)
Endothelial thickness (nm)	130 ± 8	186 ± 9*
Fenestration count (per 10 μm)	9.4 ± 0.9	5.5 ± 0.7*
Fenestration diameter (nm)	58.2 ± 1.3	69.5 ± 2.3*
Endothelial Porosity (%)	4.2 ± 0.5	2.4 ± 0.4*
Presence of basal lamina	0%	40%
Presence of extracellular matrix	25%	100%**

*P ≤ 0.001; **P = 0.07.

Baboons provide substantive opportunities for liver research. The model has been used extensively in the study of alcoholic liver disease, diabetes and pregnancy (Harewood et al., 2000; Heffernan et al., 1995, 1996; Mak et al., 1984; Mak and Lieber, 1984, 1986, 1988; Miyakawa et al., 1985). The life span of *Papio hamadryas* is approximately 30 years therefore the animals we studied are old and the changes we have demonstrated are likely to be related to the primary aging process and senescence. Ultrastructural changes in the liver sinusoid may prove to be an important biomarker outcome for studies of caloric restricted non-human primates and other species.

In conclusion, there are significant changes in the liver sinusoidal endothelium and space of Disse that occur with old age in non-human primates. We also identified a novel aging change in the stellate cell that has not been reported previously. Hepatic pseudocapillarization is a widespread aging phenomenon, now identified in several species including man.

Acknowledgements

This work was supported by grants from the Department of Veterans Affairs (#211085), the National Health and Medical Research Council (#990937). We acknowledge the support and assistance of the Ageing and Alzheimers Research Foundation, the Medical Foundation of the University of Sydney, the Electron Microscopy Unit of the University of Sydney, Professors J Thompson and A Hennessy of the Royal Prince Alfred Hospital, Dr S Thomson and S Heffernan from the National Baboon colony and Dr Sun Young Kwun for his assistance with the immunohistochemistry.

References

Birrell, A.M., Hennessy, A., Gillin, A., Horvath, J., Tiller, D., 1996. Reproductive and neonatal outcomes in captive bred baboons (*Papio hamadryas*). J. Med. Primatol. 25, 287–293.

Braet, F., Zanger, R.D., Baekeland, M., Crabbe, E., Vandersmissen, P., Wisse, E., 1995. Structure and dynamics of the fenestrae-associated cytoskeleton of rat liver sinusoidal endothelial cells. Hepatology 21, 180–189.

Cassader, M., Gambino, R., Ruiu, G., Marena, S., Bodoni, P., Pagano, G., 1996. Postprandial triglyceride-rich lipoprotein changes in elderly and young subjects. Aging 8, 421–428.

Cogger, V.C., McLean, A.J., Le Couteur, D.G., 2001. The Liver Sinusoidal Endothelium in Ageing, Second International Conference on Hepatic and Splanchnic Circulation in Health and Disease Dunedin, New Zealand,.

Fraser, R., Dobbs, B.R., Rogers, G.W.T., 1995. Lipoproteins and the liver sieve: the role of the fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis, and cirrhosis. Hepatology 21, 863–874.

Harewood, W.J., Gillin, A., Hennessy, A., Armistead, J., Horvath, J.S., Tiller, D.J., 1999. Biochemistry and haematology values for the baboon (*Papio hamadryas*): the effects of sex, growth, development and age. J. Med. Primatol. 28, 19–31.

Harewood, W.J., Gillin, A., Hennessy, A., Armitstead, J., Horvath, J.S., Tiller, D.J., 2000. The effects of the menstrual cycle, pregnancy and early lactation on haematology and plasma biochemistry in the baboon (*Papio hamadryas*). J. Med. Primatol. 29, 415–420.

Heffernan, S., Phippard, A., Sinclair, A., McLennan, S., Hennessy, A., Gillin, A., Horvath, J., Tiller, D., Yue, D., Turtle, J., 1995. A baboon (*Papio hamadryas*) model of insulin-dependent diabetes. J. Med. Primatol. 24, 29–34.

Heffernan, S., James, V., Zilkens, R., Kirwan, P., Birrell, A., McLennan, S., Hennessy, A., Gillin, A., Horvath, J., Tiller, D., Yue, D., Turtle, J., 1996. Changes of extracellular matrix in a baboon (*Papio hamadryas*) model of insulin dependent diabetes: studies using electron microscopy and X-ray diffraction techniques. Diabetes Res. Clin. Pract. 34, 65–72.

Higashi, N., Imai, K., Sato, M., Sato, T., Kojima, N., Miura, M., Wold, H.L., Moskaug, J., Berg, T., Norum, K.R., Roos, N., Wake, K., Blomhoff, R., Senoo, H., 2002. Intralobular distribution of vitamin-A storing lipid droplets in hepatic stellate cells with special reference to polar bear and artic fox. In: McCuskey, R.S., (Ed.), The 11th International symposium on the cells of the hepatic sinusoid and their relation to other cells Tucson, Arizona.

Jansen, P.L., 2002. Liver disease in the elderly. Best Pract. Res. Clin. Gastroenterol. 16, 149–158.

Le Couteur, D.G., McLean, A.J., 1998. The aging liver. Drug clearance and an oxygen diffusion barrier hypothesis. Clin. Pharmacokinet. 34, 359–373.

Le Couteur, D.G., Cogger, V.C., Markus, A.M., Harvey, P.J., Yin, Z.L., Ansselin, A.D., McLean, A.J., 2001. Pseudocapillarization and associated energy limitation in the aged rat liver. Hepatology 33, 537–543.

Le Couteur, D.G., Fraser, R., Cogger, V.C., McLean, A.J., 2002. Hepatic pseudocapillarisation and atherosclerosis in ageing. Lancet 359, 1612–1615.

Mak, K.M., Lieber, C.S., 1984. Alterations in endothelial fenestrations in liver sinusoids of baboons fed alcohol: a scanning electron microscopic study. Hepatology 4, 386–391.

Mak, K.M., Lieber, C.S., 1986. Portal fibroblasts and myofibroblasts in baboons after long-term alcohol consumption. Arch. Pathol. Lab. Med. 110, 513–516.

Mak, K.M., Lieber, C.S., 1988. Lipocytes and transitional cells in alcoholic liver disease: a morphometric study. Hepatology 8, 1027–1033.

Mak, K.M., Leo, M.A., Lieber, C.S., 1984. Alcoholic liver injury in baboons: transformation of lipocytes to transitional cells. Gastroenterology 87, 188–200.

McLean, A.J., Cogger, V.C., Chong, G.C., Warren, A., Markus, A.M., Dahlstrom, J.E., Le Couteur, D.G., 2003. Age-related pseudocapillarization of the human liver. J. Pathol. 200, 112–117.

Miller, R., Kubier, P., 1997. Blocking of endogenous avidin-binding activity in immunohistochemistry: the use of egg whites. App. Immunohistochem. 5, 63–66.

Miyakawa, H., Iida, S., Leo, M.A., Greenstein, R.J., Zimmon, D.S., Lieber, C.S., 1985. Pathogenesis of precirrhotic portal hypertension in alcohol-fed baboons. Gastroenterology 88, 143–150.

Popper, H., 1986. Aging and the liver. Prog. Liver Dis. 8, 659–683.

Schmucker, D.L., 1998. Aging and the liver: an update. J. Gerontol. Series A, Biol. Sci. Med. Sci. 53, B315–B320.

Vestal, R.E., 1989. Aging and determinants of hepatic drug clearance. Hepatology 9, 331–334.

Wisse, E., Zanger, R.B.D., Charels, K., Smissen, P.V.D., McCuskey, R.S., 1985. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Hepatology 5, 683–692.

Wisse, E., Braet, F., Luo, D.Z., Dezanger, R., Jans, D., Crabbe, E., Vermoesen, A., 1996. Structure and function of sinusoidal lining cells in the liver. Toxicol. Pathol. 24, 100–111.

Yang, M.C., McLean, A.J., Le Couteur, D.G., 2002. Age-related alteration in hepatic disposition of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and pesticides. Pharmacol. Toxicol. 90, 203–207.