Temporal and Spatial Distribution of Spontaneous Iron Pigment Overload in the Liver of Han Wistar and Sprague-Dawley Rats

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ABSTRACT

The authors performed a retrospective study to determine and describe the incidence of spontaneous pigment overload in the liver of control Han Wistar and Sprague-Dawley rats. Data was collected from 1170 control animals (550 Han-Wistar and 620 Sprague-Dawley) from control dose groups from long term regulatory studies (104-week carcinogenicity studies). Further 628 control animals (300 Han-Wistar and 328 Sprague-Dawley) from control dose groups from short term regulatory studies (13-week and 4 weeks studies) evaluated at the authors' laboratory between 2009 and 2011. Livers from Han Wistar and Sprague-Dawley rats were re-evaluated using special stains to identify the nature of the pigments. In the periportal hepatocytes and in scattered sinusoidal Kupffer cells, the predominant pigment was identified as haemosiderin and a diagnosis of spontaneous iron overload was made. A comparison between the two strains revealed higher incidences of iron overload in Han Wistar rats than Sprague-Dawley rats. A significant sex difference was observed in both strains but was greater in Han Wistar rats. An age-related increase in the incidence and severity of pigment deposition was also apparent. Since there is little compiled data on spontaneous pigment overload in the liver the aim of this report was to summarize and discuss the incidence, distribution and factors affecting the occurrence of this background finding in control rats on toxicity studies.

Keywords: pigment, iron overload, spontaneous, liver, haemosiderin, rats.

INTRODUCTION

Cellular non-heme iron is stored and sequestered in the form of ferritin and hemosiderin[1]. Ferritin micelles result from the association of iron with the protein apoferritin and represent the ubiquitous form of iron storage occurring in the normal cellular metabolism. With local or systemic iron overload, ferritin forms haemosiderin granules, which represent an insoluble end stage product of ferritin degradation[2]. In the liver the periportal hepatocytes are the first to be affected by iron hemosiderin overload, followed by deposition in hepatocytes throughout the lobule and also in Kupffer cells, bile duct epithelial cells, and sinusoidal cells[3,4]. Most cases of iron haemosiderin overload do not result in toxic effects but progressive accumulation of iron as occurs in inherited haemochromatosis in man and experimental dietary iron overload in rats may cause liver damage with fibrosis and oval cell proliferation[3-5]. Iron is a catalyst of free radical mediated reactions on biological systems, and oxidative damage to cellular lipids, proteins and nucleic acids may result in cellular organelle damage and dysfunction, involving lysosome, mitochondria, and plasma membrane. A possible mechanism for liver damage from chronic iron overload may be therefore mediated by free radicals formation and lipid peroxidation[3]. Spontaneous iron overload in the liver has been reported in the SpragueDawley rat[4]. However, in literature there is paucity of specific information on the incidence of spontaneous iron overloading in the liver of Han Wistar rats[6]. Since this incidental finding may resemble drug-induced lesions, it is important for the pathologist to have available some reference material such as a historical control database with incidences of spontaneous findings in this strain. In a review of toxicological studies carried out at our laboratories during the period 2009-2011 we observed significant iron overload in the liver of control rats of different ages and in both sexes in two different strains, Han Wistar and Sprague Dawley rats. The aim of this study was therefore to characterize the nature of the pigment and to describe the occurrence, incidence and distribution of the pigment overload in young and aged control Han Wistar rats, compared to Sprague-Dawley rats.

MATERIAL AND METHODS

Animals

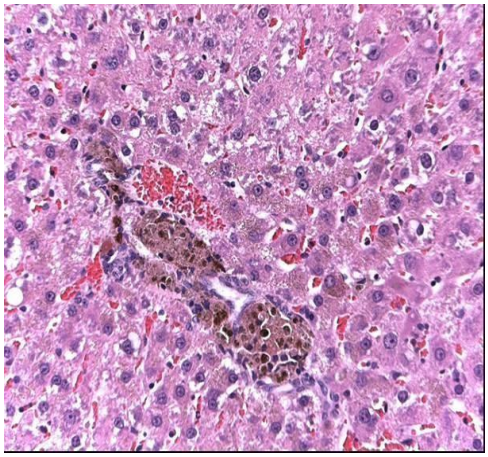


Figure 1: Moderate periportal Kupffer cells and periportal hepatocytes pigment deposition in a young control female Han Wistar rat from a 28 days study H&E X200.

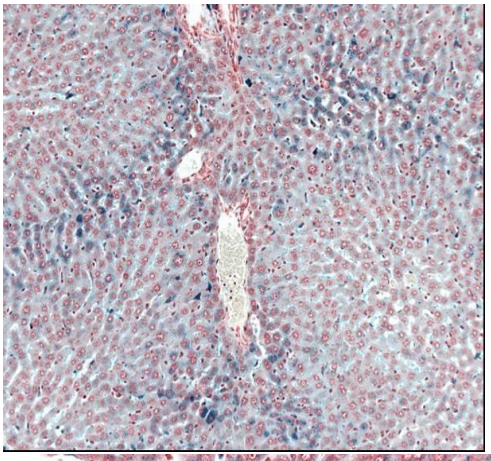


Figure 2: Moderate and predominantly hepatocellular haemosiderin pigment deposition in the periportal areas of the liver in a young control female Han Wistar rat on a 90 day study. Perls' Prussian blue reaction X100.

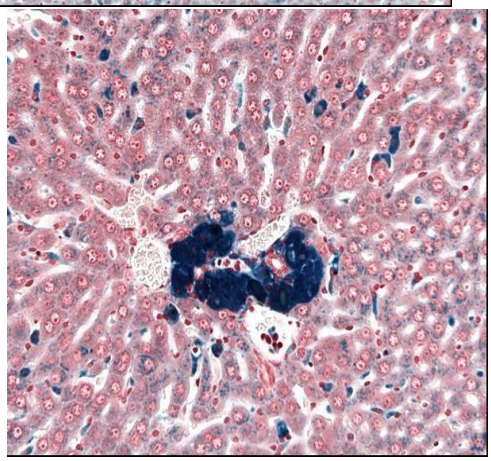


Figure 3: Moderate haemosiderin pigment deposition in periportal Kupffer cells of the liver in a young control female Han Wistar rat on a 90 days study. Perls' Prussian blue reaction X200.

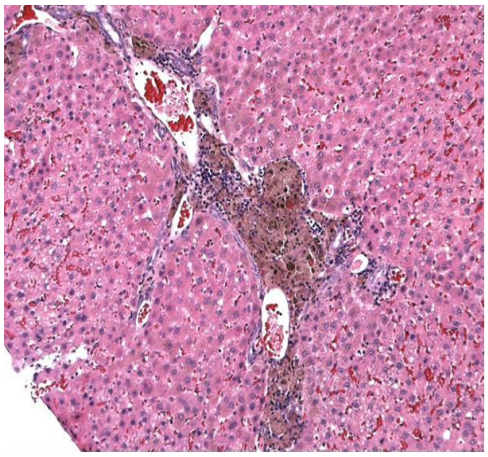


Figure 4: Moderate to marked haemosiderin pigment deposit in periportal Kupffer cells and mild deposits in periportal hepatocytes in a control female Han Wistar rat killed after 80 weeks. Note the large distension (engorgement) of Kupffer cells/macrophages with pigment, expansion of portal tracts

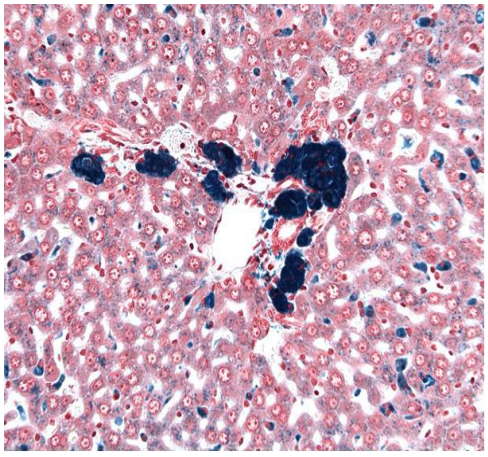


Figure 5: Perl's Prussian blue reaction positive pigment in periportal Kupffer cells control female Han Wistar rat from a carcinogenicity study X200.

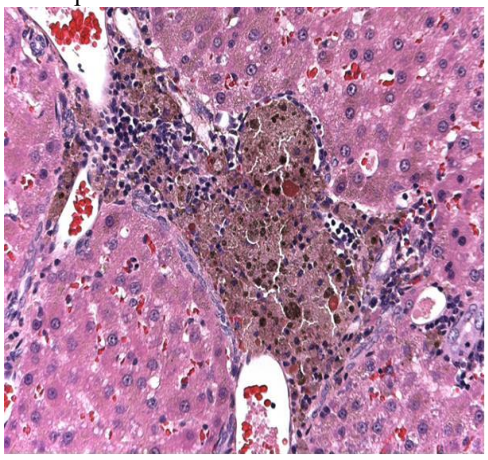


Figure 6: A higher magnification of figure 4 demonstrating haemosiderin pigment-engorged periportal Kupffer cells/macrophages and the expansion of portal tracts. H&E X200.

and a few mononuclear inflammatory cells. H&E X100.

Table 1: Incidence and cellular distribution of pigment deposits in the liver of Han Wistar rats in short term studies.

Study Duration	Number of animals/Sex	*Number of animals with pigment	Number of animals for pigment	Males				Females			
				Pigmented Liver tissues with pigment deposits				Pigmented Liver tissues on studies for pigment			
				Kupffer cells	hepatocytes	Number of tissues	% of animals	Kupffer cells	hepatocytes	Number of tissues	% of animals
28 Days	10	5	50	-	-	0	0	0	3	3	6
90 Days	10	6	100	1	-	1	1	2	4	4	4

* Includes incidental pigment present in non control animals.

Table 2: Incidence and cellular distribution of pigment deposits in the liver of Sprague-Dawley rats in short term studies.

Study Duration	Number of animals/Sex	*Number of animals with pigment	Number of animals for pigment	Males				Females			
				Total affected livers				Total affected livers			
				Kupffer cells	hepatocytes	Number of tissues	% of animals	Kupffer cells	hepatocytes	Number of tissues	% of animals
28 Days	10	2	60	-	-	0	0	1	-	1	1.6
90 Days	10	6	104	-	1	1	0.96	-	1	1	0.96

* Includes incidental pigment present in non control animals.

full-barrier buildings and fed a commercially available standard diet (pelleted Rat and Mouse No. 1 Maintenance Diet, Dietex International, UK) *ad libitum.* All studies were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986, which conforms to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, Council of Europe).

Histology

Liver slides from a total of 1170 animals (550 Han-Wistar and 620 Sprague-Dawley) were obtained from control animals assigned to 10 recent and ongoing carcinogenicity studies (2009-2011); a further total of 628 control livers from 40 short-term studies (twenty 4-week and twenty 13week studies) conducted between 2010 and 2011 at Huntingdon Life Sciences, UK were analysed. The morphological evaluation was made on the untreated control animals of each study. The numbers of animals per sex and per group in each study were; 50-65 animals for the carcinogenicity studies, 5-10 animals for the 28 days studies and 10-12 for the 13 weeks studies. Liver sections were evaluated for the presence and distribution of pigment. Tissues were histopathologically evaluated by one pathologist in order to maintain consistency in recording, and severity grades 1-5 representing, minimal, slight, moderate, marked and severe, respectively, were applied. The number of liver samples for each animal varied according to the type of study and the protocol. Sections were stained with hematoxylin and eosin routinely and, in some animals, with Perls' Prussian blue reaction (1% potassium ferrocyanide in 1% HCI) for iron and Schmorl's stain for lipofuscin. The slides were examined and photographed using a Leica light microscope.

RESULTS

Data presented in table 1-4 summarise the incidence and tissue distribution of pigment deposits in young and aged animals of both the strains, Han-Wistar and SpragueDawley rats.

Young animals

The incidence of pigment deposition in young animals on short term studies was very low in both strains and sexes, but occasional livers with up to moderate hepatocyte or Kupffer cell pigment were observed in female Wistar rats (Figure 1). Pigment deposition in young female Han Wistar rats was predominantly periportal, and in both hepatocytes and Kupffer cells, but minimal grades were mainly confined to hepatocytes (Table 1-2). The pigment was present as dark-brown finely granular material within hepatocytes and coarse granular material within Kupffer cells, with mild engorgement /swelling of the pigment laden (Kupffer) cells. All pigment deposits in young control animals stained positive for iron with the Perls' Prussian blue reaction (Figure 2 and Figure 3).

Aged animals

The results summarised in Tables 3 and 4 indicate that in aging control animals iron and haemosiderin overload occurs more commonly in Han Wistar rats. Both the incidence and severity of this change were higher in Han Wistar than in Sprague-Dawley rats. In both strains, females were more affected, but the sex difference was

Table 3: Incidence, cellular and zonal distribution of pigment deposits in the liver of Han Wistar rats in 104 weeks carcinogenicity studies.

Number of Animals/ Sex/Study	Males				Females					
	Pigment in periportal hepatocytes	Pigment in Kupffer cells	Pigment in centrilobular hepatocytes	Liver tissues with pigment deposits	Pigment in periportal hepatocytes	Pigment in Kupffer cells	Pigment in centrilobular hepatocytes		Liver tissues with pigment deposits	
				Number of tissues	% of animals				Number of tissues	% of animals
55	2	5	0	5	9	15	23	8	31	56
55	1	4	1	5	9	14	18	11	25	45
55	4	8	-	8	15	8	17	6	22	40
55	2	9	1	9	16	10	15	2	27	49
55	2	6	-	6	11	11	22	4	26	47
Total n=275/sex	11	32	2	33	12	58	95	31	131	48

Table 4: Incidence, cellular and zonal distribution of pigment deposits in the liver of Sprague-Dawley rats in 104 weeks carcinogenicity studies.

Number of Animals/Sex/Study	Males				Females					
	Pigment in periportal hepatocytes	Pigment in Kupffer cells	Pigment in centrilobular hepatocytes	Liver tissues with pigment deposits	Pigment in periportal hepatocytes	Pigment in Kupffer cells	Pigment in centrilobular hepatocytes		Liver tissues with pigment deposits	
				Number of tissues	% of animals				Number of tissues	% of animals
50	0	10	0	10	20	3	6	14	18	36
65	0	6	0	6	9	0	17	0	17	26
65	0	4	0	4	6	1	16	1	16	25
65	1	16	2	18	28	2	11	2	11	17
65	0	8	2	8	12	2	8	0	8	12
Total n=310/sex	11	32	4	46	15	8	58	17	70	23

greater (more than threefold) in Han Wistar rats. Up to marked grades of periportal hepatocyte and Kupffer cell haemosiderosis, some with gross correlation of dark livers were observed not uncommonly in aged female HanWistar rats (Figure 4 and 5). The most affected livers had moderate nodular development of pigment-engorged Kupffer cell/macrophages with mild distortion of periportal tracts, minimal inflammatory cells and bile duct/oval cell hyperplasia (Figure 6 and 7). While a periportal distribution of pigment deposits was apparent in female Han Wistar rats, (Figures 2 and 8) in males of the same strain and in Sprague-Dawley rats, pigment deposition was typically randomly distributed in sinusoidal Kupffer cells (Figure 9) or present in centrilobular hepatocytes, and was graded as minimal to slight grades of severity. All periportal hepatocyte pigment and Kupffer cell pigment stained positive for iron, but some centrilobular hepatocyte pigment present in aged female rats of both strains stained positive for lipofuscin pigment with Schmorl's stain (Figure 9).

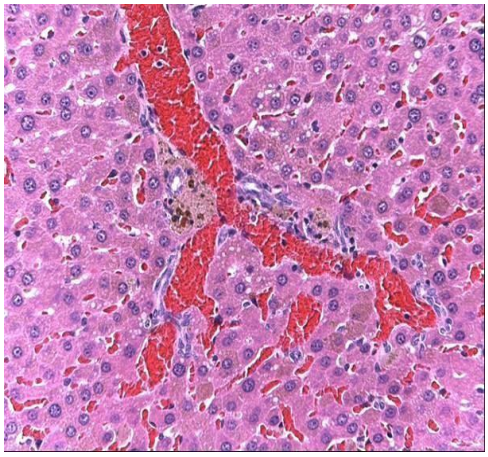
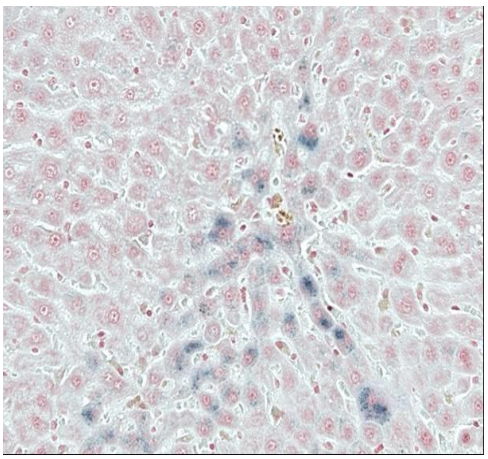


Figure 7: Moderate periportal hepatocyte and Kupffer cell pigment deposition in an aged control female Han Wistar rat killed in extremis after 72 weeks. There is an apparent decrease in the amount of intra-hepatocellular deposited pigment going towards centrilobular areas. Minimal bile duct/oval cell proliferation is also present. H&E X200.



DISCUSSION

The present study results documents the occurrence of iron overload in Han Wistar and Sprague-Dawley rats, and highlights the differences related to strain, sex and age. Haemosiderin iron deposition was more common in Han Wistar rat livers when compared to Sprague-Dawley rats. In both strains, the females were more affected and an agerelated increase in the severity and incidences of the finding was observed. The results also showed there were sex and strain differences in the distribution of the pigment across the liver lobe. In female Han Wistar rats, a clear pattern of lesion distribution with increasing age and severity could be established. In young female Han Wistar rats pigment deposits were predominantly in periportal hepatocytes with occasional involvement of periportal Kupffer cells/macrophages. Progression towards centrilobular hepatocytes and the involvement of nonhepatocyte cells increased with age and severity. This pattern is similar to that reported in Sprague-Dawley rats

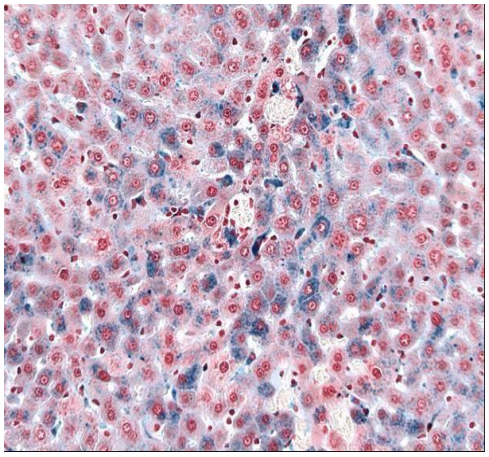


Figure 8: Moderate periportal hepatocyte pigment deposit in a control female Han Wistar rat killed after 52 weeks. Perl's Prussian blue reaction X200.

Figure 9: Lipofuscin pigment within centrilobular hepatocytes in a control female Sprague-Dawley rat sacrificed after 104 weeks. Haemosiderin pigment present in scattered sinusoidal Kupffer cells did not stain positive with Schmorl' stain. X200.

with iron overload in the literature[4,5,7]. In the present study, male animals of both strains and Sprague-Dawley rats had low grades of pigment deposition with minimal hepatocyte involvement and little or no haemosiderin-engorged periportal Kupffer cells. This report presents the incidence, morphological features and histochemical characteristics of spontaneous iron overloading that occurs in the liver in control Han Wistar rats in comparison to Sprague-Dawley rats. The results

presented from this survey highlight the importance to have a well characterized control data base to permit better assessment of potential treatment related effects that may alter the incidence or severity of a spontaneously occurring lesion such as the iron overloading in the liver.

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