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Gender and aging in the liver: preliminary data using design-based stereological methods

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Different epidemiological studies demonstrated that male gender and age are risk factors for the progression of liver disease [1]. Regarding age, it has been associated with liver mass and blood flow reduction [2], whereas in gender, the estradiol has revealed protective roles in models of liver fibrosis and of trauma-hemorrhage in rats [3]. So far, most of the studies on aging focused their attention in hepatocyte (HEP) structure and function [4-7] and few data exists pertaining to other liver cell subpopulations, including hepatic stellate cells (HSC) and Kupffer cells (KC). To the best of our knowledge, an integrative analysis (with modern stereology) of the liver cell populations, throughout aging and gender, has never been performed.
Our aim herein was to analyse, with the optical smooth fractionator, the numerical variation of the main cells involved in regeneration, fibrosis and inflammation in the liver, that is, respectively, HEP, including binuclear cells (BnHEP), HSC and KC.
We used male and female Wistar rats (n=5), of 2, 6 and 12 months. Their liver was fixed by perfusion, using 10% buffered formalin, and submitted to a systematic random sampled (SRS) cascade with a random start, according to the smooth fractionator rules; this provided a final sample of 5 fragments, on average. These were processed for paraffin embedding and exhaustively sectioned (30 μm thick) in a motorized rotary microtome. In every 30 sections, 3 consecutive ones were SRS and used for immunohistochemistry against: 1) carcinoembryonic antigen, to differentiate mononucleated hepatocytes from BnHEP; 2) glial fibrillary acidic protein, for HSC; 3) ED2, for KC.
At this point, we already completed the analysis of young male rats (Table 1), obtaining the total number (N) of HEP, HSC, KC and their proportions [8-10]. We followed the N of HSC throughout young (2 months), adults (6 months) and mature (12 months), observing a slight increase (non-significant). In adults, the first data point to a lower N of HSC in females. The coefficient of error of N estimates was low and within the limits for this type of study.
In conclusion, we show that the latest generation of stereology techniques for number estimation can be implemented in the rat liver, for quite different cell types, providing sound baseline data in normal conditions. Since those techniques rely on sampling designs, if correctly used, they are judged to grant unbiased and precise data that can be extremely useful for detecting regenerative and fibrotic responses in rat liver models, from its very early onset. The preliminary data on the N of HSC points to a lower N in female rats, corroborating the lower fibrotic responses observed in these animals; this also substantiates the existing epidemiological data [11]. Regarding aging effects, the groups studied still did not reveal statistical significant differences — we hypothesize that these should occur in older rats (beyond the 12 months here studied).

Table 1 – Total number (N) and number per gram of liver (N/g) of all the cells (*i.e.*, stromal and parenchymal), mononucleated (MnHEP) and binucleated hepatocytes (BnHEP), hepatic stellate cells (HSC) and Kupffer cells (KC). Data given as: mean (CV). CE: coefficient of error.

CELL TYPE	N	N/g	CE (N)	% of all cells
All liver cells	3.25×10^9 (0.22)	216.8×10^6 (0.14)	0.02	100
MnHEP	1.44×10^9 (0.23)	96.2×10^6 (0.17)	0.03	44.5
BnHEP	520×10^6 (0.31)	33.0×10^6 (0.24)	0.05	15.2
HSC	207×10^6 (0.17)	13.9×10^6 (0.14)	0.04	6.4
KC	283×10^6 (0.18)	19.4×10^6 (0.25)	0.05	9.0

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