

Mechanisms of Aging and Liver Functions

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Key Words

Aged liver, stress response · Aging liver · Liver regeneration · Mast cells

Abstract

Background/Aims: Morphofunctional studies suggest that the liver, compared with other organs, ages fairly well. Its success is ascribable to its lasting ability to regenerate, even if the potential of the cells to replicate progressively declines with age. The aim of this study was to analyze some aspects of the early phases of liver regeneration, its capacity to mount a stress response, and the inflammatory response in the early stage of an acute injury. **Methods:** Rats aged 2, 6, 12 and 19 months received a single intraperitoneal injection of CCl₄, and morphological, biochemical and molecular evaluations were done 2 and 24 h later. **Results:** AST and ALT, starting at age 12 months, were significantly higher than in the younger rats after CCl₄. Histological modifications were already detectable after 2 h in rats aged 12 and 19 months, thereafter becoming more diffuse and marked, whereas they become evident only 24 h after the intoxication in rats aged 2 and 6 months. Albumin, c-fos, c-myc, hepatocyte growth factor, transforming growth factor- α and HSP70 mRNA levels decreased 24 h after CCl₄ starting at age 12 months. Mast cell density was higher in the young rats than the old ones. **Conclusion:** Our results point to: (a) a basically

preserved regenerative response of the aged liver, although somehow weaker and slower, with reduced ability to counteract agents inducing cell necrosis; (b) a decrease in the HSP70 response suggesting a reduction in homeostatic capacity, and (c) a lower inflammatory response during aging.

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Aging affects organs, tissues and cell functions to different extents, resulting in a decline in the capacity of the whole organism to cope with its environment. Morphofunctional studies suggest that the liver, compared with other organs, ages fairly well [1].

This concept, for a long time more anecdotal than scientifically proven, was firmly demonstrated several years ago by means of a rat transplant model [2]. Livers from young (5 months) and old (28 months) syngeneic BN/BiRij rats were transplanted into young recipients, and there were no differences in the survival curves. Analysis of the transplanted aged livers showed reduced weight of the organ, hepatocyte degeneration, bile duct proliferation, pigment deposition, and mild to moderate fibrosis [2, 3]. All these features were perhaps well known, but certainly not the unexpected ability of the liver to survive far beyond the rats' maximum life span [2]. More evidence comes from the finding that under physiological

conditions the liver's overall functions do not appear seriously affected by aging, as suggested by a full analysis of serum tests in subjects of different ages [4]. However, wide interindividual variability is the main characteristic of aged people [1, 4].

The successful aging of the liver is probably ascribable to its relatively well-preserved ability to regenerate, even though the potential of the cells to replicate progressively declines with age [5]. This statement is based on the observation that partially resected livers in old animals need longer to regain their original volume, compared to the young. This can also be elicited experimentally by an acute chemical treatment with CCl₄ [6–8], providing a useful model to investigate aspects such as: (a) the triggering mechanisms in the early phase of regeneration; (b) the capacity to mount a stress response to a noxious stimulus, and (c) the inflammatory response in the early phase of an acute injury.

Here we report and review these aspects in the *in vivo* model of a single intraperitoneal injection of CCl₄ to young (2 months), adult (6 and 12 months) and aged rats (19 months). All the evaluations, biochemical, morphological and molecular, were done 2 and 24 h after liver intoxication, in comparison with age-matched untreated animals.

Liver Regeneration

Under physiological conditions the liver is a largely quiescent organ in terms of cell proliferation since only a very small percentage of hepatocytes undergoes mitosis at any given time. Cell necrosis or surgical resection induces the hepatocytes to proliferate and replicate, thus restoring the previous functional capacity as well as the original mass [9].

Cell death caused by CCl₄ drives hepatocytes through two main steps: a 'priming' phase that gives them replicative competence, and a 'progression' phase in which primed cells undergo DNA replication [6, 9]. This transition requires the expression of some genes whose products regulate key events during the G₀ and G₁ phases. Among these early genes, *c-fos* and *c-myc* [6, 10] act as transcription factors, encoding nuclear phosphoproteins believed to function as regulators of cell proliferation.

Regeneration is strictly governed by growth factors and cytokines, as suggested *in vitro* and *in vivo* [11] and, in this context, up-regulation of hepatocyte growth factor (HGF) and transforming growth factor (TGF)- α exert a positive control [6, 10] acting, respectively, in an auto-

crine and paracrine fashion. The whole process ends once the liver mass reaches a species- and age-specific fraction of total body mass, indicating that negative regulatory influences are needed and, in fact, a stop signal is encoded by the TGF- β_1 gene [12].

All this machinery in the senescent phenotype has been comprehensively investigated by analyzing serum transaminases, liver histology, GSH content, albumin, *c-fos*, *c-myc*, HGF, TGF- α and TGF- β_1 gene expression [13]. AST and ALT levels, biochemical markers of hepatic injury, are not affected by aging *per se*, but are significantly higher than in young rats after CCl₄, starting at age 12 months and more in the oldest rats, since there is more cell necrosis.

The histological findings indicate a very similar morphological appearance in control rats, but treated rats have localized cell necrosis in the central region (zone 3) with an inflammatory reaction, the portal tracts being almost spared. Hydropic degeneration, with a clear cytoplasm, vacuolization or ballooning, and fatty changes from few droplets to diffuse involvement, are the main features in the pericentral zone (fig. 1). These findings are evident 24 h after the intoxication in rats aged 2 and 6 months, but are already detectable after 2 h in those aged 12 and 19 months, thereafter becoming more diffuse and marked. All this points to a lower capacity to counteract the free radical toxicity subsequent to CCl₄ metabolism. The reduced GSH content of aged livers probably explains the susceptibility to oxidative stress [13, 14]. However, the recent finding of changes in the sinusoidal endothelium and spaces of Disse, termed 'pseudocapillarization', that restrict the availability of oxygen and other substrates [15], also marks the lower energy status of the aged liver as a major determinant of impaired xenobiotic detoxification.

At the molecular level this model discloses several features of the aging process. In both young and aged animals there was a clear reduction of albumin gene transcription 24 h after CCl₄ intoxication but, 2 h after the treatment, this was true only in the old rats, since this gene was up-regulated in the young ones, pointing to a more efficient transcription machinery [13, 16].

Analysis of the *c-fos* and *c-myc* gene offers information on the entry of the priming quiescent hepatocytes into the cell cycle and of the better ability of young and adult cells, compared to senescent one, 2 h after CCl₄ (fig. 2a). The persistence of *c-fos* transcripts for 24 h only in 6-month-old rats might correspond to a second round of replication, because only hepatocytes of this age are able to replicate through more than one cell cycle

Fig. 1. Microphotographs showing histological changes in liver tissue. Two-month-old control rat (a), or 2 h (b) and 24 h after CCl₄ (c); 19-month-old controls (d), or 2 h (e) and 24 h after CCl₄ (f). Sections were stained with hematoxylin and eosin. Orig. magnif. ×200.

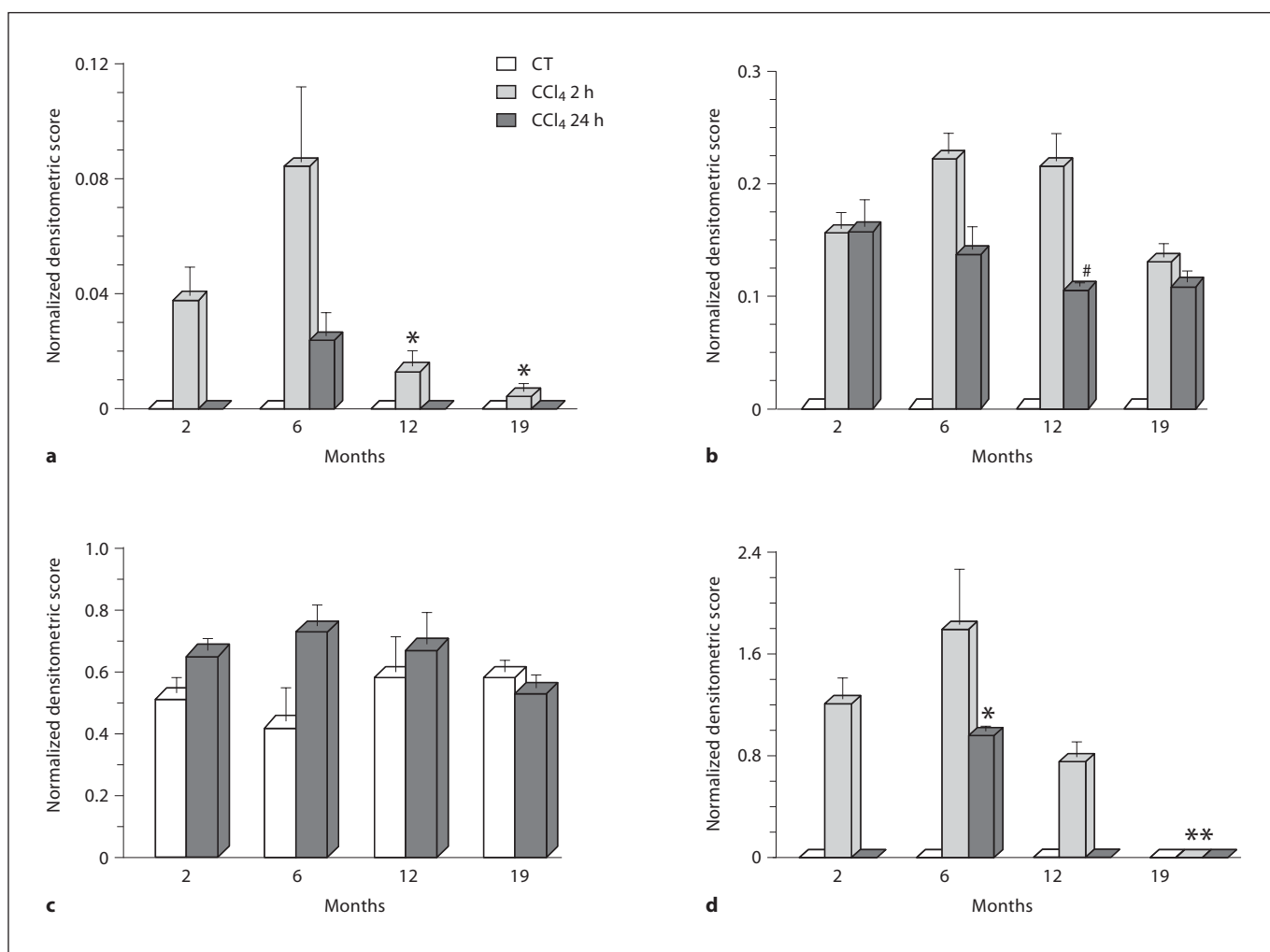
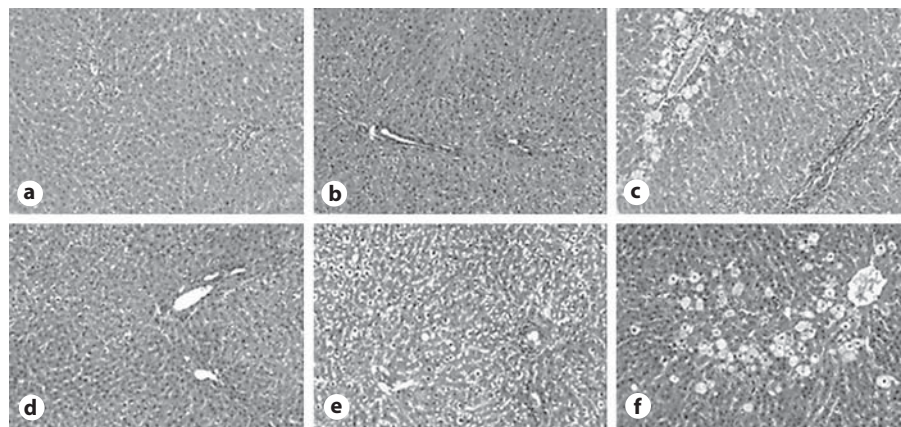


Fig. 2. Bar graph illustrating the mRNA steady-state levels in liver homogenates from control and treated rats aged 2, 6, 12 and 19 months. Changes in mRNA signal of c-fos (a), c-myc (b), TGF-α (c) and HSP70 (d) are expressed as optical densities normalized on GAPDH gene expression. Values are means ± SEM (4 animals per age and treatment group). * p < 0.05 vs. 6 months old, CCl₄ 2 h; #p < 0.05 vs. 12 months old, CCl₄ 2 h; ** p < 0.05 vs. 2, 6 and 12 months old, CCl₄ 2 h.

[9]. At the same time, the fact that c-myc mRNA levels were highest in young-adult rodents (fig. 2b) suggests some facilitation of proliferation, either by enhancing the responsiveness of growth factors [17] or by shortening the cell cycle, accelerating the G0-G1 transition [18].

All together the c-fos and c-myc data show that senescent hepatocytes are triggered for a delayed, weaker regenerative response because they are blocked in G1 phase, as already demonstrated in fibroblasts [19].

In this experimental context, the HGF gene transcript was always more abundant in the old animals, possibly because of their more limited ability to maintain homeostasis, as suggested by a positive relationship between plasma HGF levels, hepatic necrosis and systemic inflammation [20, 21].

The involvement of TGF- α seems different from that of HGF: TGF- α mRNA levels were increased after 24 h in rats aged 2, 6, and 12 months, but not in the old animals (fig. 2c).

One of the most powerful stop signals to liver growth is exerted by TGF- β_1 [12]. Again, in this model, the tendency to a progressive age-dependent decrease of its transcripts suggests that the aged liver takes longer to complete regeneration, as also indicated by the longer cell proliferation phase after treatment with a neutralizing TGF- β_1 antibody [22].

Stress Response of Aged Livers

The heat-shock response is a highly conserved defense mechanism against noxious agents, including high temperature, alcohol, heavy metals, xenobiotics, oxidants, and radiation, and is mediated by the transient transcription of genes belonging to the family of heat-shock proteins (HSP) [23]. This provides a state of increased cell resistance, because HSP form transient complexes with other cellular proteins, promoting their folding into the correct secondary structures thus preventing denaturation.

As described by several authors [24–27], aging is associated with a decrease in HSP70 response, and this is assumed to be one of the main features of aging, which involves a reduction in homeostatic capacity. Therefore, it is not surprising that 2 h after CCl₄, HSP70 mRNA levels were raised only in young-adult rats, and almost undetectable in the oldest ones (fig. 2d). Induction of the gene still persisted at 24 h only in the adult animals (6 months). The ability to produce the protective HSP70 is

unbalanced with increasing age and thus may explain, at least in part, the increased susceptibility of aged livers to stress- and age-associated diseases. Furthermore, the knowledge of the highly integrated and complex responses of aged organisms to environmental stress will provide a basis for developing therapies related to aging-associated pathophysiological conditions.

Inflammatory Response

Inflammation involves a complex set of interactions among soluble factors and cells, and identifies a finely-tuned local protective response, because its deficiencies or excesses may cause morbidity and shorten life span [28]. This response is modified in the elderly, where both function and phenotype of pivotal cells, i.e. monocytes and granulocytes, are affected [29].

Much attention has focused on the recruitment of leukocytes from the blood. However, a rapid response requires *sentinel cells* pre-stationed in the tissues, such as macrophages and mast cells (MC) [30]. MC are a heterogeneous family of cells containing different enzymatic proteins, such as tryptase, chymase, vasoactive amines, neutral proteases, carboxypeptidase and cathepsin E, whose content depends on the animal species and stage of development. MC maturation can be induced by a wide range of stimuli, including multivalent antigens (allergens), neuropeptides, growth factors, complement components (serum glycoproteins) and various drugs [31–34]. Their activation results in *degranulation*, with secretion of preformed mediators stored in the cytoplasmic granules, *synthesis* of pro-inflammatory lipid mediators, and the *synthesis* and *secretion* of cytokines and chemokines [35]. All these events lead to both immediate and late-phase responses. Recently, Nguyen et al. [36] have suggested the ability of prostaglandin E₂ (PGE₂) to initiate MC degranulation changes in the aging animal, and therefore elevated PGE₂ levels might provide an important pathway by which MCs are engaged in inflammatory responses in the elderly patient.

MCs are critical effectors in many pathological conditions, including acute liver injury and, since their tissue accumulation can change significantly in the course of the inflammatory response, they have been assigned a role as an early indicator of this process.

MCs have recently been studied to investigate their involvement in the mechanisms leading to age-related chronic diseases [37, 38]. In the experimental model described, MCs were evaluated as a quantitative parameter

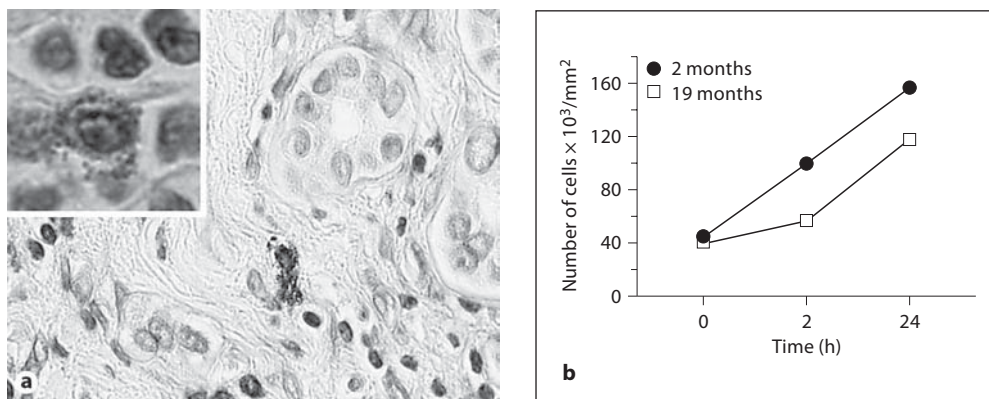


Fig. 3. a Localization of mast cells (MC) in rat liver: MCs are mainly recognizable in the connective tissue near the portal tracts and centrilobular veins. These cells are usually histochemically stained with toluidine blue, which metachromatically stains the glycosaminoglycans of MC granules (toluidine blue, $\times 40$; inset, $\times 100$). **b** MC densities in rats aged 2 and 19 months, untreated and 2 and 24 h after CCl_4 . MC density was considerably raised in young rats 2 and 24 h after CCl_4 : the difference between untreated rats and treated 2-month-old rats 24 h after intoxication was significant ($p < 0.0001$). In the 19-month-old rats, MC density increased less than in the young rats, but there were significant differences between the untreated rats and the treated rats 2 h after CCl_4 ($p = 0.032$), and between the densities after 2 and 24 h ($p = 0.037$).

of acute inflammation, in order to detect any age-dependent changes of their density in the liver [37, 38].

Histological analysis (fig. 3a) showed that although MCs were mainly located in the connective tissue near the portal tracts and centrilobular veins, there were no real differences between the various zonal arrangements of the hepatic cells and the microcirculatory system. MC density rose considerably in young rats 2 and 24 h after CCl_4 intoxication, whereas this increase was less marked in the old animals. The changes in MC density in rats aged 6 and 12 months fell between those in the rats aged 2 and 19 months (fig. 3b).

These findings, confirming a role for MC as a marker of acute liver inflammatory reaction, show that there is higher and faster recruitment of these cells in injured tissue in young rats, suggesting that this inflammatory response is lowered during aging.

Conclusions

These findings point to a basically preserved regenerative response of the aged liver, though somewhat weaker and slower than in younger rats. This was coupled with a reduction in the ability to counteract agents promoting cell necrosis.

From a clinical point of view, these results provide further evidence that: (1) acute liver failure is definitely more severe in old than young subjects; (2) the progression to chronicity and the clinical course of chronic viral hepatitis is worse in aged patients when the infection arises late in life; (3) nevertheless, livers of donors aged >65 years, when carefully evaluated, could offer a substantial source of organs, helping satisfy the increasing number of patients listed for liver transplant.

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