Review

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Aging Liver

A Review

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Aging process, liver · Reactive oxygen species · Replicative senescence

Abstract
Aging is characterized by a progressive decline of cellular functions. The aging liver appears to preserve its function relatively well. Aging is associated in human liver with morphological changes such as decrease in size attributable to decreased hepatic blood flow. Ultrastructural analysis of the human liver has revealed that the integrity of mitochondria and enzymatic activity remain mostly unchanged with aging. Reactive oxygen species (ROS) are involved in the aging process and result mainly from nonenzymatic processes in the liver. Endogenous free radicals are generated within mitochondria and suspected to cause severe injury to mitochondrial DNA. This damaged DNA accumulates with aging. In addition, polyunsaturated fatty acids, highly sensitive to ROS, decrease in liver mitochondria from human centenarians, a feature acquired during evolution as a protective mechanism to favor longevity. Diet is considered the main environmental factor having effect on lifespan. It has a major impact on aging liver, the central metabolic organ of the body. The ubiquitin proteolytic pathway in the liver serves to destroy many proteins, among them p21 which is encoded by abundant mRNA in senescent cells, can inhibit cell proliferation and favors DNA repair. Drug therapy in the elderly may be complicated by several factors such as decline in body weight, renal function, liver mass and hepatic blood flow, making adverse drug reactions more frequent. Hepatic drug metabolism is mainly mediated by the cytochrome P_{450} system and drug interactions in the elderly are likely related to the progressive decline of this system after the fifth decade of life and another decrease in individuals aged >70. Antihypertensive therapy in the elderly depends upon either hepatic or renal function and should be adjusted accordingly. Finally, telomerases are the biological clocks of replicative lifespan. Shortening of telomeric ends of chromosomes correlates with aging and decline in the replicative potential of the cell: replicative senescence. Telomere DNA of human somatic cells shortens during each cell division thus leading to a finite proliferation. Transfection of the telomerase reverse transcriptase gene results in elongation of telomeres and extension of lifespan. This also applies to humans. Replicative senescence in human cells evolved as a mechanism to protect them from continuous divisions leading to multi-

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ple mutations. Longer-lived species such as humans had to develop replicative senescence to ensure that they would have the increased protection that their longevity necessitates.

Introduction

Getting old is a normal event in the life of virtually all organisms with the exception of germinal cells and malignant cell lines. Aging is characterized by a progressive decline of cellular functions that eventually results in death because cells progressively lose their capacity to respond successfully to injury. It now seems that senescence-specific genes or alterations of growth-regulator genes may underlie some of the mechanisms leading to aging and shortening of lifespan. Furthermore, shortening of the telomeres of chromosomes might lead to aging and early cell death. Aging can be prevented by malignant transformation. Cultured human fibroblasts have a limited lifespan before they deteriorate, become senescent, lose the capacity to divide and finally die. The number of cell population doublings achievable exhibits a linear negative correlation with donor's age [1]. The original assumption that population doublings are inversely proportional to donor's age has been challenged recently by at least three studies [2–4]. None of these studies found a significant negative correlation between the two parameters.

The liver appears to preserve its function relatively well with aging and has an excellent capacity to regenerate either after partial hepatectomy or transplantation. The present review will discuss the main age-related alterations in the liver. The primary focus is on human studies supplemented with reports on experimental animals when necessary or specifically relevant.

Morphological Changes in Aging Liver

Aging is associated with a variety of morphological changes in the liver, but the mechanisms of these structural changes have remained unclear. In the past, human studies of the aging liver were limited and relied on postmortem samples and often used elderly subjects with liver disease. A study based on 1,582 subjects, aged between 20 and 80, was published a decade ago [5]. According to this study, the liver progressively decreased in size with aging and experienced a marked decline in volume occurring after the 6th decade of life as detected by ultrasonography [5]. Wynne et al. [6] showed a significant negative correlation between liver volume and age in 65 volunteer subjects aged 24–91. They [6] explained this feature by an age-dependent decrease in hepatic blood flow of about 35% in subjects over 65 years when compared to those under 40 years. The most recent study by Zoli et al. [7] has confirmed this finding.

The classic gross appearance of the liver in old persons is known as 'brown atrophy'. Similar appearance is also seen in younger patients with malnutrition and cachexia. The brown color is due to accumulation of lipofuscin (ceroid) within hepatocytes. Human liver tends to show macrohepatocytes and polyploidy with increase in nuclei and nucleoli during aging [8, 9] especially around the terminal hepatic veins. In humans aged 86–92 years the polyploidization is increased by up to 27% when compared to the young [8].

Ultrastructural variations of the organelles of hepatocytes are normally observed with considerable heterogeneity among the different cells even within the same lobule. The matrical density and number of mitochondria are decreased with aging in rats and humans [8, 9], but the integrity of the mitochondria and enzymatic activity may remain unchanged with aging [8, 9].

The rough endoplasmic reticulum (RER) seems to be diminished in aged rats [8], coupled with a decreased production of glucose-6-phophatase and increased production of γ-glutamyltransferase in periportal hepatocytes. The decline with aging in RER may represent a change in the protein synthesizing capacity of the hepatocytes [8]. The smooth endoplasmic reticulum (SER) shows a decline with age that correlates with the decline in the yield of liver microsomal protein [8]. Because the mixed-function oxidases (MFOS) exhibit a diurnal variation [10], the differences during aging have not been tested.

The number of lysosomes is increased conspicuously in aged compared to young male rats, i.e. predominantly secondary lysosomes and residual bodies [8]. Lysosomal function, however, does not appear to be increased.

Liver Function during Aging

Aging has been shown to be associated with multiple changes in hepatic function, however the clinically relevant biochemical parameters of liver function remain generally normal in the elderly. Thus, abnormalities of these parameters should be evaluated for the presence of liver disease.

Albumin Synthesis

The synthesis of albumin represents a major component of protein production in the liver. Animal studies have shown contradictory results regarding age-related changes in albumin synthesis. Some have reported age-related decline in the hepatic biosynthesis of albumin and decreased plasma concentration of albumin [11], while others have not [12]. Serum concentration of albumin may decrease by as much as 10–20% in aged rats. This could be due to decreased expression of the albumin gene [13]. The gene has a DNAse I hypersensitive site which is distinctly less sensitive in old rats. Its 5′-CCGG-3′ sequences are more methylated in the old, in which the rate of transcription is also lower. However, some studies have shown a decrease in the synthesis of intracellular proteins and increase in the concentration of extracellular protein (albumin) in the aging liver [12]. This has been attributed to an increase in albumin mRNA content in the liver [12]. On the other hand, during normal aging in humans, serum albumin exhibits a slight decline or remains normal [14]. In institutionalized aged humans with nonpsychiatric disorders, low levels of serum albumin have shown a striking correlation with mortality [15].

Lipids

Hepatic triglycerides and cholesterol contents rise with aging while phospholipids quantitatively remain unchanged [16]. Serum levels of cholesterol, high-density lipoprotein cholesterol and triglycerides increase with age, but decrease in individuals ≥90 years of age [14]. There is a decline of 35% in older subjects in the metabolism of low-density lipoprotein (LDL) cholesterol accompanied by a decrease in LDL receptors [17]. These changes may contribute to the observed elevation of serum cholesterol levels during aging of certain human populations. No doubt, this could contribute to the increased incidence of coronary artery disease in elderly subjects. In contrast, one study [18] of an aged institutionalized human population revealed that patients with serum levels of cholesterol <150 mg/dl exhibited a mortality rate of 63% when compared to 9% in patients with cholesterol levels >150 mg/dl. Another study from the same group [19] by multivariate analysis of a geriatric population, showed that men with serum cholesterol levels of ≤156 mg/dl and hematocrit of ≤41%, died at a rate 42 times to those of men with values above both thresholds.

Enzymes

It has been reported [14] that elevation in the activities of serum γ-glutamyltransferase and hepatic alkaline phosphatase occur, especially in humans aged >90. The information available from previous reports did not accept these as true findings; thus, the subject remains controversial. On the other hand, it is accepted by most authors today that aminotransferases and serum bilirubin remain normal in the aged. Caloric restriction (CR) can decrease enzymatic capacity for glycolysis and increase the enzymatic capacity for hepatic gluconeogenesis and the disposal of byproducts of muscle protein catabolism [20]. Hormonal inducibility of mitochondrial enzymes is reduced and respiratory chain enzymes can decrease with aging [21].

Reticuloendothelial System Function

It appears that in rat liver Kupffer cell function declines with age, but endocytosis by endothelial cells remains unchanged [22, 23]. The implications of those changes in humans are unknown because studies of the macrophage function of Kupffer cells with aging are rare. In one study performed in rats [23], there was no change in the volume density of Kupffer cells between 2 and 24 months of age. This suggests that the size of the Kupffer cell population does not increase with age. Furthermore, the capacity of Kupffer cells to phagocytize and degrade radiolabeled mitochondria is less efficient [23]. Their role in the production or degradation of cytokines has not been studied during aging. The possible variations of stellate cells with aging have not been investigated.

The Role of Free Radicals in Aging Liver

Reactive oxygen species (ROS) are now widely accepted as being one of the mechanisms involved in the aging process as well as in numerous human disorders where they have either a primary or a secondary role. Oxygen radicals have also been implicated in alterations of DNA and apoptosis [24]. Free radicals form as a result of ionizing radiation and nonenzymatic processes in the liver [25]. Aging has been shown to be associated with increased superoxide anion, hydrogen peroxide and hydroxyl radical resulting in oxidative protein damage in the liver [26–30]. Mitochondria appear to be the major source of the oxidative lesions that accumulate with age [28], and these lesions have been proposed as the major cause of cellular aging and death [31, 32].

The liver has a battery of enzymes with antioxidative functions, i.e. Mn-superoxide dismutase (SOD) in mitochondria; Cu, Zn-SOD and glutathione (GSH) peroxidase in the cytosol, and peroxisomal catalase. Some of these are

dependent on dietary factors, such as GSH concentration and vitamin E, while others, like SOD, are less diet-dependent [25, 33]. The activity of liver enzymes like SOD, catalase and GSH peroxidase are positively correlated with the lifespan of different animal species, whereas P_{450} and GSH concentrations are negatively correlated [25].

The rate of oxidative DNA damage in mammalian species is directly related to the metabolic rate and inversely correlated with lifespan [24]. Normally it seems that during aging oxidative damage to nuclear and mitochondrial DNA is extensive [24]. Studies with several animal species demonstrated that humans, being the longest-lived species under consideration, have the highest tissue levels of SOD [34].

Endogenous free radicals mostly produced within mitochondria are associated with a physiological decline in aging organisms [28]. Furthermore, aging is accompanied by degenerative processes [28] and dietary antioxidants are known to protect against them. ROS generated within mitochondria are suspected to cause damage to mitochondrial macromolecules. Mitochondial DNA (mtDNA) mutations and deletions have been described with aging [28]. It seems that the more severely damaged mtDNA may accumulate with aging. In addition it has been shown that the content of cytochrome oxidase exhibits a progressive decline which correlates with age-associated decline in mtRNA synthesis in brain, liver, heart, lungs and skeletal muscle [28]. Further evidence indicates that oxidative damage to mitochondrial proteins and cell membranes occur with aging, a feature consistent with the age-associated rigidity in membranes [32]. Polyunsaturated fatty acids (PUFAs) are highly sensitive to ROS [32]. It has been previously described that liver mitochondria from human centenarians have a lower degree of PUFAs and a decreased sensitivity to lipid peroxidation [35]. Thus, it is likely that during evolution a low degree of PUFAs in liver mitochondria may have been selected during longevity in mammals as a protective mechanism against oxidative damage [35].

The Influence of Diet in Aging Liver

The main environmental factor having an effect on lifespan has been shown to be diet. CR slows the rate of the aging process and extends maximum lifespan in experimental animals and possibly in humans [25, 36, 37]. In experimental animals, CR prevents development of age-related autoimmune diseases and cancers [25, 37]. While CR initiated at younger age is most beneficial, CR initiated at middle age can also have similar effects [34, 36].

The possible mechanisms involved in the effects of CR include: attenuation of cumulative oxidative damage [25, 37] to macromolecules such as proteins, lipids and DNA that have a major role in aging. In addition, CR increases the activities of SOD, catalase and GSH peroxidase at older age [25, 33], delays cancer incidence and strengthens DNA repair [25] which may be a reflection of fewer mutations induced by the oxidation process.

The liver is the central metabolic organ of the body, therefore dietary changes can have a major impact on aging liver and on general health. The total ubiquitin proteolytic pathway (UPP) is operative in all eukaryotic cells as a highly conserved 76-amino-acid protein that covalently binds proteins destined for degradation by 26S proteasomes [38, 39]. The apparent role of the UPP is to degrade normal proteins with short half-lives and abnormal or damaged proteins. Ubiquitin mediates the destruction of several important regulatory proteins, such as the tumor-suppressor protein p53, the cell cycle regulatory protein, cyclin, its kinase inhibitor CDK and p21. These enzymes play a paramount role in the different phases of mitosis [40]. In particular, p21 is interesting in the context of aging because it has been shown, in the form of SDI-1, that p21 encodes abundant messenger RNA in senescent cells that can inhibit cell proliferation [41, 42]. Moreover, certain ubiquitynated proteins appear to function in DNA repair, as well as in ribosome and peroxisome biogenesis [38]. CR prevents the age-related increases in the levels of endogenous ubiquitin and ubiquitin-protein conjugates [43]. But there are no age- or diet-related differences in the ability to degrade oxidized ribonuclease (RNAse).

Dhahbi et al. [44] have shown that male rodents exhibit a decrease in catalase gene transcription in the aging liver. CR has been shown to retard this effect. Two previously available studies [45, 46] have shown an age-dependent decline in catalase activity in male rat liver but an increase in female rat liver. In female rodents, aging can increase the translational efficiency of hepatic catalase mRNA, but this effect is preventable by CR.

Dietary content of nucleotides contributes to the hepatic RNA pool without affecting the DNA pool in rats. However, starvation or CR decrease the DNA pool especially in adult and older rats but not in the young. Both deprivation of dietary nucleotides and especially starvation decrease hepatic RNA pool in young and adult rats but not in the aged rats [47].

In a study by Higami et al. [48] in Fischer 344 rats, aging was associated with increase in the ordinary form of Fas mRNA, but not the variant form of Fas mRNA, along with enhanced Fas immunoreactivity in the hyperplastic bile duct epithelium and hepatocytes. CR significantly decreased the ordinary form of Fas mRNA in advanced age with suppression of Fas immunoreactivity. Fas protein, particularly the ordinary form of Fas, is probably involved in the age-associated apoptosis of hepatocytes. Thus, CR suppresses Fas overexpression, resulting in a reduction of the age-enhanced susceptibility to apoptosis.

Drug Handling by the Aging Liver

During the past decade, people over the age of 65 constituted 12% of the US population. Yet this segment of the population is the recipient of approximately 33% of all prescription drugs (at a cost of USD 3 billion annually) [49] and 40% of all nonprescription drug consumption. Drug therapy in the elderly can be complicated by many factors. Multiple disease processes, environmental influences and genetic variations are combined with the physiologic effects of aging itself. Apart from the underlying disease processes, oftentimes it is physiologic aging that determines the pharmacokinetics of the drugs [50].

Physiologic changes known to play a role in the pharmacokinetics of a drug in the elderly include [50, 51]: decline in total body size, total body water, lean body mass, kidney mass and function, liver mass, liver blood flow and function, serum albumin and increase in body fat stores. Polypharmacy, drug interactions and compliance also play a major role in drug therapy in the elderly. The incidence of adverse drug reactions in old patients is 2–3 times that of younger people, a feature likely related to above factors [49].

Decrease in liver mass with aging is thought to be responsible for the decline in hepatic drug metabolism, especially for low extraction drugs like phenytoin, alcohol and theophylline. The decrease in clearance of high extraction drugs such as hydralazine, nitrates, lidocaine, verapamil, propranolol and morphine correlates with the decline in hepatic blood flow [5].

Liver Drug-Metabolizing Enzymes

Hepatic metabolism of drugs depends on phase I and phase II reactions [51]. Phase I reactions comprise oxidation, reduction, demethylation and hydrolysis, rendering the compounds less lipophilic. Phase II reactions additionally serve to expose or to add functionally reactive sites to which polar groups may be conjugated by reactions of glucoronidation, methylation, sulfation, or acetylation [51].

Phase I reactions have been the most widely studied. They are usually catalyzed by the cytochrome P_{450} system in the SER of hepatocytes [11]. Using inbred male rats [5, 8] it has been shown that there is a negative correlation between chronological age and in vitro hepatic microsomal drug-metabolizing enzymes. The mechanism is attributed to a decrease in SER and total phospholipid content with an increase in cholesterol/phospholipid ratio. An interesting study [52] has shown that a drug-metabolizing enzyme, benzo(a)pyrene hydroxylase, exhibits a higher activity in young male rats than in females of similar age. This enzyme decreases with aging resulting in the loss of sex differences after the age of 25 months. The male-specific form of cytochrome P_{450} in these animals disappears in old age and becomes replaced completely by the female type of P_{450}. Thus, cytochrome P_{450} isozyme changes are sex-related, regulated by androgens and estrogens (testosterone stimulates). This may explain the differences observed during aging.

The majority of the drug interactions in the elderly humans appear to be related to hepatic metabolism by multiple enzyme systems including cytochrome P_{450} [53]. The human liver CYP_{450} protein family consists mainly of three groups of compounds: CYP_1 (CYP_{1A2} 5%), CYP_2 (CYP_{2C} and CYP_{2D6} together 45%) and CYP3 (CYP3A 50–55%). Genetic polymorphisms do exist for these enzymes, and many drug therapies can either inhibit or induce these enzymes [54]. A reduction in vitro of drug metabolism has been shown to occur with aging but this topic in humans has not been properly investigated. However, recently [53] cytochrome P_{450} in human livers has been studied. A large population of subjects between ages 20 and 70 years were investigated with respect to the activity and metabolic capacity of P_{450}. These individuals had biopsy-proven liver conditions that ranged from fatty liver with or without fibrosis to hepatitis or cirrhosis. A control group of 20 patients with normal liver histology were included for comparison. Both sexes were included, and the testosterone levels were also determined. This study showed a progressive decline in drug metabolism with aging. Specifically, P_{450} levels declined in individuals during the fifth decade of life when compared to those in the third decade. Furthermore, P_{450} levels remained unaltered between ages of 49 and 69 years and declined again in individuals aged >70 years. Testosterone levels in males declined progressively with age starting in the fourth decade and were much lower beyond the age of 70

years. Similar trends were observed in women but without reaching statistical significance. In summary, this study [53] on 226 human subjects showed a 30% decline in hepatic drug metabolism after 70 years of age. This finding correlated with similar reduction in liver cytochrome P_{450} content. On the other hand, studies in primates [55, 56] and in humans [54] do not seem to show a complete correlation with the findings of this study [53]. Thus, the entire issue needs further evaluation using, if possible or available, analysis of the liver of normal humans, i.e. individuals without significant pathology in order to determine whether the drug interactions and adverse effects observed in the elderly are related to alterations or decline in hepatic drug metabolism.

Other phase I enzymes are less well studied. Ethanol undergoes oxidation by cytosolic alcohol dehydrogenase (75–80%) and by the microsomal ethanol oxidizing system (20–25%), but age has little effect on its metabolism [57]. However, some reports suggested an increase in hepatic cytosolic alcohol dehydrogenase activity with age in male rats [58]. The significance of this finding in humans is unknown, and extrapolation of the results to humans are probably not justified.

The role of acetaldehyde metabolism in humans has not been investigated during aging, in part because a substantial number of alcoholics do not survive beyond the sixth decade of life. In addition, epidemiologic evidence suggests that ethanol per se or its main metabolite, acetaldehyde, are the cause of hepatic abnormalities leading to end-stage cirrhosis [59, 60]. This stage can only be treated with liver transplantation with variable results.

Studies dealing with phase II enzyme activities are few, conflicting and limited to some selective drugs. Some reports suggest no change in conjugation rates while others report a mild decline in conjugation with age [5]. GSH concentration in human liver is unaffected by aging, but activity of glutathione-S-transferase (GST) has not been studied in man. Cytosolic GST tends to increase in male rats during aging, whereas in females it remains unchanged [61]. Cytosolic epoxide-binding proteins, but not acetylation of isoniazid, are reduced. Furthermore, in a human study [62], the conjugation of paracetamol by the liver was similar in fit young and fit elderly subjects, but paracetamol glucoronide was significantly reduced in elderly frail subjects.

Enzyme Inhibition and Induction

Inhibition of drug-metabolizing enzymes appears to be similar in young and elderly subjects [5]. This has been shown to be especially true for cimetidine and ethanol. Induction of enzymes is less clear. Some authors showed age-related decline in MFOS inducibility, while others show no change in rat liver. Many studies have shown that, in mice, there may be a higher inducibility in the old than in the young [63].

Individual Classes of Drugs

Non-Steroid Anti-Inflammatory Drugs (NSAIDs)

The therapy with NSAIDs in older patients has been reviewed by Girgis and Brooks [64]. These drugs are predominantly cleared by hepatic metabolism to inactive metabolites with the exception of aspirin, which is metabolized to salicylate. Piroxicam and ibuprofen, metabolized predominately by oxidation, have longer plasma half-lives in the elderly. There is evidence that drugs undergoing phase II reactions in the elderly are more slowly eliminated (like naproxen), and the glucoronide of the NSAID can be hydrolyzed back to the parent compound. Aspirin has significant first-pass metabolism and salicylate can accumulate causing significant toxicity. Hepatotoxicity has been considered by the FDA as a characteristic of all NSAIDs, although serious abnormalities of hepatic function are uncommon and unpredictable [65, 66]. Conditions such as acute or chronic hepatitis with cholestasis have been reported in the elderly especially in females with abnormal renal function [67].

Antihypertensive Drugs

Several considerations apply during antihypertensive therapy in the aged. This is more so because some drugs in the elderly depend on renal function while others depend upon the status of liver function. In general, dosage of drugs with predominant hepatic metabolism should be reduced in the elderly and titrated according to clinical response [68]. Such is the case with calcium antagonists [69]. Thiazide diuretics, ACE inhibitors and some β-blockers in the elderly should be adjusted according to renal function rather than age [70, 71]. These features are summarized in table 1.

Anticonvulsant Therapy

The reduced hepatic oxidative metabolism affects carbamazepine, phenytoin and phenobarbital but there is no specific recommendation for dosage changes in the elderly [72]. Valproic acid undergoes conjugation with glucoronide. Diazepam, chlordiazepoxide and flurazepam undergo oxidative metabolism to yield active metabolites. They all have a very long half-life in the elderly. This has

Table 1. Antihypertensive therapy in the elderly

Drugs	Significant first pass metabolism	Predominant hepatic metabolism	Predominant renal clearance	Comments
Diuretics				
Thiazide, furosemide, triamterene	–	–	+	Should be adjusted according to renal function
Ca^{2+} Antagonists				
Diltiazem, verapamil, nifedipine	+	+	–	Dosage should be reduced in the elderly and patients with liver disease
ACE inhibitors				
Lisinopril, enalapril, captopril	–	–	+	Dosage should be reduced with low creatinine clearance and concomitant diuretic therapy
β-Blockers				
Atenolol, acebutolol, nadolol, pindolol	–	+	+	Acebutolol has significant (15–20%) renal excretion, thus needs careful titration
Propranolol	+	+	–	Propranolol dosage needs to be reduced in the elderly

been attributed to increased bioavailability and enhanced accumulation of the lipophilic drugs in an increased volume of distribution, characteristic of the body of the elderly [73]. Thus in the elderly, lorazepam and oxazepam should be preferred because they have shorter half-lives and undergo hepatic conjugation reactions, which are usually unaltered by aging [74].

Psychiatric Medications

Age-related changes in the liver are associated with increase in the elimination half-life of psychotropic drugs, i.e. desipramine and imipramine, by 2–3 times, compared with younger patients [75]. A study in man of the pharmacokinetics of risperidone, an antipsychotic agent, was investigated in healthy young and elderly patients and in cirrhotics or patients with renal insufficiency [76]. The result was similar in the elderly, cirrhotic and young subjects. However, the clearance of the metabolite 9-hydroxy-risperidone decreased by about 30% in the elderly, a fact attributed to age-related decline in renal function. Selective serotonin reuptake inhibitors (SSRIs) mediate drug-drug interactions through inhibition of the P_{450} isoenzymes. This applies to fluvoxamine, fluoxetine, sertraline and paroxetine. Citalopram appears to have little, if any, effect on the P_{450} isoenzymes. Patient care should be assessed on an individual basis when using drug combinations to avoid potentially dangerous effects [77]. Citalopram and sertraline, because of their linear pharmacokinetics [78], are the preferred SSRIs in the elderly.

Gastrointestinal Medications

Cimetidine is known to inhibit various P_{450}-metabolizing enzymes, but there are no dosage recommendations in the elderly. The aging liver causes no significant changes in the pharmacokinetics of the proton-pump inhibitors. The 'ulcer-healing' efficacy of H_2-blockers and proton-pump inhibitors appears to be similar in both the young and old persons [79]. Some hepatic dysfunction occurs due to H_2-blockers and proton-pump inhibitors, but no special dosage recommendations in the elderly have been proposed [79].

Antituberculous Medications

Isoniazid is metabolized in the liver mainly by acetylation (which is genetically determined) and dehydrazination. Rifampin is rapidly eliminated in the bile, and undergoes enterohepatic circulation, during which progressive deacetylation occurs, reducing intestinal reabsorption. Pyrazinamide is hydrolyzed in the liver to its major active metabolite, pyrazinoic acid, that is hydroxylated to the main excretory product, 5-hydroxypyrazinoic acid. It is well documented that the risk of hepatotoxicity increases with age for isoniazid, rifampin and pyrazinamide [80–83]. The rise in aminotransferases is 2–3 times more common in the elderly taking isoniazid and rifampin [80]. The risk of progressive liver disease with isoniazid is up to 2.3% in those over 50 years of age. However, there are no recommendations for alteration in dosage schedule for either prevention or treatment. It is recom-

mended that clinical assessment and baseline liver function tests be performed before the administration of isoniazid, rifampin and pyrazinamide in the elderly, and repeated monthly [80]. Clinical symptoms and signs of hepatitis are an indication for discontinuation of medications.

Telomerases, Replicative Capacity, and Aging Liver

Certain mammalian cells in culture after a number of cell divisions, stop dividing permanently, a process called replicative senescence. It is postulated that replicative senescence in human cells evolved as a mechanism to protect them from continuous cell divisions leading to multiple mutations with deleterious effects.

Telomerases are the biological clocks of replicative lifespan [84, 85]. Telomerase is an RNA-dependent DNA polymerase that adds DNA base pairs (bp) (TTAGGG/CCCTAA repeats) to the telomeric ends [86, 87] of chromosomes. Shortening of telomeric ends has been correlated with aging and decline in the replicative potential of the cell [84, 85]. Telomerase activity is usually undetectable in adult human tissues and is positive in embryonic tissues and in cancers [88]. In humans, telomerase activity is lost or persists at very low level soon after birth but becomes elevated in nonmalignant hepatocyte proliferations such as in the regenerative nodules of cirrhosis and possibly hepatocellular carcinoma, irrespective of the age of the individual [88]. In rodent liver, telomerase activity remains at a constant level throughout life [86, 88]. It increases 48 h after partial hepatectomy and is 5–10 times higher in ascite hepatoma cells when compared to normal rat liver.

Telomere reduction in human liver has been studied recently [89]. Telomere DNA of human somatic cells shortens by 50–150 bp during each cell division thus leading to a finite proliferative capacity. When telomere repeats in the form of terminal restriction fragments shorten to 5–6 kilobase pairs, human fibroblasts stop proliferation and enter into senescence. However, human skin fibroblasts from 100-year-old individuals retain terminal restriction fragments at a length of 6–7 kilobase pairs and replicative capacity. If transfection of the telomerase reverse transcriptase gene is performed in human somatic cells, it results in elongation of telomere length and extension of lifespan [85]. This is also true of human lifespan since aging in normal individuals and in those with inherited disorders of premature aging, abnormal telomere loss is observed [89]. Furthermore, human hepatocytes are known to replace each other slowly under normal conditions, i.e. once a year. One study [89] showed that telomere restriction fragments in normal liver were reduced by about 120 bp per year and remain longer than 5 kilobase pairs in 80-year-old patients. In addition, telomere repeats were shorter in chronic liver diseases than in age-matched normal livers.

Telomere loss is thought to control entry into senescence. Telomerase-expressing clones obtained by transfection of the human telomerase reverse transcriptase subunit were compared in vitro with telomerase-negative epithelial cell clones. Telomerase-expressing clones were found to have a normal karyotype, had already exceeded their normal lifespan by at least 20 population doublings, exhibited elongated telomeres, divided vigorously, and showed reduced β-galactosidase activity, a biomarker of senescence.

Rat liver has an extraordinarily high capacity for regeneration after partial hepatectomy, compared to human liver [88]. High telomerase activity in rat liver is correlated with its high regenerative capacity [88] and high replicative potential as evidenced by complete restoration of hepatic mass even after resection of over two-thirds of the liver [90]. It is assumed that the hepatocytes themselves are capable of replication by compensatory hyperplasia, which is much faster in rodents than humans [88, 90]. It has also been suggested that adult liver contains precursor cells with 'stem cell' properties, which come into play in the regenerative process when mature hepatocytic proliferation is delayed or prevented by liver injury. Hepatocytes have been shown, after hepatectomy, to have a capacity for at least 69 population doublings and perhaps as many as 86 doublings [90].

Clinically, slow regeneration with increasing age is also important considering the fact that a higher mortality in the elderly occurs after partial hepatic resection. The benefit of resection has to be weighed against postoperative risk while considering partial hepatectomy in elderly patients [8].

Old livers appear to regenerate much better when transplanted to a youthful environment and in humans, old livers have been an important source of donor organs accounting for about 17 % of the total liver transplants in the USA. However, some have shown that the rate of graft failure doubles when the donor is 40–65 years of age, with an accelerated increase after 65 [91]. This point has been debated [92, 93]. In one study [92] reporting on 184 consecutive liver transplants, the 1-year graft and patient survival were 65–71% in recipients of older grafts and 69–

76% in recipients of younger grafts. In another study with 30% female donors [93] reporting on 365 donor hepatectomies (ages 8–69), obesity and liver failure were the main complications but age >50 did not influence the outcome. Some investigators have suggested that graft failure is much higher in female liver donors because of hormonal factors, while others attribute this to smaller liver size. Although this point is still controversial, it should be taken into consideration when parent-children donations are attempted.

Human dividing cells by shortening their telomeres during each cell division count the number of divisions they have undergone in order to stop dividing [94] after 50–90 cell population doublings. This process is mediated by the telomerases. The shortening of the telomeric ends with aging leads to a loss of the ability of the cell to mask the chromosomal ends that normally are recognized as broken DNA. This broken DNA induces activation of the checkpoints where p53-dependent damage can lead to growth arrest. Replicative senescence in human cells [95] evolved as a mechanism to protect them from continuous divisions leading to multiple mutations. Longer-lived species had to develop replicative senescence to ensure that they would have the increased protection that their longevity necessitated and thus be able to resist the deleterious effects caused biologically by the aging process.

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