

# Hepatic drug metabolism and ageing

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The hepatic clearance of many drugs is reduced in the elderly. **Contrary to previously held views we suggest the following:**

1. Specific activities of several cytochrome P-450 Phase I enzymes are not reduced with age *per se* in primate and man, nor is enzyme affinity for substrate changed.

2. **An important contribution to the reduced hepatic elimination is made by reduction in liver size and blood flow with advancing age.**

3. Major changes in liver drug metabolism may occur in frail old people. Future studies of hepatic drug metabolism and ageing must closely define the subject groups under study.

The increased risk of adverse drug reactions in the elderly population has been extensively investigated.<sup>1-5</sup> Whilst it appears that at least part of this apparent excess may be due to increased drug usage<sup>6</sup> epidemiological studies suggest that **geriatric patients are up to three times more likely to suffer from a drug side effect;** and Type A, dose dependent reactions appear to be especially frequent.

Part of the explanation for this phenomenon may be increased pharmacodynamic sensitivity to drugs, but it has also been shown that **the systemic elimination of many agents is decreased in the elderly,** leading to higher blood concentrations and hence a higher possibility of Type A adverse effects.<sup>1,3</sup> Since Kato and colleagues<sup>7</sup> had shown in 1964 that the specific activities of the most important hepatic drug metabolising enzymes, the monooxygenases, **declined with age in male rats,** it has seemed that **a simple explanation for impaired drug metabolism in ageing humans exists.**

Recent work from San Francisco<sup>8-10</sup> and Newcastle Upon Tyne<sup>11-13</sup> has, however, cast doubt on this assumption, and this review will discuss the effect of ageing upon the hepatic elimination of drugs and other foreign compounds.

## BASIC PRINCIPLES OF HEPATIC DRUG ELIMINATION

The Hepatic Clearance ( $CL_H$ ) of a drug can simply be described by the formula:

$$CL_H = Q \cdot \frac{C_a - C_v}{C_a} = Q \cdot E$$

where  $Q$  is hepatic blood flow,  $C_a$  and  $C_v$  are the arterial and venous concentrations of the compound respectively, and  $E$  is the steady state organ extraction ratio. From this equation it can be seen when  $E$  approaches unity,  $CL_H$  will approach and be limited by hepatic blood flow. Such drugs are termed flow limited.<sup>14</sup> By contrast, if  $E$  is small,  $CL_H$  will vary according to hepatic extraction and will be relatively independent of flow. Such agents are termed capacity limited.<sup>14</sup> Hepatic extraction is a variable dependent upon liver size, uptake into hepatocyte, and affinity and activity of drug metabolising enzymes. When considering hepatic drug elimination in age it is therefore important to address the following variables:

1. Activities of drug metabolising enzymes and their induction and inhibition
2. Liver size
3. Liver blood flow and hepatic uptake

## METABOLIC PATHWAYS OF DRUG METABOLISM

The metabolic pathways involved in the biotransformation of foreign molecules may be divided into two phases.<sup>15</sup> Phase 1 reactions comprise oxidative, reductive or hydrolytic processes. In general they render the compound less lipophilic, but additionally serve to expose, or to add, functionally reactive sites to which polar groups may be conjugated. Such conjugations, which include glucuronidation, methylation, sulphation, acylation and mercaptan formation are called Phase 2 reactions. Compounds which already possess suitable reactive sites may undergo Phase 2 conjugation without prior Phase 1 conversion. Thus paracetamol, which has an

accessible hydroxyl group, is glucuronidated and sulphated; and the sulphonamides, which have amine groups, are acetylated.<sup>16</sup>

Much attention has been focused on Phase 1 oxidations and ageing, especially those mediated by the hepatic microsomal monooxygenase enzyme system (MMO). Other Phase 1 reactions, and Phase 2 reactions, have been relatively neglected.

## PHASE 1 REACTIONS AND AGEING

### **In vivo studies of hepatic drug clearance**

The most widely used marker of hepatic drug oxidation has been antipyrine.<sup>17,18</sup> This drug is rapidly and almost completely absorbed from the gut and has no significant pre-systemic metabolism. It is distributed to body water, and has protein binding of under 10%. 95% is metabolised by hepatic microsomal monooxygenase enzymes, the three major products being norphenazone, 3-hydroxymethylantipyrine and 4-hydroxyantipyrine. In one of the first studies of pharmacokinetics in aged man O'Malley and colleagues<sup>19</sup> showed in 1971 that the half life of antipyrine was lengthened by 45% in the elderly and that this was principally due to a decreased total plasma clearance. Several other studies have since produced comparable results<sup>20-22</sup> and have also shown that the changes may be less marked in women. There is no doubt, that factors other than age are important in determining hepatic drug elimination; and at least some of the apparent age related decline can be accounted for by factors other than chronology. For example Vestal and colleagues<sup>25</sup> have shown, in a study of 307 healthy males, that whilst there is a significant negative correlation between antipyrine clearance and ageing, only 3% of the total variance depended on age itself, 12% being related to different smoking habits in young and old individuals.

Many other careful pharmacokinetic studies of drugs eliminated by hepatic Phase I metabolism have been carried out (see Greenblatt et al.<sup>25a</sup> for review). These have shown highly variable decreases in the systemic clearance of drugs with advancing age between 0-50%. Lauterberg and Schnegg<sup>25b</sup> compared the clearance of aminopyrine and caffeine—two established markers of hepatic microsomal function with galactose elimination, said to reflect functioning liver cell mass, in healthy subjects over age 70. They found a parallel decline in clearance of aminopyrine and caffeine on the one hand and galactose on the other; they speculated that this might be due to reduced liver cell mass.

There has been a suggestion that some isoenzymic forms of the microsomal monooxygenases may be selectively impaired with ageing, and Posner et al.<sup>26</sup> have described a specific reduction in N-demethylation capacity with age. However, using theophylline as substrate no similar reduction in N-demethylation has been shown by others<sup>27,28</sup> and it is as yet premature to suggest that specific cytochrome P450 forms are preferentially lost with advancing age in humans.

### *Other Phase 1 enzyme systems*

Less attention has been paid to the effect of age on other Phase 1 enzyme systems, although they are of considerable importance.

Ethanol for example undergoes oxidation by both cytosolic alcohol dehydrogenase (75–80%) and by the microsomal ethanol oxidising system MEOS (20–25%). Although age may influence the volume of distribution of ethanol resulting in rather higher blood levels after acute dosing, the metabolism of the drug is little affected by ageing.<sup>29,30</sup>

Esterases are another important group of drug metabolising enzymes,<sup>31</sup> which until recently had been neglected by gerontologists. We have however recently investigated the effect of age upon plasma aspirin esterase, an enzyme which is responsible for the hydrolysis of aspirin to acetate and salicylate, and whose activity correlates well with the pharmacodynamics of aspirin. In addition, activity of the plasma enzyme correlates well with that in liver.<sup>31</sup> Ageing had no effect on the activity of the enzyme in healthy elderly individuals. However, physical frailty, as defined by Woodhouse et al.<sup>32</sup> was associated with a significant decline in plasma esterase activities.<sup>33</sup> Furthermore it appears that, as with MMO enzymes, the affinity of esterases is unaffected by ageing, or indeed by frailty.<sup>34</sup>

## **In vitro studies of liver enzyme activities**

### *Microsomal monooxygenases (MMO)*

**Rodents.** An enormous body of work exists concerning age related declines in activities of MMO enzymes in animal strains, and these data have been fully reviewed by Schmucker.<sup>35</sup> As early as 1964 Kato and co-workers<sup>7</sup> demonstrated a negative correlation between chronological age and in vitro hepatic MMO activities, using inbred male rats. Similar findings have been reported from

other laboratories.<sup>11,36</sup> Several mechanisms behind this decline have been postulated, and include:

a) A reduction in smooth endoplasmic reticulum in aged rat liver.<sup>37</sup>

b) Alterations in the lipid profile of membranes. This comprises a decline in total phospholipid content and an increase in the cholesterol/phospholipid ratio.<sup>38-41</sup> The relative proportions of specific phospholipid classes appear to change little during ageing,<sup>38,39,42</sup> although the fatty acid composition of the microsomal membrane may alter.<sup>43</sup> The significance of these possible changes in membrane composition remains unclear and reports of ageing effects on membrane fluidity are contradictory.<sup>43,44</sup>

c) Alterations in cytochrome P450 isozymes. There is now good evidence that most of the changes in MMO activities which are seen in elderly animals are sex related. Age associated declines in MMO activities in female rats are much less marked,<sup>68</sup> and it appears that MMO activities are increased by testosterone in young males. Ageing results in an impaired hypothalamic pituitary testicular axis, manifesting in reduced MMO activities and loss of male specific cytochrome P450 forms.<sup>45</sup>

*Man and primates.* Although well described in male rodents, and widely extrapolated to man, there is no good evidence of an age related decline in MMO activities in humans and non human primates. Our own studies, using aldrin, 7-ethoxycoumarin, and 7-ethoxyresorufin as probe substrates have shown no age related alterations in MMO specific activities in histologically normal human liver.<sup>12,13</sup> Similarly others have found that both microsomal protein and cytochrome P450 content is unaffected by age or sex in rhesus monkeys,<sup>9</sup> and similarly studies of P450 content, NADPH-cytochrome C reductase and benzo(a)pyrene metabolism in microsomes from livers of female pigtailed macaque monkeys (*Macaca nemestrina*) have shown no change from age 2 to 21 years.

For drugs metabolised by 1st order kinetics, specific activities ( $V_{max}$ ) of the MMO enzymes are only one determinant of reaction rate. Equally important is the affinity of enzyme for substrate, of which apparent  $K_m$  is good index. Using 7-ethoxyresorufin and aldrin as probe substrates, we have demonstrated similar  $K_m$  values and hence enzyme affinities in microsomes from young and old rat livers,<sup>11</sup> and have confirmed the lack of ageing effect in human livers of varying ages using 7-ethoxycoumarin as a

substrate.<sup>12</sup> Similarly, age has no effect on the affinity of rat liver microsomal NADPH cytochrome C reductase for substrates.<sup>46</sup>

Overall therefore it is safe to say that age related declines in MMO specific activities do occur in rodent liver, but that these changes are sex and species specific. Similar reductions in ageing human or primate enzyme activities have not been shown. Furthermore age has no apparent effect on the affinities of MMO enzyme systems for substrates in rat or human liver.

## PHASE 2 REACTIONS AND AGEING

In contrast to studies of oxidative drug metabolism in the elderly, conjugation reactions have been relatively neglected. It is widely stated in the literature that conjugation rates are unaffected by ageing, and indeed some work is available to support this,<sup>47-50</sup> however other authors have reported a small decline in drug conjugation with age,<sup>51-53</sup> and clearly further work is required to clarify this issue.

The effect of ageing upon Phase 2 conjugation reactions *in vitro* has also been less extensively studied, and such data as are available are confusing, because of marked species and sex differences in drug conjugation.<sup>54</sup> Several authors have examined the effect of age upon UDP-glucuronyl transferase activities (responsible for glucuronidation) and have reported no change in Fisher 344 rats or minimal changes only (10-20%) in Wistar rats.<sup>55-58</sup> Similarly sulphation of paracetamol in isolated hepatocytes was reported to be slightly decreased in old rats but not in old mice,<sup>59</sup> when compared to young adults. No data are available on the effects of age on these enzymes in human liver.

Glutathione conjugation is an important detoxification mechanism. The concentrations of glutathione in human liver are unaffected by ageing<sup>13</sup> but the activities of the glutathione transferases have not been studied in man. In animal liver however, the activities of these enzymes show either no change, or minor changes only with ageing, depending upon the substrates chosen.<sup>60-63</sup>

## INDUCTION AND INHIBITION OF DRUG METABOLISING ENZYMES

The inhibition of drug metabolising enzymes appears to be similar in young and elderly subjects. Two separate studies have investi-

gated the effect on cimetidine of drug oxidation, and found that the magnitude of inhibition was not related to the age of the patient.<sup>64,65</sup> Similarly studies from this laboratory have shown that acute doses of ethanol result in a similar percentage reduction in acetanilide clearance in young and healthy aged volunteers.<sup>30</sup>

The relationship between ageing and induction of drug metabolism is less clear. Whilst some authors have shown some age related declines in MMO inducibility in some animal strains<sup>66,67</sup> others have reported no age related changes.<sup>68,69</sup>

It has also been suggested that induction of drug metabolism by environmental factors and other drugs is decreased in elderly *humans*, although it has to be said that the evidence for this is inconclusive.<sup>25,70</sup>

## AGEING AND LIVER SIZE

One of the earliest and largest studies of liver size in relation to age was undertaken in 1933.<sup>71</sup> 1582 subjects aged between 20 and 80, and dying of acute injury or accident were studied at autopsy. Liver size fell by 18% in women and 24% in men between these ages, with the most marked changes occurring after the sixth decade. In another autopsy study<sup>72</sup> a 25% fall in liver weight was observed between the ages of 20 and 70 years, with a more marked reduction in the very elderly—a fall of 46% between the third and tenth decade. However autopsy studies are difficult to interpret, in that the dead are not representative of the general population, even if death was accidental. For example in the elderly, injury is often a manifestation of physical illness.

Several non invasive methods of measuring liver volume have therefore been developed, including computerised tomography,<sup>73,74</sup> radionucleotide scanning<sup>75–77</sup> and ultrasonography.<sup>78–80</sup> Of these, sonography is the most acceptable as it involves no exposure to ionizing radiation. Using ultrasound, several studies relating age to liver size have been performed. In 1978, Swift and colleagues<sup>81</sup> examined liver volume using ultrasound in 15 volunteers in the third decade and 11 healthy older subjects aged 75–86 years. Mean liver volume was 990 ml in the aged individuals compared to 1303 ml in the young volunteers—a 24% decline. In our own studies,<sup>80</sup> comparing 65 healthy community based volunteers aged 24–91, we have shown a significant negative correlation between liver volume and age. Linear regression analysis of our results suggests average liver volumes of

1474 ml at age 24 compared to 934 ml at age 91—a fall of 37%. Furthermore the changes were slightly more marked in females than males, the percentage decline in liver volume being 44% in women and 28% in men.

The significance of a reduction in liver volume of between 24% and 44% with ageing is considerable, as it is of similar magnitude to the age related reduction in antipyrine clearance described earlier and to the reported 30–40% decline in the clearance of imipramine<sup>82</sup> and theophylline.<sup>83</sup> We feel therefore that changes in liver size alone are sufficient to explain age related decrements in the clearance of low extraction drugs, without invoking changes in drug metabolising enzymes.

Marchesini and colleagues<sup>84</sup> have also examined liver volume with ageing in man using an ultrasound technique. Estimated liver volume fell by about 33% between ages (approx.) 30 and 85 years. They compared this with galactose elimination in 50 normal subjects and found a close correlation between galactose elimination and estimated liver volume. Using partial correlation analysis they suggested that while the decline in liver volume with age would explain most of the decline in galactose elimination there was possibly also an age related, liver volume independent, decline in this marker of 'intrinsic metabolic capacity'.

## AGEING AND LIVER BLOOD FLOW

For many years, hepatic blood flow has been said to fall with age. However, until recently, such assertions were based on a study which was not specifically designed to investigate this parameter,<sup>85</sup> and which estimated liver blood flow using a bromsulphthalein (BSP) clearance. Unfortunately, BSP clearance is determined by relative storage capacity ( $S$ ) and transport rate maximum ( $T_M$ ), and studies have shown that both may be modified independently by ageing.<sup>86,87</sup> This agent is therefore not suitable as a model for studies of ageing and liver blood flow.

Indocyanine green (ICG) a dye with flow dependent clearance and which has been widely used in studies of blood flow, is probably a better model drug to use, as its hepatic extraction is unchanged between young adulthood and senescence, at least in rodents.<sup>88</sup>

The clearance of ICG, and hence apparent liver blood flow, does fall with ageing in humans.<sup>89</sup> Our own studies of ICG clearance in 65 healthy volunteers aged between 24 and 91 years



(and assuming a constant hepatic extraction ratio of 0.74) showed a decline in apparent liver blood flow of 35% in those over 65 years compared to those under 40 years, even allowing for changes in body weight. In addition, liver perfusion (liver blood flow per unit liver volume) also fell over this age range by a factor of 11%.<sup>80</sup>

As with liver size, these age related changes in liver blood flow are probably of utmost importance. Reductions in systemic clearance of several high extraction drugs (flow limited) have been reported in the elderly, including propranolol (45% fall),<sup>90</sup> chlo-methiazole (30% fall),<sup>91</sup> and morphine (35% fall)<sup>92</sup>—all of which are compatible with the decline in apparent liver blood flow. In addition presystemic elimination of such drugs has been reported to be reduced with age.<sup>93,94</sup> This may, however, be less marked in fit elderly than in hospitalized subjects.<sup>95</sup> Factors other than liver size and blood flow are important in determining presystemic elimination including uptake into hepatocytes, functional 'shunting' and saturation of enzymes—all of which could theoretically be affected by ageing, and the relative contributions of these factors to the change in presystemic elimination must be speculative.

## CONCLUSIONS

In summary we can draw the following conclusions regarding hepatic drug metabolism and ageing:

1. The total capacity of the liver to metabolise many drugs declines with age in man
2. This decline is very variable, from drug to drug and from person to person
3. Much of the variance may be accounted for by the influence of disease states and 'frailty', environmental factors, co-administration of other drugs, and possibly by genetic factors
4. Liver size falls by about 35% between early adulthood and late old age in humans
5. Liver blood flow also falls in elderly humans, probably to a slightly greater extent than liver size. This reduction in liver size and blood flow with age may well offer a further major explanation for the decrease in hepatic clearance of many drugs in old age
6. Whilst monooxygenase enzyme activities decline in aged rats, this is not the case in humans or non-human primates, although indirect evidence suggests that a few, specific cytochrome P-450 forms may selectively decline

7. Age has no effect on the affinity of monooxygenase enzymes for substrate in rat or human at least for the substrates so far studied, or on plasma aspirin esterase affinities in humans
8. Conjugating enzyme activities are little affected by age in animals, and the same probably applies to humans but data are scarce.

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