Vitamin K status in human tissues: tissue-specific accumulation of phylloquinone and menaquinone-4

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We measured the vitamin K status in postmortem human tissues (brain, heart, kidney, liver, lung, pancreas) to see if there is a tissue-specific distribution pattern. Phylloquinone (K_1) was recovered in all tissues with relatively high levels in liver, heart and pancreas (medians, 10·6 (4·8), 9·3 (4·2), 28·4 (12·8) pmol(ng)/g wet weight tissue); low levels (< 2 pmol/g) were found in brain, kidney and lung. Menaquinone-4 (MK-4) was recovered from most of the tissues; its levels exceeded the K_1 levels in brain and kidney (median, 2·8 ng/g) and equalled K_1 in pancreas. Liver, heart and lung were low in MK-4. The higher menaquinones, MK-6–11, were recovered in the liver samples (*n* 6), traces of MK-6–9 were found in some of the heart and pancreas samples. The results show that in man there are tissue-specific, vitamin-K distribution patterns comparable to those in the rat. Furthermore, the accumulation of vitamin K in heart, brain and pancreas suggests a hitherto unrecognized physiological function of this vitamin.

Vitamin K: Phylloquinone: Menaquinone

We reported previously on the distribution of vitamin K in the rat (Thijssen & Drittij-Reijnders, 1994). A tissue-selective distribution of phylloquinone (K_1) was observed, with high levels in liver and heart tissue, and low levels in, for instance, brain. Surprisingly, all tissues examined contained the K-vitamin menaquinone-4 (MK-4). MK-4 differs from K_1 in that the phytyl side-chain of the latter is replaced by an *all-trans* poly (*n* 4) isoprenoid chain. Exocrine organs like pancreas and salivary gland, particularly, were found to contain high levels, but brain, kidney and cartilagenous tissue also contained MK-4 levels which exceeded K_1 stores. Furthermore, the study showed dietary K_1 to be a source of tissue MK-4. The vitamin K distribution in rat tissues only partly coincides with the presence of known aspects of vitamin K metabolism. For example, enzymes of the vitamin K cycle, vitamin-K-dependent carboxylase and vitamin K epoxide reductase, have not been detected in heart and brain tissue (Friedman & Smith, 1977; Thijssen & Baars, 1991). The typical distribution pattern may suggest an, as yet, unknown physiological function of vitamin K. So far it is known that K-vitamin forms function as essential cofactors in the posttranslational carboxylase reaction converting glutamate residues of distinct substrate proteins into γ-carboxyglutamates (Gla; Vermeer, 1990). Gla-containing proteins are known to originate from liver (the coagulation proteins II, VII, IX, X, and the anticoagulation factors protein C and protein S), from bone (bone Gla protein (BGP), matrix Gla protein (MGP), and protein S), and from endothelium (protein S) (Fair *et al.* 1986). It is probable that renal tissue also forms Gla proteins which are secreted in urine (Gallop *et al.* 1980).

Here we report on the vitamin-K status in postmortem human tissue samples. The observational findings show vitamin K distributions comparable to those observed in the

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rat, strengthening the suggestion of a, hitherto unrecognized, additional function(s) of this vitamin.

MATERIALS AND METHODS

Human tissue samples were from autopsies. The samples were obtained through the Department of Pathology of the University Hospital. The samples were from autopsies performed within 6 h of death. Autopsy samples were stored at −70° until analysed. Table 1 summarizes the histories of the autopsy samples. Tissues were homogenized in phosphate-buffered saline, one part in three volumes, using an Ultra Turrax blender at 20000 rev./min. A 0·5 ml portion of each homogenate was made up to 1 ml with water. The mixture was thoroughly mixed with 2 ml ethanol containing 400 pg 2′,3′-dihydrophylloquinone (a gift from Hoffmann-La Roche, Basel, Switzerland) as internal standard. The final mixture was extracted with 3·5 ml n-hexane. The hexane phase was evaporated to dryness under a gentle stream of O_2-free N_2 at 30°. The residue was taken up in 2 ml n-hexane and absorbed onto a silica gel column (500 mg silica gel 60, 40–63 μ, Merck, Darmstadt, Germany). The column was washed with 5 ml ethylacetate in n-hexane (2 ml/l) whereafter the vitamin-K-containing fraction was eluted with 3 ml ethylacetate in n-hexane (20 ml/l). The recovered fraction was evaporated to dryness and the residue taken up in 0·025 ml propan-2-ol. The K-vitamins were assayed by fluorescence detection (excitation, 244 nm; emission, 420 nm) following HPLC separation and post-column reduction as described previously (Thijssen & Drittij-Reijnders, 1994). The recovery and precision of the assay were estimated from spiked homogenates of vitamin K-deficient rat liver. The percentage recoveries for MK-4 and K_1 were 85 (SD 21) and 103 (SD 10), 88 (SD 5) and 90 (SD 16), 97 (SD 10) and 106 (SD 3) for wet weight liver tissue contents of 0·6, 2, and 4 ng/g respectively (n 4). For MK-6–9 the percentage recoveries were 88 (SD 13), 83 (SD 6), 83 (SD 9), and 81 (SD 10) for 2 ng/g tissue contents, 85 (SD 5), 81 (SD 5), 80 (SD 11), and 86 (SD 4) for 4 ng/g tissue contents (n 4).

K_1 and MK-4 were purchased from Sigma Chemicals (St Louis, MO, USA). MK-6–9 and 2′,3′-dihydrophylloquinone were a gift from Hoffman La Roche (Basel, Switzerland). To analyse the contents of MK-10 and -11 the following approach was employed: (1) the retention times of MK-10 and MK-11 were predicted from the linear relationship between n, the number of isoprenoids in the side chain, and the logarithm of the capacity factor k′ ($= (t_R - t_O)/t_O$, where t_O and t_R are the retention times of the void volume and the component respectively). The relationship was estimated for MK-4, and MK-6–9; r 0·999, $P < 0{\cdot}0001$; (2) the fluorimetric responses per mole of the K vitamins, i.e. K_1, MK-4, MK-6–9, following the post-column reduction were found to be equal. The same was assumed to hold for MK-10 and MK-11.

RESULTS

Typical examples of chromatograms are given in Fig. 1. In the absence of postcolumn reducing conditions the detector signal at the positions of the vitamin-K peaks returned to baseline level except for MK-6 where some of the liver samples showed an endogenous peak.

The observed tissue K_1 and MK-4 levels are summarized in Fig. 2. K_1 was recovered in all tissue samples examined; MK-4 was also present in most of the samples. As can be seen, the levels of the vitamins varied per tissue: heart, liver, and pancreas had relatively high K_1 levels (medians: 9·3 (4·2), 10·6 (4·8), 28·4 (12·8) pmol(ng)/g tissue), lung kidney and brain were low in K_1 (medians: 1·5 (0·7), 0·9 (0·4), 1·5 (0·7) pmol(ng)/g). Relatively high MK-4 levels were found in pancreas, kidney and brain (medians: 21·6 (9·6), 6·3 (2·8), 6·3 (2·8) pmol(ng)/g). MK-4 levels in liver were lower, except for one sample where MK-4

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Table 1. *Autopsy samples: donor histories*

No.	Sex	Age (years)	Tissues	Medical history
1	M	36	B, H, K, Li, Lu, P	Amyotrophic lateral sclerosis
2	M	84	H, K, Li, Lu, P	Atherosclerosis, bronchopneumonia
3	M	54	B, H, K, Li, Lu, P	Lung emboly, myocarditis
4	F	46	Li	Sepsis
5	F	83	H, K, Li, P	Sepsis, bronchopneumonia
6	F	50	B, H, K, Li, P	Myocardial infarction, bronchopneumonia

B, brain; H, heart; K, kidney; Li, liver; Lu, lung; P, pancreas.

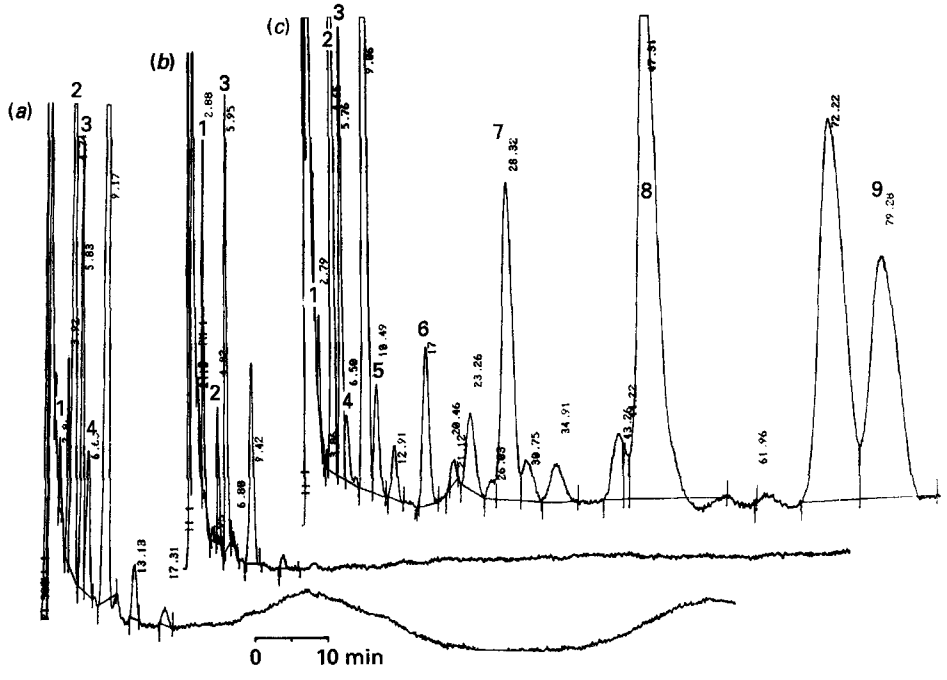

Fig. 1. Chromatograms of vitamin-K forms obtained during the analysis of human tissue samples by HPLC. (a), Heart; (b), brain; (c), liver. Bold numbers on the chromatograms refer to: 1, menaquinone-4 (MK-4); 2, phylloquinone; 3, internal standard; 4, MK-6; 5, MK-7; 6, MK-8; 7, MK-9; 8, MK-10; 9, MK-11.

amounted to 21·0 pmol/g wet weight tissue. Heart-tissue samples were low in MK-4, and only one of the three lung samples contained detectable MK-4. Except for liver (r 0·972, $P < 0·01$, n 6), there were no significant correlations between tissue K_1 and MK-4 contents. However, if the liver sample with the high K_1 and MK-4 contents (50·8 and 21·0 pmol/g respectively) was excluded, the correlation for liver tissue was not significant either. The results show that brain contained more MK-4 than K_1, MK-4: K_1 ratios were 2·4, 3·4 and 13·3. For kidney, three out of five samples contained higher MK-4 levels, ratios > 10. Pancreas appeared to contain about equal amounts of the two forms of vitamin. For the other tissues there was less MK-4 than K_1. The ratios are given in Table 2.

In agreement with reports from others (Shearer *et al.* 1988; Usui *et al.* 1989), liver contained considerable amounts of higher menaquinones, MK-8–11 (Table 3). Great

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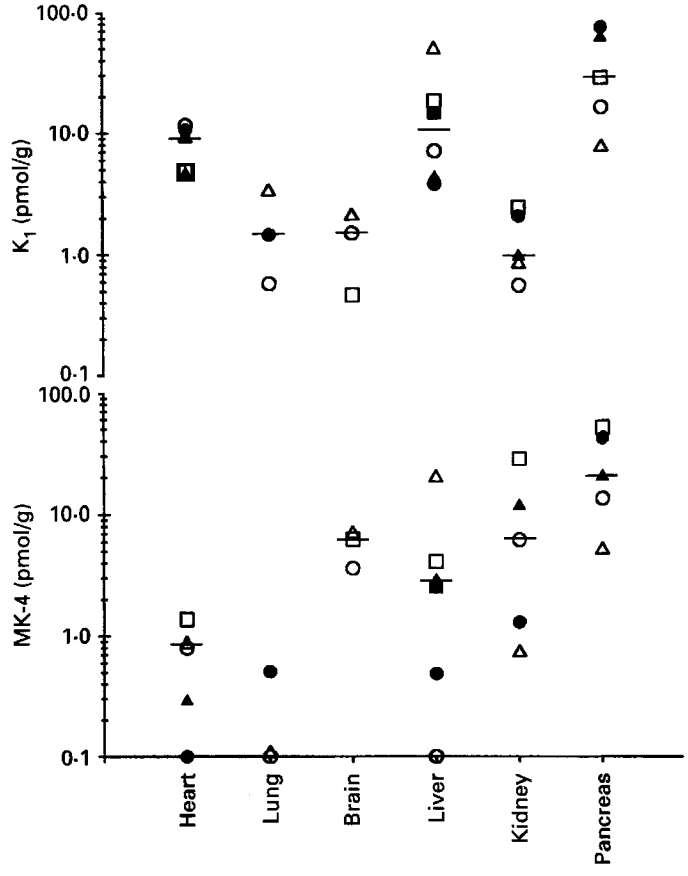

Fig. 2. The distribution of phylloquinone (K_1) and menaquinone-4 (MK-4) in human tissues obtained at autopsy. Postmortem samples from the same donor are represented by the same symbol. Horizontal lines depict the median value. Values below the level of quantitation are arbitrarily given the value of 0·1 pmol/g.

Table 2. *Menaquinone-4 (MK-4): phylloquinone (K_1) ratios in human tissues obtained at autopsy*

Tissue	*n*	MK-4:K_1 Mean	SD
Heart	4	0·1	0·1
Liver	5	0·3	0·2
Kidney	5	7·3	6·0
Brain	3	6·4	6·0
Pancreas	5	0·9	0·6

differences between the liver samples were apparent, some samples (livers 2 and 6) contained high amounts (more than 50% of the total K vitamin store) of MK-10 and MK-11, while others were intermediate or low in these species. The total vitamin-K store was found to range between 40 and 125 pmol/g wet weight tissue, with K_1 comprising

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Table 3. *Levels of phylloquinone (K_1) and menaquinones 4–11 (MK-4–11) (pmol/g wet tissue) observed in human liver samples obtained at autopsy*

Vitamin	Liver sample					
	1	2	3	4	5	6
K_1	7·1	3·8	50·8	14·6	4·4	18·5
MK-4	< 0·25	0·5	21·0	2·8	2·9	4·2
MK-6*	—	5·4	4·5	4·9	2·2	1·2
MK-7	1·1	8·4	1·1	3·4	2·9	2·4
MK-8	2·3	6·2	8·7	11·2	5·9	4·9
MK-9	2·6	6·3	9·2	1·8	10·1	15·4
MK-10	22·6	39·0	7·8	3·2	15·5	46·6
MK-11	8·3	34·5	2·0	3·2	12·6	30·1
Total	44·0	104·1	105·4	45·1	86·1	123·2

* The presence of an endogenous peak makes the quantitation of MK-6 in some of the samples less reliable (see p. 122).

4–45% of it. Traces of MK-7–9 (< 2 ng/g) were also observed in some of the heart and pancreas samples but not in any of the other tissues.

DISCUSSION

This observational study on vitamin K contents in human tissues demonstrates tissue-selective distributions of K_1 and MK-4 which, generally, are comparable to the distribution patterns observed previously in the rat, i.e. relatively high K_1 levels in heart and liver, low levels in other tissues. MK-4 levels were found to be 'high' in brain, kidney and pancreas. Human pancreas appears to be rich in K_1 also. Dietary K_1 is transported through the body via chylomicrons and their remnants (Saupe *et al.* 1993). Little is known about the uptake by tissues. If the tissue K_1 distribution was a partitioning process merely based on lipophilicity, high levels in brain but not in heart or pancreas would be expected. Thus, the K_1 accumulations in, for example, heart and pancreas suggest some specific tissue-related phenomena. Alternatively, the observed tissue vitamin-K contents might be the result of the continuous accumulation of small amounts during the lifetime. Compared with young rats, elevated K_1 and MK-4 levels in old (> 24 months) rats have been observed (M. J. Drittij-Reijnders, unpublished results). For the small number of samples in the present study no relationship was apparent with the age of the donors.

One can only speculate about the source of MK-4 in human tissues. MK-4 is only a minor form of bacterial menaquinones (Kindberg *et al.* 1987; Conly & Stein, 1992), and is not abundantly present in normal food products, e.g. cows' milk (Shirahata *et al.* 1991) and meat (Hirauchi *et al.* 1989). We showed previously that K_1 can be a source of MK-4 in rats (Thijssen & Drittij-Reijnders, 1994), either through the intervention of intestinal bacteria that may remove the phytyl side chain to release menadione (Billeter *et al.* 1964) or by tissue specific conversion of K_1 to MK-4. The latter would indicate an essential cellular function for MK-4 which cannot, or can only poorly, be replaced by K_1. Alternatively, the human body is exposed to menadione which in some tissues is converted to MK-4. This also suggests a cellular need for MK-4. It is not known if human dietary products contain free menadione. More likely, menadione will be a product of the intestinal flora, formed from dietary K_1 (Billeter *et al.* 1964) or by synthesis.

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The estimated K_1 levels in the liver are comparable with data reported previously (Shearer *et al.* 1988; Usui *et al.* 1989; Thijssen & Drittij-Reijnders, 1993). The presence of bacterially-derived menaquinones in liver, particularly MK-10 and MK-11, has been reported by others (Shearer *et al.* 1988; Usui *et al.* 1989). The higher menaquinones may even comprise the major part of the liver store. It is not clear whether they contribute to the biochemical function of vitamin K-dependent γ-glutamyl carboxylation. The fact that these higher menaquinones are mainly compartmentalized in the mitochondrial fraction (Usui *et al.* 1989) may point to the contrary. In rat liver, also, the higher menaquinones were found to be mainly present in the mitochondrial fraction (Thijssen & Drittij-Reijnders, 1994). The liver seems to be the main organ containing the higher menaquinones; none, or only traces, of these K-vitamins were recovered in other tissues. This is also the case in the rat (Thijssen & Drittij-Reijnders, 1994). Hodges *et al.* (1993) reported the presence of MK-6–8 in human bone in amounts equal to or lower than the amount of K_1.

In summary, the present study shows that some tissues accumulate K_1 and/or MK-4. In all probability MK-4 is synthesized in the tissues. Whether all these tissues produce specific, as yet unknown, Gla-containing proteins or whether there is a common Gla protein, e.g. matrix Gla protein (Fraser & Price, 1988) or the recently discovered vitamin-K-dependent cell-growth regulating protein (Manfioletti *et al.* 1993), remains to be investigated. As, for example, heart and brain are devoid of, or have low levels of vitamin-K-dependent carboxylase and vitamin-K (epoxide) reductase activity, the data suggest that additional physiological functions for vitamin K are still to be found.

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