

Radiofrequency Fields, Transthyretin, and Alzheimer's Disease

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Abstract. Radiofrequency field (RF) exposure provided cognitive benefits in an animal study. In Alzheimer's disease (AD) mice, exposure reduced brain amyloid- β ($A\beta$) deposition through decreased aggregation of $A\beta$ and increase in soluble $A\beta$ levels. Based on our studies on humans on RF from wireless phones, we propose that transthyretin (TTR) might explain the findings. In a cross-sectional study on 313 subjects, we used serum TTR as a marker of cerebrospinal fluid TTR. We found a statistically significantly positive β coefficient for TTR for time since first use of mobile phones and desktop cordless phones combined ($P = 0.03$). The electromagnetic field parameters were similar for the phone types. In a provocation study on 41 persons exposed for 30 min to an 890-MHz GSM signal with specific absorption rate of 1.0 Watt/kg to the temporal area of the brain, we found statistically significantly increased serum TTR 60 min after exposure. In our cross-sectional study, use of oral snuff also yielded statistically significantly increased serum TTR concentrations and nicotine has been associated with decreased risk for AD and to upregulate the TTR gene in choroid plexus but not in the liver, another source of serum TTR. TTR sequesters $A\beta$, thereby preventing the formation of $A\beta$ plaques in the brain. Studies have shown that patients with AD have lowered TTR concentrations in the cerebrospinal fluid and have attributed the onset of AD to insufficient sequestering of $A\beta$ by TTR. We propose that TTR might be involved in the findings of RF exposure benefit in AD mice.

Keywords: Blood-cerebrospinal barrier, cordless phone, DECT, dementia, mobile phone, nicotine, plexus choroideus

INTRODUCTION

Alzheimer's disease (AD) is one of the most common diseases in older persons with a prevalence that nearly doubles in every 5-year age cohort [1]. About one in six men and one in three women will suffer from AD in their lifetime [2]. The global prevalence of dementia in persons ≥ 65 years of age has been estimated to be 7.1%, 95% confidence interval (CI) = 6.8–7.4 [3]. It is a leading cause of death, and AD mortality increased 31% from 1999 to 2004 in the US [4]. The etiology of AD is poorly understood although causal risk factors include age and family history of dementia. The apolipoprotein E $\epsilon 4$ genotype increases the risk and,

after age, is the most important risk factor (for further discussion, see [5]).

Recently it was reported that exposure to radiofrequency fields (RF), 918 MHz, 250 mW/kg, provides cognitive benefits for both normal and transgenic mice [6]. In Alzheimer's disease (AD) mice, long-term exposure reduced brain amyloid- β ($A\beta$) deposition through decreased aggregation of $A\beta$. It was proposed that the mechanism might be increased $A\beta$ clearance from the brains of Alzheimer's disease mice, increased neuronal activity, and increased cerebral blood flow.

Based on our findings of increased serum concentrations of transthyretin (TTR) among long-term users of wireless phone, i.e., mobile phone and cordless phone [7] and in a provocation study [8] we postulate that TTR might be involved in the preventive effect on AD by exposure to RF.

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TTR or prealbumin is produced in the liver, retina and choroid plexus. It constitutes about 20% of the proteins synthesized and 50% of the proteins secreted by choroid plexus [9,10] and is secreted only into the cerebrospinal fluid (CSF). TTR synthesized in the liver does not contribute to TTR in the CSF.

Choroid plexus has the highest concentration of TTR mRNA in the body and choroid plexus is only present in the epithelial cells [11]. TTR is involved in the transport of thyroid hormone and retinol binding hormone into the brain [12]. It has been regarded to be a negative acute phase protein in the plasma since it inversely correlates to e.g. inflammation, malnutrition, trauma and surgery [13]. This regulation is independent of the synthesis in choroid plexus. Thus, under these conditions, TTR is decreased in the liver, but not in choroid plexus [9]. Clinically, TTR has also been used as an indicator of postoperative recovery and nutritional status [14].

In the context of exposure to RF TTR is interesting since it sequesters $A\beta$, thereby preventing the formation of $A\beta$ plaques in the brain [15]. However, amyloid fibrils can also be formed in the choroid plexus and ependyma as a conjugate of TTR and amyloid peptides. Several studies have shown that patients with AD have lowered TTR concentrations in CSF and have attributed the onset of AD to insufficient sequestering of $A\beta$ by TTR [16,17]. TTR availability is inversely related to $A\beta$ insolubility, plaque formation and dementia [18].

RF might thus be of interest in the prevention of AD based on our TTR studies [7,8] and on animal findings [6,19]. Costa et al. showed in a study on protection against cognitive impairment and decreased $A\beta$ deposition in AD transgenic mice given long-term environmental enrichment that the gene, which was most up-regulated in animals exposed to enrichment was in fact TTR [19]. Another interesting aspect is that clinical studies have shown that nicotine increases the synthesis and secretion of TTR in choroid plexus [20]. The TTR gene in the choroid plexus is thereby up-regulated independently of the TTR gene in the liver [9]. This may be the clinical link between epidemiological findings between cigarette smoking and protection from dementia [21].

In the following we give a short presentation of our two studies on exposure to RF and serum TTR concentrations, for further information see the published articles [7,8] and a medical thesis [22]. The ethical committee approved both studies.

MATERIALS AND METHODS

Cross-sectional study

In total, 500 men and 500 women aged 18–65 years were randomly recruited from the Örebro municipality in Sweden using the population registry [7]. They were asked to give informed consent to leave blood and answer a postal questionnaire on e.g. use of mobile phones and cordless phones and some health and life-style related factors. The blood was stored in a so-called Biobank and later analysed for serum concentration of TTR. Standard immunonephelometric techniques using commercially available reagents were used. All analyses were performed without knowledge of exposure data. The detection limit of TTR was 0.02 g/l and all results are expressed in g/l.

Provocation study

In this study 44 volunteers aged 18–30 years were recruited from the municipality of Umeå, Sweden [8]. Of these, 35 were randomly recruited from the population registry and 9 by personal invitation at the Department of Radiation Sciences, Umeå University, Sweden. Certain factors such as use of wireless phones were assessed by a structured questionnaire. The final sample consisted of 41 persons since one woman was excluded because of pregnancy, another due to loss of blood pressure during blood sampling and a third could not participate for technical reasons in the statistical pair-wise analyses. Exposure was to an 890 MHz GSM signal during 30 min, for technical details see Söderqvist et al. [8]. A homogenous specific absorption rate (SAR_{1g}) of 1.0 Watt/kg to the temporal area was applied.

Blood was drawn at four occasions; the first 10–15 min after arrival to the hospital, the second after 30 min rest including study information and just before the provocation, the third immediately after 30 min exposure, and the fourth 60 min after ending of the exposure. Blood was stored in a Biobank and the same method for analysis of TTR as in the cross-sectional study was used.

STATISTICAL METHODS

Cross-sectional study

Frequency tables were produced and explanatory factors were analyzed by the Wilcoxon rank-sum test.

Table 1
Linear regression analysis of serum transthyretin and years since first use of wireless phone use adjusted for age and gender

Group	n	Standardized β coefficient	95% CI	P
<i>Years since first use, mobile phone/DECT</i>				
Mobile phone + DECT	309	0.13	0.02 to 0.24	0.03
–Men	133	0.19	0.01 to 0.38	0.04
–Women	176	0.10	–0.05 to 0.25	0.20

Age was adjusted for in all analyses while adjustment for gender was applied only in analyses of total samples.

Data for years since first use of mobile phone/DECT are missing for one person (a user of both mobile phone and DECT).

Following log-transformation of TTR values to normalize the distribution, linear regression was used to test an association between long-term trends in wireless phone use and serum concentrations of TTR. Standardized β coefficients were used throughout. The same method was used for analysis of current and previous smoking and use of oral snuff.

Long-term use of wireless phones was examined by comparing years since start of use and serum concentrations of TTR, as well as fractions of total duration for the different phone types. To avoid multicollinearity in the latter, the fraction of digital phone (GSM), which was the most frequently used, was omitted in the linear analysis of long-term use.

Provocation study

The four blood samples were compared using repeated-measures ANOVA with Huynh-Feldt correction followed by linear contrasts for pairwise comparisons between sample 1 and sample 2, sample 2 and sample 3, sample 3 and sample 4. Since these a priori chosen comparisons did not exceed the degrees of freedom between samples no multiple comparison adjustments were performed. All analyses were done using StataSE 10.1 (Stata/SE 10.1 for Windows; StataCorp., College Station TX).

RESULTS

Cross-sectional study

Of the invited persons 314 agreed to participate and answered the questionnaire and gave blood. The analysis of one sample failed so the final study encompassed 313 subjects, 133 men and 180 women. The median age was 47 years. Serum TTR was significantly higher in the oldest group (> 47 years), mean = 0.284,

median = 0.280 (range 0.180–0.470), compared with in the younger one (≤ 47 years) 0.271, 0.260 (range 0.170–0.480), respectively; $P = 0.02$.

Men had statistically significantly higher TTR concentrations than women. The mean TTR concentration for men was = 0.307, median = 0.300 (range 0.180–0.480) as compared with women = 0.256, median = 0.250 (range 0.170–0.400); $P < 0.0001$. Time during the day (hour), weekday or month of the year (March–October) for drawing blood were not determinants of the concentration in either men or women.

A statistically significantly positive β coefficient was found for time since first use of mobile phones and DECT combined ($P = 0.03$), Table 1. When stratifying on gender, positive β coefficients were found for both men and women, although statistically significant only for men ($P = 0.04$). Additional analyses with adjustments for fraction of use of the different telephone types did not change the overall results in the linear regression analysis on time since first use (data not in Table). The results of fraction analyses also showed that neither of the different types of wireless telephones altered the relative level of trend for time since first use for mobile telephone and DECT, combined. Analyses were also performed on extent of use, i.e. cumulative number of hours, but no statistically significant correlation was found ($P = 0.11$; β coefficient = 0.08).

We analyzed smoking habits, Table 2. Statistically significantly higher concentrations of TTR were found among persons that had quit smoking. Analysis on gender showed no differences in men but previous smokers among women had statistically significantly higher TTR concentrations than never smokers.

We grouped both current and previous smoking in number of cigarettes smoked per day without any statistically significant findings for TTR concentrations (data not in Table). Linear regression analysis of serum TTR and current and previous smoking yielded positive standardized β coefficients, although not statistically significant, Table 3.

Table 2
Transthyretin levels (g/l) for current and previous smoking and use of oral snuff

	n	Mean	SEM*	Median	Min	Max	P**
Total							
Smoking							
–Current	45	0.276	0.008	0.270	0.180	0.440	0.65
–Previous	98	0.289	0.006	0.280	0.200	0.480	0.03
–Never	169	0.271	0.004	0.260	0.170	0.420	
Use of oral snuff							
–Current	33	0.321	0.012	0.320	0.200	0.480	< 0.0001
–Previous	28	0.296	0.008	0.285	0.200	0.380	0.002
–Never	251	0.270	0.003	0.260	0.170	0.440	
Men							
Smoking							
–Current	19	0.304	0.014	0.290	0.220	0.440	0.89
–Previous	45	0.315	0.009	0.300	0.210	0.480	0.49
–Never	69	0.302	0.006	0.300	0.180	0.420	
Use of oral snuff							
–Current	27	0.331	0.014	0.320	0.200	0.480	0.04
–Previous	22	0.296	0.009	0.285	0.200	0.380	0.73
–Never	84	0.302	0.006	0.300	0.180	0.440	
Women							
Smoking							
–Current	26	0.256	0.008	0.260	0.180	0.330	0.39
–Previous	53	0.266	0.006	0.250	0.200	0.400	0.03
–Never	100	0.250	0.004	0.245	0.170	0.390	
Use of oral snuff							
–Current	6	0.273	0.016	0.265	0.230	0.330	0.19
–Previous	6	0.298	0.016	0.290	0.250	0.350	0.01
–Never	167	0.254	0.003	0.250	0.170	0.400	

* = Standard error of the mean.

** = Wilcoxon rank-sum test.

Table 3

Linear regression analysis of serum transthyretin and current and previous smoking and use of oral snuff adjusted for age and gender

Group	n	Standardized β coefficient	95% CI	P
Smoking				
–Current	45	0.01	–0.10 to 0.11	0.92
–Previous	98	0.06	–0.05 to 0.17	0.31
Use of oral snuff				
–Current	32	0.15	0.04 to 0.26	0.01
–Previous	28	0.04	–0.07 to 0.14	0.47

The concentration of TTR in relation to use of oral snuff was statistically significantly higher for current and previous use, Table 2. Most persons that used oral snuff were men and in that group the median concentration of TTR was 0.320 compared with 0.300 for men that had never used oral snuff ($P = 0.04$). In women both current and previous use of oral snuff yielded higher concentrations of TTR, but the results were based on low numbers ($n = 6$ in each group). Linear regression analysis of serum TTR and use of oral snuff gave for current use β coefficient = 0.15, 95% CI = 0.04 to 0.26, whereas for previous use the coefficient was not statistically significantly increased, Table 3. The same analysis for number of years of current use

Table 4

Linear regression analysis of serum transthyretin and years of current use of oral snuff adjusted for age and gender

Group	n	Standardized β coefficient	95% CI	P
Years				
–1–15 years	13	0.08	–0.02 to 0.18	0.13
≥ 15 years	18	0.14	0.03 to 0.24	0.01

Information missing for one person.

of oral snuff yielded highest β coefficient in the group with > 15 years use ($P = 0.01$), Table 4.

Provocation study

Repeated-measures ANOVA yielded statistically significant differences between all four samples of TTR, Table 5. A statistically significant decrease was seen between samples 1 and 2. No statistically significant increase was evident immediately after the provocation in sample 3, whereas in the last sample after 60 min of rest, the trend changed to a statistically significant increase between samples 3 and 4 ($P = 0.02$). Overall, similar results were seen for men and women.

Table 5
Serum concentrations of transthyretin between all the blood samples in the provocation study and linear contrasts for a priori pairwise comparisons ($n = 41$)

	Mean	SEM*	Median	Min	Max	P
<i>Repeated-measures ANOVA**</i>						0.0003
Sample 1	0.230	0.006	0.234	0.146	0.310	
Sample 2	0.225	0.006	0.230	0.129	0.307	
Sample 3	0.224	0.006	0.230	0.132	0.309	
Sample 4	0.227	0.006	0.235	0.132	0.310	
<i>Linear contrasts</i>						
Sample 1 vs Sample 2						0.001
Sample 2 vs Sample 3						0.33
Sample 3 vs Sample 4						0.02

* = Standard error of the mean.

** = Huynh-Feldt correction.

DISCUSSION

In the study by Arendash et al. [6] it was concluded that long-term exposure to electromagnetic field (EMF) protects against and reverses cognitive impairment and A β neuropathology in AD transgenic mice. As proposed by the authors, one possible mechanism could be findings of decrease in brain A β deposition and increase in soluble A β levels combined with suppression of A β aggregation and oligomerization in homogenates of hippocampus *in vitro*. The biological explanation for these animal results is unknown, although reduced A β deposition, increased body temperature and increased neuronal activity by EMF were discussed.

Based on the results both in our cross-sectional study [7] and provocation study [8] on humans we propose transthyretin to be a candidate that might explain the protective effect on AD by exposure to RF in the animal study. We found in our cross-sectional study higher concentrations of TTR for long-term use of wireless phones. In the linear regression analysis of total samples, statistically significant β coefficients were obtained indicating higher TTR concentrations for mobile phone and cordless phone combined the longer these phones had been used.

It should be mentioned that in the cross-sectional study we also analyzed serum TTR in relation to cumulative (number of hours) of use of wireless phone, but no statistically significant correlation was found. Whether that reflects the true absence of a correlation or simply a somewhat more pronounced non-systematic error related to recall of use (as compared to first year of use) is difficult to say. While cumulative use did not correlate significantly ($P = 0.11$ vs. $P = 0.03$ for years since first use) with TTR, the β -coefficient had the same direction, though with weaker slope for mobile phone and DECT combined.

TTR synthesis in choroid plexus and liver is up-regulated by estrogen in mice [23,24]. Estrogen replacement therapy is common among elderly women, but elevated TTR levels have also been reported in women using hormonal contraceptive drugs [25]. Since use of hormonal drugs is expected to be low in men we made gender specific analyses, although ideally intake of hormones should have been assessed in both men and women.

Malnutrition and inflammatory processes can affect TTR produced by the liver [14], but this would probably not associate with long-term use of wireless phones. Body mass index (BMI) is a proxy for nutritional status, and predicted indeed serum concentrations of TTR in our study. Adjusting for BMI in the regression analysis, however, did not significantly change the results.

In the provocation study each participant acted as his or her own control. However, no sham exposure was used and all study subjects were aware of the exposure condition. Serum TTR decreased in the second blood sample compared with the first. This could be due to the relaxed situation after arrival to the hospital with more information about the study and after the insertion of the needle for drawing blood. A statistically significant increase of TTR was found between the 3rd and 4th samples, i.e. 60 min after exposure. This might indicate an effect by the RF, in that case it might be due to increased cerebral blood flow. However, a biological compensatory increase (feed-back) in TTR over time following the previous decrease can of course not be excluded.

In our cross-sectional study higher TTR concentrations were found in subjects who had used oral snuff. As nicotine has been reported in animal studies to up-regulate the synthesis and secretion of TTR in the choroid plexus, but not in the liver [9] these results support our choice of method using serum TTR as a marker of TTR in CSF.

We also found statistically significantly higher serum concentration of TTR among previous smokers, Table 2. This was partly explained by the fact that ex-smokers instead used oral snuff. In the linear regression analysis no association was found for smoking whereas current use of oral snuff yielded a statistically significantly increased standardized β coefficient, Table 3. This was only found for long-term (≥ 15 years) use of oral snuff, Table 4. The increase was of the same magnitude as for long-term use of wireless phones.

In a study that used urinary cotinine levels of smokeless tobacco as a marker for nicotine exposure, three times higher concentrations were found than for smokers [26]. Snuff products are capable of rapidly delivering high doses of nicotine with large variation between different brands [27,28]. In the light of these results our finding of larger impact on TTR concentrations among users of oral snuff than among smokers seems to be of relevance.

Nicotine has been shown to improve behavioral impairment and to be neuroprotective in AD transgenic mice [29,30]. Different pathways have been proposed such as up-regulation of the nicotinic acetylcholine receptor [31,32]. Another explanation could be up-regulation of the TTR gene in plexus choroideus [20].

TTR levels in the cerebrospinal fluid have been shown to be selectively decreased in AD patients [33, 34]. This suggests that a deficient $A\beta$ -binding capacity in the cerebrospinal fluid may contribute to the amyloidogenic process in AD. TTR has also been shown to degrade aggregated forms of $A\beta$ peptide [35]. Thus, TTR may be a useful therapeutic agent for preventing or retarding the cerebral amyloid plaque formation in AD pathology.

In a review of five studies on exposure to electric and extremely low frequency electromagnetic fields it was concluded that there is an association between exposure to these fields and increased risk of AD [36]. The same conclusion was made in a more recent meta-analysis [37]. These results have been corroborated by a study on AD among residents near power lines [38]. Residence < 50 from a 220–380 kV power line yielded odds ratio = 2.0, 95% CI = 1.2–3.3. The mechanism for this is not known, although secretion of $A\beta$ peptide from glioma cells has been suggested in a laboratory study [39].

It should also be noted that in a study on young male Wistar rats exposed to 900 MHz mobile phone signal 2 h/day on 5 days/week, exposure for 5 weeks significantly improved the rate of learning [40]. The whole body average specific energy absorption rates were 0.3

or 3.0 W/kg or sham. No degenerative changes, dying neurons or effects on leakage of the blood-brain barrier were detected. In a meta-analysis of human neurobehavioural effects of RF fields emitted by GSM mobile phone it was concluded that cognitive performance measured by subtraction task was mildly facilitated from exposure [41]. Nineteen studies were considered and ten were included in the meta-analysis based on the inclusion criteria.

The findings of increased risk for AD in subjects exposed to extremely low frequency (ELF) EMF [36–38] are in contrast to the results in AD transgenic mice by Arendash et al. [6], although based on only one study. On the other hand, the latter study used RF exposure, which in terms of frequency differs far from ELF EMF. As such, there is no conflict between the epidemiological and experimental animal studies. It should also be mentioned that microwave exposure has been shown to promote amyloid fibril formation under non-physiological conditions with 24 h exposure of bovine insulin at 60°C in a TEM cell [42]. The authors concluded, however, that moderate microwave exposure might even prove beneficial by activation of heat shock protein. An increased blood flow might be part of a beneficial effect.

Arendash et al. [6] suggest that increased blood flow might be one mechanism for decrease in brain $A\beta$ deposition in AD transgenic mice. Sensations of warmth on the ear and around the ear used for mobile phone calls has been reported [43]. This is probably associated with increased blood flow.

We propose that TTR might be involved in the findings of RF benefit in AD mice. Furthermore, based on our results on RF exposure and use of oral snuff we suggest that this is a long-term effect whereby an up-regulation of the TTR gene in choroid plexus might be involved, although an increased blood flow resulting in increased TTR secretion cannot be ruled out. Further studies are needed to collaborate these findings, to elucidate the biological mechanism and to find a therapeutic use in AD patients of RF fields, if any. In contrast to this it must be pointed out that several studies indicate an increased risk for brain tumours for persons with long-term use (≥ 10 years) of mobile (and when included cordless) phones taking into account which side of the head the handset has been mostly used. This has been further discussed in other articles [44–47].

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