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ENVIRONMENTAL RADON DAUGHTERS REVEAL PATHOGNOMONIC CHANGES IN THE BRAIN PROTEINS AND LIPIDS IN PATIENTS WITH ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE, AND CIGARETTE SMOKERS

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This paper presents an investigation of the retention of environmental radon daughters, ^{210}Po (alpha particle emitting radio-nuclide) and ^{210}Bi (beta particle emitting radio-nuclide), in lipid and protein fractions of the cortical grey and subcortical white matter from the frontal and temporal brain lobes of patients who had suffered from Alzheimer's disease or Parkinson's disease, of cigarette smokers, and of control subjects. ^{210}Po and ^{210}Bi radioactivity increased tenfold in the cortical grey and subcortical white protein fraction in patients with Alzheimer's disease and smokers, and tenfold in the cortical grey and subcortical white lipid fraction in patients with Parkinson's disease. Free radicals generated by radon daughters may add to the severity of the radio-chemical injury to the brain astrocytes. The pathognomonic distribution of radon daughters to lipids in patients with Parkinson's disease and to proteins in patients with Alzheimer's disease was attributed to high chlorine affinity of radon daughters. The changes in the membrane protein pores, channels, and gates in patients with Alzheimer's disease and in the lipid bilayer in patients with Parkinson's disease are at the core of what the authors think are two systemic brain diseases.

Key words:
cell membrane impairment, chlorine hypothesis, radio-chemical injury, systemic brain disease

Radon (^{222}Rn) is a highly lipid-soluble, radioactive, and noble gas, ubiquitous in the human environment (1). When inhaled, environmental ^{222}Rn has a preference to accumulate in the lipid tissue throughout the body with the highest concentration in the brain, and bone marrow (2, 3). *Pohl and Pohl-Ruling* (4) found that one third of the inhaled radon decay products passes from the lungs into the bloodstream, which indicates that radon gas does not flow quickly in and out of the lungs, but can linger

long enough to allow radiation damage the body. Indeed, *Lykken and co-workers* (5, 6) showed that inhaled ^{222}Rn was rapidly absorbed from the lung into the body where it tended to accumulate in the cranium, resulting in increased ^{214}Bi gamma-ray emissions and altered EEG signals.

Radon is chemically inert; however, it is radioactive and decays through a distinct series of radioactive daughters before it ends up stable ^{206}Pb (Table 1) (7). When

Table 1 *Major products of ^{222}Rn decay series and their characteristics (22)*

		Major radiation emissions (MeV)		
Radionuclide	**Half-life**	**Alpha**	**Beta**	**Gamma**
^{222}Rn	3.823 d	5.49	0.670	0.295
^{218}Po	3.05 min	6.00	0.730	0.352
^{214}Pb	26.8 min		1.02	
^{214}Bi	19.7 min		1.0	0.609
			1.51	1.12
			3.26	1.764
^{214}Po	164 μs	7.69		
^{210}Pb	22.3 y	0.015	0.047	
			0.061	
^{210}Bi	5.01 d	1.161		
^{210}Po	138.4 d	5.305		
^{206}P	Stable			

decaying, radon and its daughters (RDs) release high-energy alpha and beta particles, or gamma radiation, strong enough to damage the cell (8). One should bear in mind that as soon as the alpha particle is emitted from the decaying radon and the radon daughter is born a chemical transmutation will take place. Indeed, all RDs, including the biologically most relevant ^{210}Pb, ^{210}Bi, and ^{210}Po, down to stable lead, are heavy metals that are highly neurotropic and neurotoxic (9). In contrast to mother radon which is a lipid-soluble gas, freely moving in and out of the brain despite the blood-brain barrier, none of the transmuted heavy metal RDs are lipid-soluble. Hence, they remain trapped in the brain where they emit additional gamma radiation and alpha and beta particles over their lifetime, thus adding up chemical to the radiation damage to the brain.

The effect of constant low-level radiation exposure to large alpha particles of high linear energy transfer such as ^{222}Rn and ^{210}Po on cognitive functions in the human brain is unknown. Studies on children of atom bomb survivors in Japan who had been irradiated *in-utero* showed that they scored lower on IQ tests (10). The deleterious effects of high-energy radiation on the brain are already recognised in the classification of the Diagnostic and Statistical Manual of Mental Disorders and coded as »294.1 dementia due to intracranial radiation« (11). Indeed, subjects exposed to lethal radiation doses showed profound psychological impairment without subsequent pathological changes in the brain (12).

Relying on the original observation on radon accumulation in the cranium by *Lykken and co-workers* (5, 6), the objective of this study was to comparatively analyse the pattern of distribution of natural environmental RDs – ^{210}Po as a strong alpha particle emitter (5.3 MeV) and ^{210}Bi as a moderately strong beta particle emitter (1.2 MeV) – in the protein and lipid fractions of the cortical grey and subcortical white matter from the frontal and temporal lobes of the brains taken from deceased patients who had suffered from Alzheimer's disease (AD) or Parkinson's disease (PD), and from deceased smokers and nonsmokers (controls) who had never shown a sign of neurological disease. Because the concentration of RDs is high in cigarette smoke (13), we placed the brains from cigarette smokers without a neurological disease to a separate category.

SAMPLES AND METHODS

Brain samples

Brain samples from deceased patients who had suffered from Alzheimer's disease (AD) or Parkinson's disease (PD), those who had smoked, and those who had shown no known neurological disease in their lifetime were kindly provided by the Alzheimer's Research and Treatment Center in St. Paul, MN. The pathological diagnosis of AD relied on the presence of an age-adjusted moderate to severe number of plaques in the neurocortex (14). We received samples from the frontal and/or temporal lobes from the brains: 11 AD, six PD, four smokers, and eight nonsmokers (controls). Age and sex distribution was similar between the groups except for the cigarette smokers who were slightly younger (Table 2). We altogether analysed 45 brain lobe samples of 29 subjects, 25 from the frontal lobe and 20 from the temporal lobe. In each group there were four subjects from whom we had both frontal and temporal lobe available for the analysis (Table 2). Before the analysis, the brain slices weighing approximately 80 g had been frozen in liquid nitrogen and stored for ten years on average. Long enough to reach secular equilibrium between the ^{210}Po formed in the brain from ^{210}Bi directly and from ^{210}Pb indirectly *via* ^{210}Bi, as ^{210}Bi and ^{210}Po can come about only through the decay of ^{222}Rn.

We separated cortical grey and subcortical white matter from each frontal and temporal lobe. One gramme of each sample was fractionated into protein and lipid content before we assessed ^{210}Bi and ^{210}Po activity. Subcortical white matter was sampled 0.5 cm orthogonally below the sampled cortical grey matter area. Three replicates of each lipid and protein fraction were prepared from grey and white matter from every brain lobe.

Trichloracetic acid soluble fraction

Unless specified otherwise, all chemicals used in this study were reagent grade and supplied by Fisher Scientific, Ithaca, IL. Only distilled H_2O was used.

Table 2 *Brain proteins (mg/g). Subjects are arranged according to their age at the moment of death*

			Brain lobe protein (mg/g brain tissue)					
			Temporal			Frontal		
No.	**Age**	**Sex**	**Grey**	**White**	**W/G**	**Grey**	**White**	**W/G**
CONTROL								
1	52	M	148.3	70.7	0.477	159.6	93.7	0.587
2	53	M	150.6	72.3	0.480	181.4	74.4	0.410
3	60	M				105.3	97.3	0.924
4	70	F	149.7	71.2	0.476	170.5	89.8	0.527
5	70	M	150.9	69.8	0.462			
6	76	F	152.1	71.4	0.469	138.5	86.5	0.624
7	79	M				217.4	71.4	0.328
8	88	M	233.8	123.2	0.527			
ALZHEIMER'S DISEASE								
9	62	F				126.8	42.7	0.337
10	66	M				128.1	63.2	0.493
11	71	F	189.7	68.8	0.363	172.1	94.4	0.548
12	73	M				206.0	54.4	0.264
13	80	F				213.5	48.4	0.227
14	82	M				158.8	70.1	0.441
15	82	F	160.4	78.4	0.489	191.7	49.0	0.256
16	84	F	89.9	44.7	0.497	126.2	59.8	0.474
17	85	M	233.8	102.2	0.437			
18	86	F	100.4	51.2	0.510	108.4	53.4	0.493
19	93	F	120.6	59.1	0.490			
PARKINSON'S DISEASE								
20	68	M	148.4	107.8	0.726	158.7	73.5	0.463
21	71	M	199.6	56.6	0.283	224.0	60.9	0.272
22	73	F				181.4	81.4	0.449
23	77	F				147.8	75.7	0.512
24	80	M	366.6	138.9	0.379	224.0	155.8	0.695
25	81	F	204.0	107.6	0.527	165.2	56.4	0.341
SMOKERS								
26	47	F	171.7	68.7	0.400	171.9	81.9	0.476
27	68	M	101.3	49.3	0.487	131.6	68.8	0.524
28	72	F	102.6	54.4	0.530	141.3	72.4	0.512
29	79	M	99.5	43.7	0.439	123.7	64.7	0.523

M – male, F – female; G – cortical grey brain matter, W – subcortical white brain matter, W/G – ratio between the White and Grey matter protein fractions

First we separated grey and white cortical matter (A. L. Politoff, personal communication). One gramme of either grey or white matter was homogenised at 4 °C in a Potter-Elvehjem glass-Teflon tissue homogeniser with 6 ml of distilled H_2O and 0.5 ml of 100% trichloracetic acid. After centrifugation at 5,000 rpm (CRU-5000 Centrifuge, Hamden Heights, MA) for 10 min, the supernatant was poured off and saved in a 20 ml plastic tube. After vigorous mixing of the precipitate in 2 ml of H_2O, the extraction procedure was repeated. The supernatant fluids were pooled and labelled as trichloracetic acid soluble fraction. This fraction contains primarily nucle-

otide and other small molecular weight compounds. They were found to be essentially free of all RDs.

Extraction of lipids

Lipids were extracted from the trichloracetic acid precipitate according to the method of *Folch and co-workers* (15) by using chloroform:methanol (2:1 vol./vol.). The precipitate was mixed with a 3 ml methanol for one minute. After 10 minutes, 6 ml chloroform was added. After centrifugation, the supernatant was filtered through sintered glass (medium porosity). The precipitate was rinsed with 4 ml of Folch reagent and the filtrates combined. Biphasic separation was achieved upon addition of 1 ml potassium acetate. The upper aqueous layer was removed and discarded. The organic phase was taken to dryness under a stream of nitrogen at room temperature. The total lipids were dissolved in a 1 ml chloroform. Any residual proteins were removed by filtration through glass wool and the lipid fraction was divided in two equal portions, one for alpha spectroscopy and other for beta-scintillation spectrometry. All calculations based on amount of total lipid extracted from one gram of tissue.

Protein fraction

The protein precipitate which remained on the sintered glass was dissolved in 1 ml 0.1 M NaOH at 75 °C over night (16). The protein fraction was divided in three equal portions; one to assess the amount of protein, and one each for alpha and beta activity analysis. The amount of protein was quantitatively assessed employing Bradford's reagent (Bio-Rad Laboratories, Hercules, CA). After addition of the reagent, absorbency was measured employing a Beckman spectrophotometer at 760 nm (Beckman Instruments Co., Fullerton, CA). Bovine albumin (Bio-Rad Laboratories, Hercules, CA) served as a standard.

Low level alpha particle counting

Alpha particles were measured with an Alpha Spectrometer System (EG&G ORTEC, Oak Ridge, TN) using a radionuclide library software package from the same manufacturer. This computer-controlled system consists of five separate surface barrier alpha spectrometers with alpha resolution of 6 KeV, which allows for accurate discrimination of ^{208}Po, ^{209}Po, and ^{210}Po isotopes. The detection limit of the instrument was 10 μBq per gramme of brain tissue. Every week, a 48-hour naturally occurring background was measured at the photo peak value for every detector and isotope, i.e., ^{208}Po (5.115 MeV), ^{209}Po (4.880 MeV), and ^{210}Po (5.305 MeV) (17).

Silver disk sample spiking and plating

We essentially followed the method of P.O. Jackson, which was developed at Battelle Pacific Northwest Laboratories (18). Polonium has a high selective affinity for silver. Hence, one-inch silver disks (HH Lucas-Milhaupt, Inc, Cudahy, WI) were painted on one side with black Krylon spray paint (Home of Economy, Grand Forks, ND); the other side was polished, rinsed and placed in the plating apparatus described below.

To determine recovery (percent), the samples were »spiked« with a standard stock solution of ^{208}Po of known activity (1606 Bq/ml, Isotope Products Laboratories, Burbank, CA). The standard stock solution was diluted with 0.4 N HCl so that each sample was spiked with ^{208}Po yielding 1 disintegration per minute (dpm) per 10 μl. The error of pipetting was $\pm$2%. The background for each polonium peak of any of the silver disks did not exceed 2 counts per 48 hours.

To destroy organic matter, the spiked samples were placed in a 250 ml beaker and digested in a 10 ml concentrated HNO_3. After heating at 85 °C for 30 min, 10 ml of 30% hydrogen peroxide and 3 ml of concentrated HNO_3 were added and the solution heated to dryness (30 min). The procedure was repeated 3 times before 5 ml of 12 M HCl and 5 ml of distilled H_2O were added to the dry sample to form polonium chloride. The solution was heated at 85 °C until dry and dissolved in 120 ml of distilled H_2O containing 10 mg of ascorbic acid. To allow for plating of the polonium on the silver discs, the pH was <2. Low pH enhances extraction of any cation. To plate the polonium, the silver disk was placed in a Teflon holder, which was immersed in the solution of the spiked sample from either grey or white matter. The beaker was placed on a stirring hot plate at 85 °C and the solution stirred at 2.5 Hz. The ^{208}Po and ^{209}Po from the spike and unknown ^{210}Po from the sample were allowed to plate out over a 6–8 hour period, after which time the disk was removed, rinsed with distilled H_2O, and counted for alpha activity for 48 hours.

Separation of ^{210}Pb from ^{210}Bi and ^{210}Po

We essentially followed the method of *Orlandini and co-workers*, which was developed at the Argonne National Laboratory (19, 20). The lipid or protein fractions were passed through a polymembrane under a negative pressure gradient (6 ml/min). The polymembrane consists of two negatively charged ion exchange membranes (Gelman Sciences, Ann Arbor, MI) which constitute a special system for absorption of ^{210}Bi and ^{210}Po. The two readily form oxichlorides which bind to the membrane, whereas ^{210}Pb is selectively filtered. The membranes were dried prior to alpha particle analysis or rinsed with 0.4 mol/L HCl and placed in a 20 ml vial for the assessment of beta emission.

Alpha radioactivity

The standard ^{208}Po and sample ^{210}Po counts under the peaks were summed separately. The background counts were subtracted from the sample counts to obtain the net counts for each isotope. ^{210}Po and ^{208}Po net counts were corrected for decay and ^{210}Po counts were extrapolated to the time of the patient's death. The final activity was expressed as ^{210}Po disintegration per minute (dpm)/g brain tissue. Two replicates of each brain sample were prepared for alpha counting.

^{210}Pb decays to ^{210}Bi which, in turn, decays to ^{210}Po; each at a different decay rate (Table 1). After 600 days a »secular equilibrium« is reached, meaning that the activities of both the ^{210}Bi and ^{210}Po in the sample are equal to the ^{210}Pb activity. Therefore, from the time of the brain sampling postmortem to the time of ^{210}Po plating on Ag disks, ^{210}Po formed in the brain from ^{210}Bi directly and from ^{210}Pb indirectly via ^{210}Bi. We used the standard Bateman differential equation of growth and decay of radionuclides in a decay chain to correct for this contribution (21, 22).

Blanks were prepared separately by spiking silver disks with 10 μL of ^{208}Po and measuring them in the same way as brain samples. The ^{208}Po recovery after plating was 85±3%. The alpha particle dpm for all the brain samples fell within ±2 SD.

Beta radioactivity

Low-level beta liquid scintillation counting (LSC) involved the following procedure; 10 ml of liquid scintillation cocktail solution (Beckman Co., Fullerton, CA) were added to the sample and the activity measured in a Beckman scintillation spectrometer (Beckman Co., Fullerton, CA). The detection limit of the instrument was 10 μBq per g of the brain tissue. As outlined above, ^{210}Bi beta activity in the sample is an excellent measure of the ^{210}Pb activity, since ^{210}Pb, ^{210}Bi, and ^{210}Po were in secular radioactive equilibrium in each brain several years after the death of the subjects. This is why the calculations for a three-component chain decay followed the same procedure as the alpha particle counting. The efficiency of binding of ^{210}Po and ^{210}Bi to the membrane was assessed with a ^{210}Pb standard (Battelle Pacific Northwest Laboratories, Richland, WA), and found to be 68% and 99%, respectively. The technique is similar to that of measuring the activity of caesium in seawater (23) and it gave the same values as those reported by the Argonne National Laboratory (20).

Statistical analysis

Measurements of alpha and beta activities were expressed as dpm/g of brain tissue. This allows for direct comparison of instruments with different counting efficiency and hence for direct comparison of two different methods of analysis, i.e., alpha and beta counting. In other words, beta (^{210}Bi) and alpha (^{210}Po) dpm served as standards for each other.

All samples were counted until the number of counts above background exceeded 400. This is well above the minimum criteria for accurate measurement at the 5% probability level (signal to noise ratio) defined here as 100% times the square root of the net counts above background and divided by the net counts. Thus the net counts exceeded 400. This satisfies the accuracy criterion that the standard error (the square root of the number of counts) does not exceed 5% of the measurement (24). *Currie* (25) has suggested that a net signal of 10 sigma background is satisfactory for quantitative measurements. However, *Currie's* signal was broad and contained a large number of counts whereas our signal was very narrow and contained only zero, 1, or 2 counts at most over a 48-hour counting period. Therefore, we choose 3 sigma background as the significant level (24). The entire data set on ^{210}Bi and ^{210}Pb is shown in Appendix I, Parts A–C.

Statistical analysis searched for possible dependence of radiation level on measurement method (alpha or beta-radiation), brain fraction (lipid or protein), brain lobe (frontal or temporal), brain matter (grey or white), subject type (AD, PD, smoker, or control), and interactions of these effects. We used multivariate analysis of variance (MANOVA) with subject type as a between-subjects effect (factor) and all other effects as within-subject effects (contrasts of the response variables). The radiation level was log-transformed to approximate normality (however, Figures 1–4 below show untransformed data).

Neither the alpha-beta main effect nor any interactions involving this factor were significant at the 0.05 level (Table 3). Hence, alpha and beta measurements were averaged and a second MANOVA was performed on the remaining factors. In this

Table 3 *Comparison between alpha ^{210}Po and beta ^{210}Bi specific activities (AB) in proteins and lipids from the cortical grey and subcortical white (GW) matter from the frontal and temporal brain lobe (FT) in patients with Alzheimer's disease, Parkinson's disease, smokers, and controls using the multivariate analysis of variance (MANOVA)*

Effect	DF	P
AB	1	NS
AB * FT	1	NS
AB * GW	1	NS
AB * PL	1	NS
AB * CAPS	3	NS
AB * FT * GW	1	NS
AB * FT * PL	1	NS
AB * FT * CAPS	3	NS
AB * GW * PL	1	NS
AB * GW * CAPS	3	NS
AB * PL * CAPS	3	NS
AB * FT * GW * PL	1	NS
AB * FT * LP * CAPS	3	NS
AB * GW * PL * CAPS	3	NS
AB * FT * GW * PL * CAPS	3	NS
FT	1	NS
FT * GW	1	<0.0001
FT * PL	1	<0.0002
FT * CAPS	3	NS
FT * GW * PL	1	NS
FT * GW * CAPS	3	<0.0001
FT * PL * CAPS	3	<0.005
FT * GW * PL * CAPS	3	NS
GW	1	<0.0017
GW * PL	1	NS
GW * CAPS	3	NS
GW * PL * CAPS	3	<0.0456
PL	1	<0.0001
PL * CAPS	3	<0.0001
CAPS	3	<0.0001

Legend:

A – alpha activitiy
B – beta activitiy
F – frontal lobe
T – temporal lobe
G – grey brain matter
W – white brain matter
NS – not significant
P – protein
L – lipids
C – controls
A – patients with Alzheimer's disease
P – patients with Parkinson's disease
S – cigarette smokers
CAPS – controls, to Alzheimer's disease, Parkinson's disease, and cigarette smokers

analysis, the subject type-lobe-grey/white and subject type-grey/white-protein/lipid interactions were significant, indicating non-additivity of effects on a log scale (Table 4). Therefore, the eight measurements defined by combinations of lobe, grey or white matter, and

protein or lipid, were examined separately as response variables in one-way analysis of variance with subject type as the independent variable. Differences among subject types were highly significant in all eight cases (P ranged from 0.001 to 0.006). Pairwise comparisons were made using Fisher's protected *t* procedure with the significance set at 0.05 (26). All analyses were run on SAS, Version 6.11 (SAS Institute Inc., Cary, NC).

Table 4 *Multivariate analysis of variance*

Effect	DF	P
FT	1	NS
FT * GW	1	<0.0001
FT * PL	1	<0.0005
FT * CAPS	3	NS
FT* GW * PL	1	NS
FT * GW * CAPS	3	<0.025
FT * PL * CAPS	3	NS
FT * GW * PL* CAPS	3	NS
GW	1	<0.005
GW*PL	1	NS
GW * CAPS	1	NS
GW * PL * CAPS	3	<0.01
PL	1	<0.0001
PL * CAPS	3	<0.0001
CAPS	3	<0.0001

Legend:
DF – degrees of freedom
NS – not significant
FT – frontal and temporal cerebral lobe
GW – grey and white brain matter
PL – proteins and lipids
CAPS – controls, Alzheimer's disease, Parkinson's disease, and cigarette smokers

RESULTS

The amount of protein in the frontal and temporal brain lobe ranged from 75.7 to 366.6 mg/g grey matter tissue and 42.7–155.8 mg/g white matter tissue. The amount of protein in the grey matter was approximately twice as high in the white matter. The average ratio of white to grey matter protein for the 45 analysed brain samples was (mean±SD) 0.470±0.126 and the coefficient of variation was approximately 25%, a reasonable value considering the biological variability of the subjects and the limits of the analytical methodology involved.

Two different radio analytical techniques showed no difference between alpha (^{210}Po) and beta (^{210}Bi) activity. Consequently, the values of ^{210}Po and ^{210}Bi were averaged for further analysis (Table 3). The analysis of these averaged measurements

revealed some three-way interactions (Table 4). Such high-order statistical interactions make interpretation difficult and are usually ignored in the analysis of the effect. In spite of non-additivity of effects, the relationships between subject type, brain fraction, and grey/white matter appear very similar in the frontal (Fig. 1) and the temporal (Fig. 2) lobes (Table 5). One-way ANOVA revealed that the deposition of ^{210}Bi and ^{210}Po in the brain has a distinct, pathologically based distribution in the protein (Fig 3)

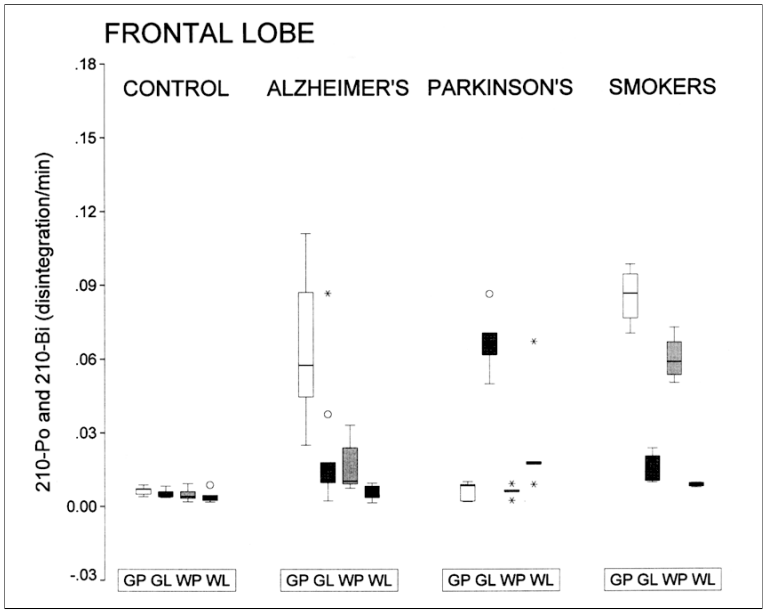

Figure 1 *Frontal lobe. ^{210}Po and ^{210}Bi in protein (P) and lipid (L) fractions from the cortical grey (G) and subcortical white (W) matter from the frontal (F) brain lobe in Alzheimer's disease, Parkinson's disease, smokers, and controls. The box-and-whisker plots. The horizontal line inside the box represents the median. The lower boundary of the box is the 25th percentile and the upper boundary is the 75th percentile. The vertical lines (whiskers) show the largest and the smallest observed values that aren't outliers. Cases with values that are more than 3 box lengths from the upper or lower edge of the box are extreme values (*). Cases with values that are between 1.5 and 3 box lengths from the upper or lower edge of the box are outliers (o) (SPSS for Windows, SPSS Inc., Chicago, IL 1993)*

and lipid (Fig. 4) fractions. The retention of the radionuclides was the lowest in the control brains. In contrast, radioactivity of both RDs in AD brains was about an order of magnitude, that is, ten times higher in the protein fraction of both grey and white matter than in the control brains. Some of the AD brains also manifested somewhat

Table 5 *Pairwise comparisons of concentrations of radioactive radon daughters in proteins (P) and lipids (L) in the cortical grey (G) subcortical white (W) brain matter from frontal (F) and temporal (T) brain lobe in Alzheimer's disease (AD), Parkinson's disease (PD), cigarette smokers (S) and controls (C)**

	FG	FW	TG	TW	FG	FW	TG	TW
C vs. AD	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	NS	NS	P<0.05
C vs. PD	NS	NS	NS	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
C vs. S	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
AD vs. PD	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
AD vs. S	NS	P<0.05	NS	P<0.05	NS	NS	NS	NS
PD vs. S	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

* Fisher's protected *t*-test

Legend: F – frontal lobe; T – temporal lobe; G – grey brain matter; W – white brain matter; NS – not significant

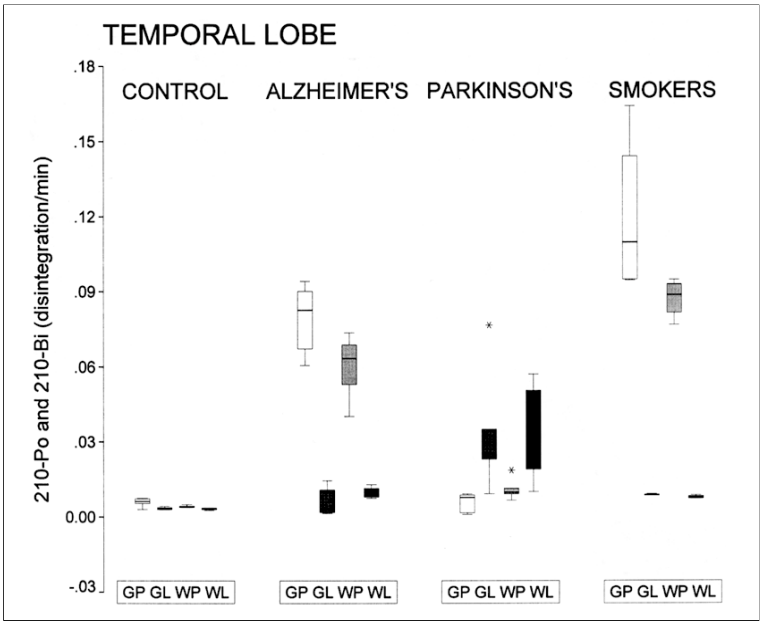

Figure 2 *Temporal lobe. ^{210}Po and ^{210}Bi in the protein (P) and lipid (L) fractions from the cortical grey (G) and subcortical white (W) matter from the temporal (T) brain lobe in Alzheimer's disease, Parkinson's disease, cigarette smokers, and controls (see Fig.1 legend for explanation)*

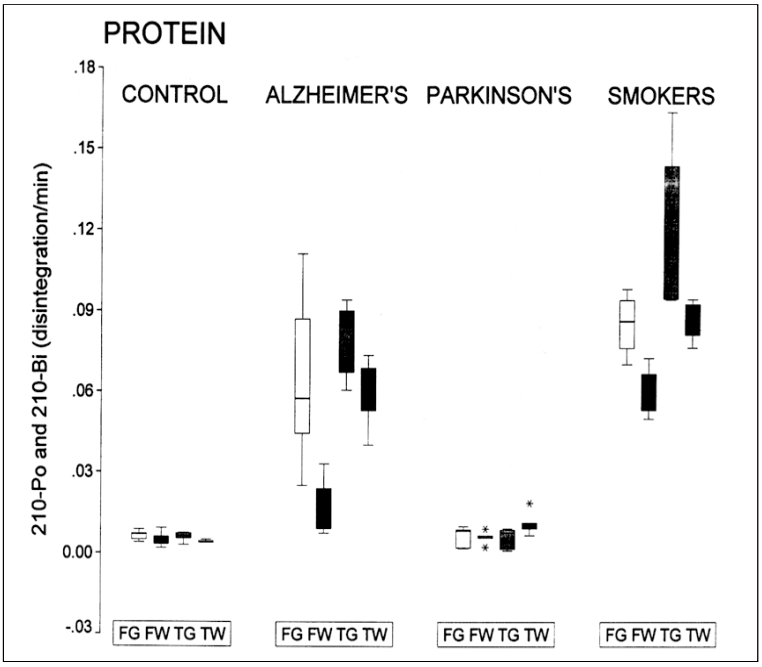

Figure 3 *Protein. ^{210}Po and ^{210}Bi in the protein fraction (P) from the cortical grey (G) and subcortical white (W) matter from the frontal (F) and temporal (T) brain lobe in Alzheimer's disease, Parkinson's disease, cigarette smokers, and controls (see Fig.1 legend for explanation)*

increased radioactivity in the lipid fraction, but the increases were much smaller and statistically significant only for the frontal grey and temporal white matter.

However, the radioactivity in the lipid fraction of the cortical grey and subcortical white matter of both the frontal and temporal lobes in PD were approximately an order of magnitude higher than that of controls, while increases in radioactivity in the protein fraction were much smaller and significant only for the white matter of the temporal lobe. As in AD, the radioactivity greatly increased in the protein fraction and somewhat in the lipid fraction of the smokers' brains. Unlike AD, however, the smokers did not show any increase of the activity in the lipid fraction of the brain grey matter (Table 5).

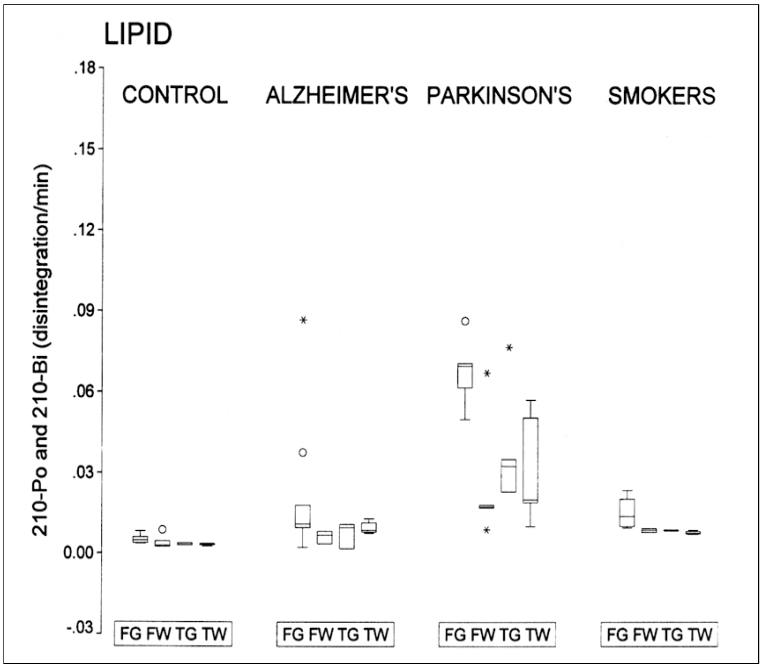

Figure 4 *Lipid. ^{210}Po and ^{210}Bi in the lipid fraction (L) from the cortical grey (G) and subcortical white (W) matter from the frontal (F) and temporal (T) brain lobe in Alzheimer's disease, Parkinson's disease, cigarette smokers, and controls (see Fig.1 legend for explanation)*

DISCUSSION

The results of our study showed that, indeed, there is residual RD radioactivity in the brain, that RDs accumulate ten times as much in the diseased AD and PD brains, and even more in those of smokers, as in the control brains, that this accumulation is not random or uniform, but shows selective, disease-dependent distribution to the protein fraction of the cortical grey and subcortical white brain matter in AD and smokers, and to the lipid fraction in PD, and that the same results were obtained by two different radioanalytical techniques, that is, one for alpha particles (^{210}Po) and the other for beta particles (^{210}Bi).

The reliability of the data and the strength of the statistical analysis are greatly enhanced because each sample was analysed in multiple replicates and above all, with two different radio-analytical methods involving the respective alpha and beta particles (27). Indeed, both analytical methods yielded the same selective pattern of RDs distribution in the proteins and lipids of cortical grey and subcortical white brain matter within the groups of subjects who suffered from AD, PD, smoking, and in the controls. It is important to note that the radioactivity in the lipids was approximately

the same for the cortical grey and subcortical white brain matter in spite of their presumably different fatty acid composition (28). The congruency of the radioanalytical results further meant that the samples were indeed in a »secular equilibrium«; yet another check of the accuracy of the radio-analytical work. These data explicate that, in fact, both AD and PD are systemic brain diseases which result from the impaired protein biochemical structure in the former and that of the lipids in the latter case. Perhaps they indicate the advanced stage of the disease in our patients (29) because, thus far, clinical AD was generally associated with changes in the cholinergic projections of the limbic system in the Papez circuit (30) and PD with degenerative changes and lack of dopamine from the substantia nigra (31). Thus, environmental radon provided a naturally occurring marker which enabled us to differentiate between the biochemical changes which underlie these two neuropathological conditions, that is, AD and PD. Our data provide some support to the idea of the underlying genetic origins of predisposition to the effect of the environmental toxicants in the aetiology of AD and PD, similar to that assumed to link smoking and increased incidence of lung cancer (32, 33).

Our further discussion will focus on an attempt to (a) elaborate more extensively the possible risk of low-level high linear energy of environmental radon and RD alpha and beta particle radiation to the brain cell population and (b) provide some logical frame which may explain the selective pattern of RD distribution in the lipid and protein fraction of the brain in PD and AD and smokers, respectively.

Our study demonstrated that radon has a capacity to infest the brain with poisonous progeny of radioactive heavy metals. Indeed, judging from data on environmental radon which reaches the brain in the course of an average, 70-year human life and from the number and size of cells in a 1.5 kg brain, there are enough high-energy alpha particles to hit and damage every single cell in the brain more than once over a lifetime (34). This adds to the tremendous potential for radiation-induced microinjury, point mutations, and deletions in gene expression (35, 36). The high-energy alpha and beta particles exhibit deleterious biological effects along their pathway in direct proportion to their initial energy (37, 38).

Along such alpha and beta particle »death path«, intensive ionisation will also generate a large number of free radicals (12), as will the heavy metals generated from the transmuted radon and its daughters. Free radicals will additionally harm the molecular biological structures via protein oxidation, lipid peroxidation, and DNA intercalation and impair the signal transduction, cell membrane function, and gene expression at the cellular level (39–43). Today, it is considered that radiation is a zero-threshold carcinogen which means that if no threshold exists, there is no dose – other than zero – at which the risk of cancer is nil (44). Hence radon and its daughters in the brain present a potential radiation hazard which may combine with other physical, chemical, and biological hazards to the brain (45). It should be noted that the health risks of therapeutic doses of irradiation of the human brain are far above those of the low-level exposure to the environmental radioactive RDs. Indeed, current limits of sensitivity to indicate overexposure from acute irradiation are about 20–30 mSv (46).

The most likely candidates for the radiation injury appear to be the brain astrocytes and other glia cells which are highly radiosensitive in contrast to the more radioresistant neurons which do not divide (47, 48). Apparently, astrocytes supply the neurons with proteins necessary for the formation of new dendritic connections (49)

which are vital for the formation of new memories (50). Indeed, the astrocyte services the neuron in numerous ways; it provides the neuron with many protein components (51), removes potassium ions from the extracellular space following neuron depolarisation (29), collaborates in presynaptic firing of the neuron (52), and helps metal ion excretion (53). The astrocyte is also peculiar in that it has many projections linking the capillary endothelium cell and the neuron (54) which show up only when the astrocyte is sessile in its location (55). When an astrocyte is injured, it becomes mobile, leaves its position, and disrupts the complex three-dimensional cytoarchitectonics of the brain (56). There is growing evidence of astrocyte involvement in AD; ApoE, the major apolipoprotein in the nervous system which is essential for the neuron function, is expressed in astrocytes and its »bad« E4 variant may interact abnormally with neuronal cytoskeletal proteins favouring microtubule degradation and the formation of neurofibrillary tangles which occur in the brains of people with Alzheimer's disease (57). Thus, the amyloid deposits and tangling observed in Alzheimer's disease may well reflect the response to injury of the astrocyte (58). This study hypothesised that there was sufficient radioactivity and free radicals accompanying the presence of radon and its daughters in the brain to act as an apogen and induce such cascade (59, 60).

Our finding of selective, disease-specific patterns of RD accumulation in AD proteins and PD lipids was quite unexpected. To paraphrase *Sir Roger Bannister* (51), we found ourselves in the position where to meaningfully interpret the data was indeed more difficult than to collect them. *Ramon y Cayal* and *Golgi* were the first to exploit the metal-binding affinity of brain cells to make them histologically visible (29). A century later, we found that nature did the same for us by marking with environmental RDs different biochemical compartments in the brain, that is, proteins of AD and lipids of PD. We found from other studies of radio-chemical affinity that both polonium and bismuth have practically exclusive affinity for the chlorine ions to form highly reactive oxichlorides over the pH range 1–10 (61, 62). We concluded that the increased concentration of chlorine ions in the proteins and lipids in AD and PD, respectively, would result in increased trapping of RDs. Indeed, chlorine is the major extracellular cation in the brain (63). The increased trapping of chlorine ions would most likely reflect the increase in chlorine intracellular concentration; via specific membrane chlorine channels or those where chlorine is passively associated with sodium transport (64).

But if there indeed is transmembrane chlorine leakage, how is that it occurs in the proteins of AD and in the lipids of PD? We inferred that, in accordance with the fluid mosaic model of the cell membrane structure (65), the pathognomonic distribution of the RDs to the lipids in PD and to the proteins in AD might reflect the disease-related changes in the brain cell membrane. The changes involve the protein-built pores, channels, and gates in AD and the lipid bilayer in PD, and are followed by increased ionic leakage, which points at the impaired membrane potential, discharge, and excitability. The nerve cells, then, do not necessarily need to be dead to allow for chlorine leakage through the membrane. In other words, the impairment may be partial and, perhaps, some of the cell membranes may self-repair and even recover temporarily. This may further explain the ill-understood mechanism of the fluctuation in the course of such systemic brain cell membrane diseases as AD and PD. It would also explain why so many different aetiologic factors, from common cold to alumin-

ium, can be associated with the onset of AD and PD. The brain requires about 20% of cardiac output (66) and produces about ten times its own weight of ATP per day. This huge amount of energy is mostly used for proper membrane function (67). It is not, then, surprising that the change of the dynamic properties of the cell membrane and transfer of the ions and neurotransmitter have already been suggested as the underlying principle of a wide range of neurological disorders (68–70). Indeed, the observed 10 times higher retention of radioactive heavy metal ^{203}Pb in the brain of a suckling, compared to that of adult rats (71), may be related to age-dependent immaturity of functional integrity of the neuron's membrane.

In contrast to the peripheral nervous system (52, 72), we were surprised to find how little is known about the supposedly cholinomimetic effects of nicotine, that is, smoking on central nervous system, except that nicotine can »excite motor cortex and stimulate our cognitive capabilities« (73). In our study, smokers had the highest activity of ^{210}Po and ^{210}Bi in the protein fraction of the brain grey and white matter. We think that this finding further supports our chlorine hypothesis, because nicotine is most likely to bind to specific protein receptors (74) and thus temporarily hyperpolarise the membrane *via* intracellular influx of chlorine ions. In other words, the effects of nicotine on the central nervous system may mimic those of the gamma-aminobutyric acid more than that of acetylcholine. Incidentally, smoking (nicotine) is contraindicated in the ulcerous gastric diseases, and nicotine receptors in the stomach are also related to the handling of the chlorine ions (75). We had only one AD patient who had also been a smoker (those radioactivity values were not included in the tables or calculations). However, his brain concentration of ^{210}Bi and ^{210}Pb was inordinately high, some 20–22 times the level found in controls. Many sub-structural subtleties in the brain protein composition which are beyond the resolving power of our method may be related to such a synergistic picture of RD accumulation. We did not study the interaction between nicotine and protein, but our results for smokers indicate that presumably easily volatile nicotine from cigarette smoke (76) may have a direct access to specific receptor proteins of the cortical grey and subcortical white brain matter.

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APPENDIX I. *^{210}Po (alpha) and ^{210}Bi(beta) specific activity in the brains from Alzheimer's disease, Parkinson's disease, cigarette smokers and controls (disintegration/min/g).*

Part A. *^{210}Po in the protein (P) and lipid (L) fraction from cortical grey (G) and subcortical white (W) brain matter from the frontal lobe (F). (a and b are replicates).*

Subject	FGPa	FGPb	FGLa	FGLb	FWPa	FWPb	FWLa	FWLb
CONTROL								
1	0.00710	0.00720	0.00493	0.00499	0.00630	0.00620	0.00420	0.00440
2	0.00470	0.00490	0.00410	0.00398	0.00372	0.00370	0.00279	0.00284
3	0.00910	0.00880	0.00814	0.00823	0.00920	0.00890	0.00880	0.00910
4	0.00480	0.00490	0.00423	0.00443	0.00443	0.00439	0.00297	0.00330
5								
6	0.00420	0.00400	0.00352	0.00354	0.00259	0.00610	0.00230	0.00240
7	0.00698	0.00690	0.00587	0.00592				
8								
ALZHEIMER'S DISEASE								
9	0.04430	0.03968	0.00193	0.00200	0.00680	0.00730	0.00300	0.00260
10	0.05780	0.06180	0.00960	0.01000	0.00890	0.00870	0.00360	0.00420
11	0.02680	0.02717	0.00960	0.01050	0.01790	0.01820	0.00770	0.00830
12	0.01100	0.00830	0.01870	0.01900	0.00940	0.01070	0.01100	0.00800
13	0.08690	0.09290	0.01090	0.01690	0.01200	0.00930	0.00710	0.00730
14	0.10830	0.10960	0.09230	0.08800	0.04100	0.03310	0.00800	0.01100
15	0.06880	0.06970	0.04410	0.03000	0.00890	0.00960		
16	0.04230	0.02990	0.00870	0.00940	0.00840	0.00790	0.00430	0.00440
17								
18	0.09600	0.09900	0.01200	0.00790	0.01960	0.02400	0.00790	0.00850
19								
PARKINSON'S DISEASE								
20	0.00880	0.00820	0.07100	0.06500	0.00560	0.00570	0.02100	0.01500
21	0.00850	0.00810	0.07300	0.05670	0.01040	0.00750	0.04870	0.05090
22	0.00850	0.00910	0.06900	0.07700	0.00630	0.00580	0.02400	0.01800
23			0.04400	0.04900			0.00940	0.00890
24	0.00190	0.00490	0.09500	0.08800	0.00870	0.00890	0.01770	0.01820
25			0.06700	0.06300	0.00580	0.00620	0.05800	0.06400
CIGARETTE SMOKERS								
26	0.07310	0.07150	0.00960	0.01450	0.04920	0.04680	0.00770	0.00820
27	0.08840	0.08940	0.01796	0.01800	0.05380	0.05430	0.00960	0.00990
28	0.07960	0.08240	0.01040	0.00810	0.07180	0.07230	0.00720	0.00710
29	0.10900	0.08750	0.01990	0.02810	0.05960	0.06030	0.00950	0.01090

APPENDIX I. *^{210}Po (alpha) and ^{210}Bi (beta) specific activity in the brains from Alzheimer's disease, Parkinson's disease, cigarette smokers and controls (disintegration/min/g).*

Part B. *^{210}Po in the protein (P) and lipid (L) fraction of cortical grey (G) and subcortical white (W) brain matter from the temporal lobe (T) (a and b controls are replicates).*

Subject	TGPa	TGPb	TGLa	TGLb	TWPa	TWPb	TWLa	TWLb
CONTROL								
1	0.00746	0.00748	0.00390	0.00390	0.00380	0.00366	0.00310	0.00289
2	0.00649	0.00651	0.00321	0.00320	0.00428	0.00430	0.00354	0.00357
3								
4	0.00589	0.00567	0.00310	0.00290	0.00398	0.00390	0.00327	0.00329
5	0.00732	0.00783	0.00449	0.00448	0.00513	0.00516	0.00369	0.00380
6	0.00510	0.00530	0.00297	0.00299	0.00390	0.00413	0.00330	0.00300
7								
8	0.00300	0.00320	0.00277	0.00279	0.00410	0.00401	0.00317	0.00331
ALZHEIMER'S DISEASE								
9								
10								
11	0.09180	0.09230	0.01480	0.01530	0.07340	0.06870	0.00960	0.01200
12								
13								
14								
15	0.07740	0.07680	0.00134	0.00111	0.05920	0.05560	0.00770	0.00860
16	0.06230	0.06180	0.00880	0.00860	0.04760	0.04920	0.00776	0.00780
17	0.08670	0.08740	0.00980	0.01190	0.06713	0.06550	0.00930	0.00880
18	0.09280	0.09330	0.00156	0.00150	0.07270	0.06870	0.01280	0.01320
19	0.06920	0.06930	0.00940	0.01400	0.05340	0.04850	0.00800	0.00670
PARKINSON'S DISEASE								
20	0.00860	0.00930	0.02970	0.03190	0.00940	0.01080	0.01780	0.01800
21	0.00890	0.00910	0.00660	0.00739	0.00890	0.00970		
22	0.00850	0.00810	0.07300	0.05670	0.01040	0.00750	0.04370	0.05290
23								
24	0.04100	0.02870	0.02300	0.01310	0.06300	0.05690		
25	0.00780	0.00830	0.02300	0.01480	0.00860	0.01090	0.01770	0.01820
CIGARETTE SMOKERS								
26	0.08700	0.10900	0.00870	0.00770	0.07600	0.08200	0.00770	0.00720
27	0.13400	0.12700	0.00850	0.00920	0.09100	0.08600	0.00830	0.00740
28	0.09300	0.08800	0.00780	0.00830	0.09150	0.08670	0.00683	0.00690
29	0.15400	0.16500	0.00920	0.00980	0.09680	0.09710	0.00930	0.00870

APPENDIX I. *^{210}Po (alpha) and ^{210}Bi (beta) specific activity in the brains from Alzheimer's disease, Parkinson's disease, cigarette smokers and controls (disintegration/min/g).*

Part C. *^{210}Bi in the protein (P) and lipid (L) fraction of cortical grey (G) and subcortical white (W) matter from the frontal lobe (F) and temporal lobe (T).*

Subject	FGP	FGL	FWP	FWL	TGP	TGL	TWP	TWL
CONTROL								
1	0.00690	0.00520	0.00580	0.00480	0.00720	0.00360	0.00410	0.00230
2	0.00950	0.00370	0.00330	0.00310	0.00660	0.00290	0.00450	0.00360
3	0.00860	0.00840	0.00950	0.00860				
4	0.00510	0.00480	0.00460	0.00260	0.00620	0.00340	0.00370	0.00310
5					0.00750	0.00420	0.00490	0.00350
6	0.00380	0.00370	0.00220	0.00250	0.00560	0.00280	0.00430	0.00290
7	0.00710	0.00610	0.00190	0.00180				
8					0.00290	0.00340	0.00380	0.00340
ALZHEIMER'S DISEASE								
9	0.04700	0.00250	0.00770	0.00310				
10	0.05520	0.01200	0.00930	0.00330	0.08500	0.00150	0.07600	0.01500
11	0.02300	0.00910	0.01900	0.00870	0.08800	0.01390	0.07600	0.01500
12	0.09700	0.01700	0.00990	0.00690				
13	0.08400	0.01800	0.00980	0.00640				
14	0.11300	0.08300	0.02900	0.00930				
15	0.07200	0.03800	0.04000	0.00140	0.08100	0.00160	0.06100	0.00890
16	0.03800	0.00840	0.00870	0.00390	0.05900	0.00920	0.03200	0.00810
17				0.08500	0.00950	0.07100	0.00840	
18	0.10900	0.00960	0.02600	0.00800	0.09500	0.00190	0.06400	0.00980
19					0.06500	0.00990	0.05500	0.00760
PARKINSON'S DISEASE								
20	0.00910	0.07300	0.00630	0.01700	0.00930	0.03400	0.01250	0.02000
21	0.00910	0.08800	0.00930	0.05100	0.00100	0.00940	0.00630	0.01080
22					0.00790	0.06600	0.00550	0.01500
23	0.00180	0.05300	0.00230	0.00850				
24	0.00800	0.08100	0.00930	0.01670	0.00160	0.03500	0.01900	0.05400
25	0.00210	0.05800	0.00710	0.07300	0.00730	0.02700	0.00970	0.02200
CIGARETTE SMOKERS								
26	0.06800	0.00920	0.05200	0.00850	0.09500	0.00930	0.07400	0.00810
27	0.09100	0.01600	0.05900	0.00920	0.11700	0.00810	0.09300	0.00690
28	0.08400	0.00990	0.07300	0.00780	0.09800	0.00870	0.08300	0.00730
29	0.09800	0.02300	0.06120	0.00810	0.16800	0.00870	0.09200	0.00820

Sažetak

RADONOVE KĆERI U OKOLIŠU KAO POKAZATELJI BIOKEMIJSKIH PROMJENA U MOZGU BOLESNIKA S ALZHEIMEROVOM I PARKINSONOVOM BOLESTI I U PUŠAČA

Odredili smo radioaktivnost radonovih kćeri, ^{210}Po (alfa čestice) i ^{210}Bi (beta-čestice) u lipidima i proteinima iz sive mase korteksa i bijele supkortikalne supstancije iz frontalnog i temporalnog režnja mozga osoba oboljelih od Alzheimerove ili Parkinsonove bolesti, pušača i nepušača bez kliničkih znakova neurološke bolesti. Ustanovili smo da je radioaktivnost ^{210}Pb i ^{210}Bi bila deset puta veća selektivno u proteinima sive i bijele moždane supstancije osoba oboljelih od Alzheimerove bolesti i u pušača. Za razliku od toga, radioaktivnost radonovih kćeri bila je selektivno deset puta veća u lipidima sive i bijele supstancije mozga osoba oboljelih od Parkinsonove bolesti. Alfa čestice visoke energije predstavile su se kao neizbježni čimbenik rizika iz prirodnog okoliša za čovjekov mozak koji zajedno s popratnim stvaranjem slobodnih radikala mogu dovesti do minimalne lokalne udružene radiokemijske ozljede moždanih stanica, najvjerojatnije astrocita. Rezultati pokazuju da patognomonična distribucija afiniteta radonovih kćeri za lipide u bolesnika s Parkinsonovom bolesti i za proteine u bolesnika s Alzheimerovom bolesti odražava povećanu prisutnost lokalno raspoloživih klornih iona za koje se radonove kćeri selektivno vežu. Mislimo da promjene u sastavu proteinskih pora, kanala i vrata ugrađenih u staničnu membranu u bolesnika s Alzheimerovom bolesti, kao i promjene propusnosti dvoslojnoga lipidnog sloja te iste membrane leže u biti tih dviju teških sistemskih bolesti čovjekova mozga.

Ključne riječi:
klorna hipoteza, oštećenje stanične membrane, proteini i lipidi mozga, pušenje, radiokemijska ozljeda, sistemska moždana bolest

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