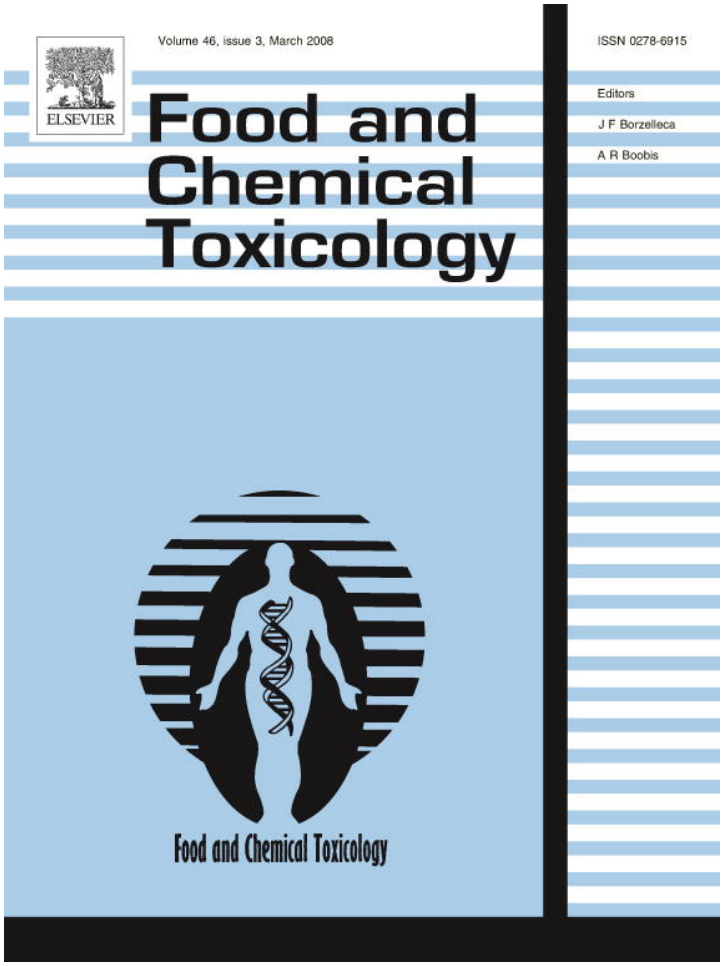

Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 46 (2008) 949–954
www.elsevier.com/locate/foodchemtox

The role of lecithin cholesterol acyltransferase and organic substances from coal in the etiology of Balkan endemic nephropathy: A new hypothesis

Nikola M. Pavlovic [a,*], William H. Orem [b], Calin A. Tatu [c], Harry E. Lerch [b], Joseph E. Bunnell [b], Gerald L. Feder [b], Emina N. Kostic [a], Valentin L. Ordodi [c]

[a] *Institute of Nephrology and Haemodialysis, Medical Faculty, University of Nis, Bul. Zoran Djindjic 48, Nis, Serbia*
[b] *US Geological Survey, 956 National Center, Reston, VA 20192, USA*
[c] *Department of Immunology, University of Medicine and Pharmacy Timisoara, Romania, Str. Oglinzilor 5 Ap.1, 300708 Timisoara, Romania*

Received 4 July 2007; accepted 22 October 2007

Abstract

Balkan endemic nephropathy (BEN) occurs in Serbia, Bulgaria, Romania, Bosnia and Herzegovina, and Croatia. BEN has been characterized as a chronic, slowly progressive renal disease of unknown etiology. In this study, we examined the influence of soluble organic compounds in drinking water leached from Pliocene lignite from BEN-endemic areas on plasma lecithin-cholesterol acyltransferase (LCAT) activity. We found that changes for all samples were the most prominent for the dilution category containing 90% plasma and 10% of diluting media. Water samples from BEN villages from Serbia and Romania showed higher LCAT inhibiting activity ($p = 0.02$) and ($p = 0.003$), respectively, compared to deionised water and non-endemic water. A secondary LCAT deficiency could result from this inhibitory effect of the organic compounds found in endemic water supplies and provide an ethiopathogenic basis for the development of BEN in the susceptible population.

Keywords: BEN; LCAT; Pliocene lignite; Organic compounds; Medical geology

1. Introduction

Balkan endemic nephropathy (BEN) occurs with a high rate of prevalence in Serbia, Bulgaria, Romania, Bosnia and Herzegovina, and Croatia (Stefanović and Cosyns, 2004). The first cases described in Serbia and Bulgaria date to the late 1950's (Danilović et al., 1957; Tanchev and Dorossiev, 1991). BEN is characterized as a chronic, slowly progressive familial tubular interstitial renal disease of unknown etiology (Ferluga et al., 1991).

The etiology of BEN has been the subject of numerous studies yielding the publication of quite a few hypotheses (Apostolov et al., 1975; Atanasova et al., 2005; Ivic, 1970; Krogh et al., 1977; Maksimovic, 1991; Toncheva et al., 1991, 1998). These hypotheses may be categorized into two main groups (Stefanovic et al., 2006) (Table 1).

Although data published on these various hypotheses presented some indication of their relevance to the etiology of BEN, none of them has provided conclusive evidence linking all facts related to BEN etiology and its clinical characteristics. Due to these inconsistencies and various features of the disease, most researchers favour the idea of a multifactorial etiology for BEN.

In this study, we propose a new idea on the possible relationship between the Pliocene lignite (Feder et al., 1991; Orem et al., 2004) and decreased LCAT activity hypotheses (Pavlovic et al., 1991) and their mutual or combined role in the etiology of BEN. The Pliocene lignite hypothesis is based on the assumption that naturally occurring toxic organic compounds in lignite, or in weathered lignite, may be leached by groundwater and thus contaminate

* Corresponding author. Tel.: +381 18 510 363; fax: +381 18 512 048.
E-mail address: nikpav@eunet.yu (N.M. Pavlovic).

0278-6915/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2007.10.033

Table 1
Classification of hypotheses on BEN etiology

Exogenous (environmental) factors	Endogenous factors
Lead intoxication	Genetic predisposition
Selenium deficiency	Genetic polymorphism
Chronic intoxication with *Aristolochia clematitis* (aristolochic acid)	Chromosomal aberrations
Ochratoxin A	Changes in enzyme activity – secondary LCAT deficiency
Chronic toxicity from drinking ground water containing organic compounds leached from Pliocene lignite	Viral disease

Exogenous (environmental) and endogenous factors are considered to be involved in the causation of the disease.

drinking water supplied by wells in BEN-endemic villages. Several classes of aromatic (including heterocyclic molecules with nitrogen and oxygen and phenolic structures) and aliphatic organic compounds have been found in larger numbers and higher concentrations in water from wells and springs in BEN-endemic villages, compared to water from control wells and springs in non-endemic villages, suggesting a possible role for these organic contaminants in the etiology of BEN. High molecular weight (HMW) organics (possibly similar to humic acids) may also play a role, as they are frequently encountered in high concentrations in the endemic village drinking water supplies. Many of these compounds are still unidentified, or only tentatively identified, but a likely candidate for a BEN-causing molecules could be among them. The absolute concentrations of the organics are typically low (individual compounds usually $<1\,\mu g/L$), which might account for the long time required for the kidney lesion to reach a critical threshold and the clinical phase of the disease to develop (Orem et al., 2004). The Pliocene lignite hypothesis emphasises the role of environmental factors involved in etiology of BEN, accounts for the distinctive geographic restriction of BEN, and provides a basis for investigating the possible wider impact of coal derived toxic organic compounds in groundwater on human health (Feder et al., 1991, 2002 Orem et al., 2004, 2002; Tatu et al., 2003).

Norum and Gjone (1967) described familial deficiency of lecithin-cholesterol acyltransferase (LCAT) (EC 2.3.1.43) in 1967. LCAT is a serum enzyme, which converts the free cholesterol of lipoprotein into esterified cholesterol. They showed that familial renal disease can develop secondary to LCAT deficiency and associated lipid abnormalities. LCAT deficiency is associated with percentage increase of free cholesterol and decrease of esterified cholesterol, and disturbances in lipoprotein particle structure. Lipid disorders affect, along with the kidneys, other organs, such as cornea and erythrocytes with clinical manifestations of proteinuria, usually associated with renal insufficiency, corneal opacities and haemolytic anaemia. The gene encoding LCAT is localized in region q21–22 on chromosome 16. Clinical manifestations of familial LCAT deficiency are highly variable, although it is characterized by no or only low LCAT activity. This may suggest that additional environmental factors and genes of minor importance modulate expression of the disease (Idzior-Walus et al., 2006; Borysiewicz et al., 1982). A study by Pavlovic in 1991 showed that a certain proportion of healthy subjects from BEN families have a peculiar form of lipid abnormalities associated with an abnormal LCAT activity, and a possible association between these abnormalities and the etiology of BEN was raised for the first time. It is well known that there are two types of LCAT deficiency i.e., primary and secondary ones. Familial type of LCAT deficiency described by Norum and Gjone in 1967 is the primary one with genetic background and there are a great number of papers published since then dealing with various patterns of lipid abnormalities, etiology of LCAT deficiency, modes of inheritance, etc. Secondary type of LCAT deficiency is encountered in chronic liver and renal diseases. What we actually propose here is a peculiar type of secondary LCAT deficiency, induced by environmental factors and leading to a peculiar type of chronic renal failure.

2. Materials and methods

2.1. Plasma sample preparation

In order to examine the possible bifactorial character of BEN etiology, we examined the influence of soluble organic compounds in drinking water supplies on plasma LCAT activity, using organic matter concentrates from drinking water collected from BEN villages and control sites. Control plasma samples were prepared from human blood. Peripheral blood samples were collected from six healthy subjects with no previous history of renal or liver disease, metabolic disorder, normal lipoprotein profile in plasma, and normal renal function. The blood samples were taken after 12 h of over night fasting in test tubes kept on ice and centrifuged after 15 min in a refrigerated (4 °C) centrifuge for 10 min at 3000 rpm. Obtained plasma samples were kept at −20 °C. Plasma samples were diluted in series with water concentrates in increasing step values of 10%. In this way, we provided nine categories of diluted media with the volume of plasma ranging from 90% to 10%.

2.2. Water sample preparation

Water samples were collected from endemic and non-endemic locations in Serbia and Romania. Two well water samples were collected from two endemic villages in Romania (Erghevita and Pietris villages, Drobeta Turnu Severin endemic area), and a well water sample and a tap water sample were collected from an endemic village (Petka) located in the Lazarevac BEN endemic area in Serbia. For each sample, 50 l of water were collected. One set of organic concentrates from a non-endemic area from Romania and another one from endemic villages from both Serbia and Romania were used in this study. Control samples were diluted in deionised water in the same manner as the test samples. Organic concentrates from drinking water samples were prepared using tangential flow ultrafiltration, with 50 liters of clear water yielding 100 ml of final volume of ultrafiltrate. While the endemic well water sample concentrates showed a yellowish or brown color, the non-endemic well water sample concentrate from Romania and the tap water sample concentrate from Serbia did not exhibit any significant color. Moreover, HPLC–MS analysis has revealed a similar qualitative and quantitative composition of the endemic well water concentrates in terms of abundances and numbers of major peaks, showing similar molecular ions (molecular weights 265, 293 and

321 Da, respectively) to those generated from a humic substances standard (Sigma–Aldrich, St. Louis, MO, USA).

2.3. Lecithin-cholesterol acyltransferase assays

LCAT activity kits (Roar Biomedical, New York, NY, USA) were used for the measurement of LCAT activity with fluorescence detection. Fluorescence was recorded using a Fluorolog-3 spectrofluorometer (HORIBA, Jobin Yvon, France) in a 1 ml quartz cuvette, at a 90° angle. Both excitation and emission slits were set to 2 nm. The excitation wavelength was 340 nm, and emission spectra were recorded between 360 and 500 nm. Spectra were online corrected for light source variations. All measurements were done in triplicate for each plasma sample and the presented data are the average values. Activity was assessed as a change in 470/390 nm emission intensity. Plasma LCAT was incubated with fluorescently labeled substrate. The intact substrate fluoresces at 470 nm. During hydrolysis by LCAT, the monomer emission at 390 nm increases. The emission intensities of 390 nm and 470 nm represent the emission of the substrate hydrolyzed (390 nm) and not hydrolyzed (470 nm). A ratio of the two emission intensities (470/390) will indicate an increase in concentration of 390 nm emitters and simultaneous decrease in concentration of 470 nm emitter in the presence of LCAT (Idzior-Walus et al., 2006).

Means and SDs were calculated for all dilution categories, and means were compared using the *T*-test in Microsoft Excel 2003 statistical tools "Analysis ToolPak."

3. Results

When we compared the average values of the LCAT inhibiting activity (390/470 nm ratio) of organic concentrates isolated from drinking water samples in various dilution categories, we found that changes for all samples were the most prominent for dilution category #1 (Table 2), containing 90% plasma and 10% of diluting media. *T*-test results for the initial measurements (category #1) with 10% of DW or water concentrates are presented in Table 3. Organic concentrates of well drinking water samples from BEN villages (BEN-S) from Serbia (Fig. 1), as well as from Romania (BEN-R) (Fig. 2) showed significantly higher LCAT inhibiting activity ($p = 0.02$) and ($p = 0.003$), respectively, compared to deionised water (DW). Tap water concentrates from Serbia (collected from an endemic area) (TW-S) (Fig. 3) showed significantly higher LCAT inhibiting activity compared to DW ($p = 0.009$), while organic concentrates of drinking water samples from non-endemic sites in Romania (Non-BEN-R) showed no significant difference compared to DW ($p = 0.098$) (Figs. 3 and 4).

Table 3
T-test values for the initial measurements (category #1) with 10% of DW or water concentrates

Samples	Specimens			
	TW-S**	BEN-S***	Non-BEN-R****	BEN-R*****
DW*	$p = 0.009$	$p = 0.002$	$p = 0.098$	$p = 0.003$
TW-S**	–	$p = 0.000$	$p = 0.134$	$p = 0.011$
BEN-S***	–	–	$p = 0.021$	$p = 0.143$
Non-BEN-R****	–	–	–	$p = 0.017$

DW*: Deionized water; TW-S**: Tap water Serbia; BEN-S***: BEN Serbia; Non-BEN-R****: Non-BEN Romania; BEN-R*****: BEN Romania.

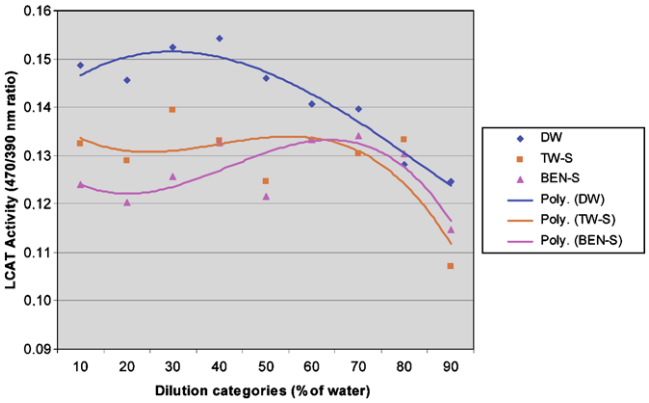

Fig. 1. LCAT activity (470/390 nm ratio) of DW, TW-S, and BEN-S samples for various dilution categories (% of water).

Category # 1 values for TW-S LCAT inhibiting activity showed significant differences compared to BEN-S ($p < 0.0001$) (Fig. 1), and BEN-R ($p = 0.011$) (Fig. 5), but no differences with Non-BEN-R (Table 3). Value of Non-BEN-R LCAT inhibiting activity showed significant differences, compared to BEN-S (Fig. 5) and BEN-R (Fig. 2), ($p = 0.021$) and ($p = 0.017$) respectively (Table 3). No significant differences between BEN-S and BEN-R values were found ($p = 0.143$) (Fig. 3, Table 3).

Dilution curves for TW-S and BEN-S samples (Fig. 1) showed a polynomial behaviour with an initial increase

Table 2
The average values and standard deviation of water concentrates' LCAT inhibiting activity (390/470 nm ratio) in various dilution categories

Category # (sample dilution percent)	Specimens				
	DW*	TW-S**	BEN-S***	N-BEN-R****	BEN-R*****
9 (10%)	0.149 ± 0.007	0.132 ± 0.001	0.124 ± 0.001	0.139 ± 0.009	0.120 ± 0.006
8 (20%)	0.146 ± 0.008	0.129 ± 0.005	0.120 ± 0.003	0.147 ± 0.007	0.132 ± 0.002
7 (30%)	0.152 ± 0.008	0.139 ± 0.009	0.126 ± 0.001	0.146 ± 0.005	0.132 ± 0.002
6 (40%)	0.154 ± 0.006	0.133 ± 0.010	0.133 ± 0.002	0.140 ± 0.001	0.129 ± 0.001
5 (50%)	0.146 ± 0.004	0.125 ± 0.005	0.122 ± 0.001	0.132 ± 0.003	0.129 ± 0.004
4 (60%)	0.141 ± 0.004	0.133 ± 0.006	0.133 ± 0.008	0.126 ± 0.005	0.130 ± 0.005
3 (70%)	0.140 ± 0.006	0.130 ± 0.010	0.134 ± 0.005	0.122 ± 0.004	0.116 ± 0.002
2 (80%)	0.128 ± 0.003	0.133 ± 0.002	0.130 ± 0.002	0.108 ± 0.003	0.111 ± 0.003
1 (90%)	0.125 ± 0.006	0.107 ± 0.013	0.115 ± 0.003	0.097 ± 0.003	0.101 ± 0.006

DW*: Deionized water; TW-S**: Tap water Serbia; BEN-S***: BEN Serbia; Non-BEN-R****: Non-BEN Romania; BEN-R*****: BEN Romania.

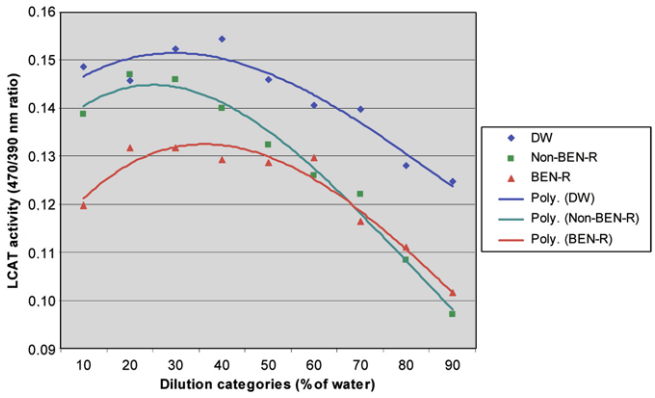

Fig. 2. LCAT activity (470/390 nm ratio) of DW, Non-BEN-R, and BEN-R samples for various dilution categories (% of water).

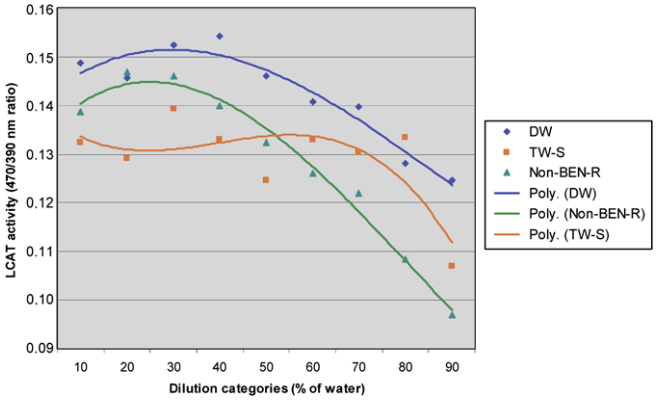

Fig. 3. LCAT activity (470/390 nm ratio) of DW, TW-S, and Non-BEN-R samples for various dilution categories (% of water).

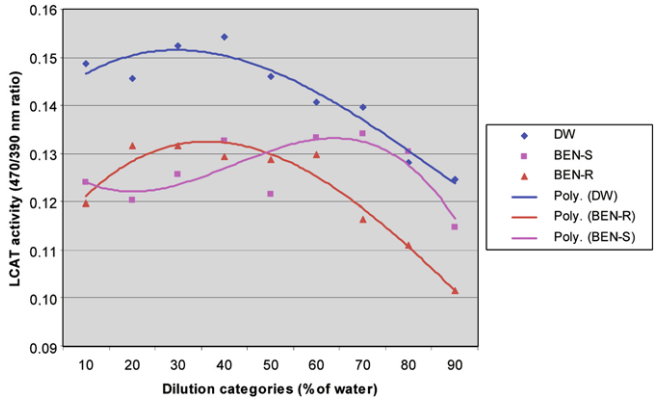

Fig. 4. LCAT activity (470/390 nm ratio) of DW, BEN-S, and BEN-R samples for various dilution categories (% of water).

of LCAT inhibiting activity followed by slight increase and then a drop to the final value, which was significantly lower compared to the initial one ($p = 0.015$ and $p = 0.004$) (Fig. 1). Dilution curves for DW, Non-BEN-R and BEN-R samples (Fig. 2) showed an initial decrease of LCAT inhibiting activity followed by steady increase reaching highly significant low final values ($p = 0.006$, $p = 0.001$, and $p = 0.012$) compared to the initial ones (Fig. 2).

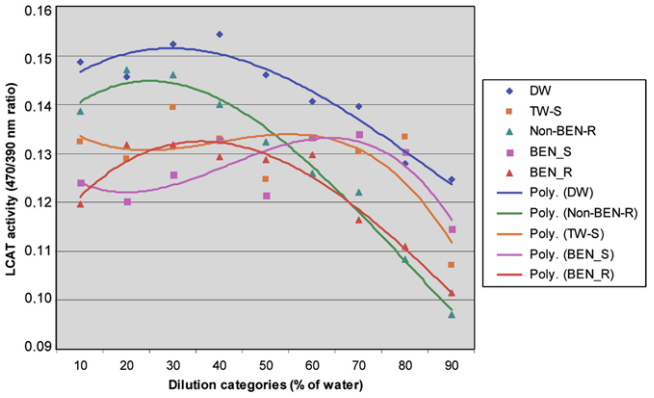

Fig. 5. LCAT activity (470/390 nm ratio) of DW, TW-S, Non-BEN-R, BEN-S, and BEN-R samples for various dilution categories (% of water).

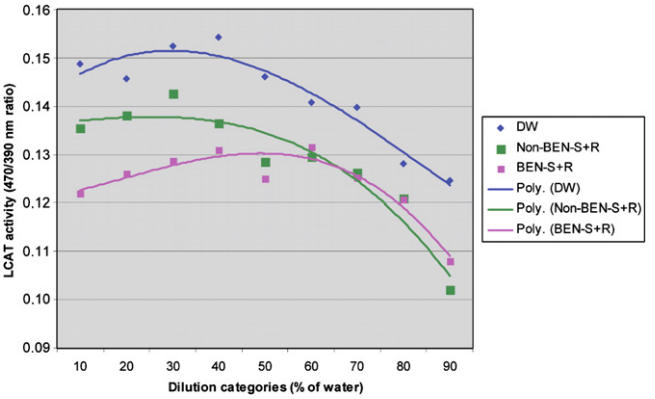

Fig. 6. Pooled LCAT activity (470/390 nm ratio) of DW, TW-S plus Non-BEN-R, and BEN-S plus BEN-R samples for various dilution categories (% of water).

In order to illustrate substantial differences between Non-BEN and BEN samples' LCAT inhibiting activities in various dilution categories were pooled into Serbian and Romanian Non-BEN samples, and samples from BEN areas (Fig. 6). Pooled Serbian and Romanian data from both Non-BEN and BEN villages (Fig. 6) showed significantly higher LCAT inhibiting activity values compared to DW ($p = 0.013$ and $p = 0.001$). Pooled data from BEN villages had significantly higher LCAT inhibiting activity compared to Non-BEN values ($p = 0.001$) (Fig. 6).

4. Discussion

The possible simultaneous role of coal-derived toxic organic compounds and decreased enzyme LCAT activity in the etiology of BEN was tested here for the first time. Results support the new multifactorial hypothesis of BEN etiology.

This new hypothesis may account for both the geographic distribution of BEN, and many of its medical features. Well water from BEN villages contains higher numbers and concentrations of both extractable and high molecular weight organic compounds compared to con-

trols (Orem et al., 2004). We presume that in this study these compounds contributed to higher LCAT inhibiting activity of Serbian and Romanian drinking water samples from both Non-BEN and BEN villages (Figs. 1 and 2). On the other hand, the higher abundance of organic compounds in drinking water samples from BEN villages caused much higher LCAT inhibiting activity compared to Non-BEN ones. This finding indicates that there is presumably a clear-cut distinction of drinking water qualities coming from those two localities, which is well documented in Fig. 6, presenting pooled Serbian and Romanian data. It is possible that the LCAT inhibitory effect to be due to the complex chemical mixtures represented by the groundwater supplies in the endemic areas, rather than to an individual compound. This would also explain, at least in part, the fact that Serbian tap water concentrates have a stronger inhibitory effect compared to distilled water. Hydrogeologically, the tap water and the well water from the endemic villages have a similar source (i.e., aquifers with more or less extensive contact with the Pliocene lignite layers), however, the former being treated and filtered through water plants before being distributed to the population. It is likely that the filtration process will remove a significant part of the organic contaminants, while the remaining ones could be responsible for the inhibitory effect. The tap water supply system was introduced in Serbian BEN villages in the 1960s when the untreated ground water supplies were suspected to carry the agent for BEN and although a slight decrease in the incidence disease has been noted since then, BEN still occurs in those villages.

On the basis of these results showing higher LCAT-inhibiting activity of organic compounds we propose here the concept of cause and effect events in the pathogenesis of BEN. We hypothesize that organic compounds in drinking water from BEN villages inhibit LCAT activity and may cause plasma lipid abnormalities and consequent changes of cellular membrane lipid composition that can trigger the pathogenic mechanisms responsible for the development of BEN. Proteins in a biological membrane are surrounded by a shell or annulus of 'solvent' lipid molecules (Lee, 2005). The structures of the solvent lipid molecules are important in determining the conformational state of a membrane protein, and hence its activity, through charge and hydrogen bonding interactions between the lipid head groups and residues in the protein, and through hydrophobic matching between the protein and the surrounding lipid bilayer (Mall et al., 1998, 2000). Membrane lipids play the major role in the complex area of cell death, comprising apoptosis and various forms of programmed cell death (Cristea and Degli Esposti, 2004). The possibility that changes in cell membrane lipids may promote or block membrane lipid alteration of mitochondria and other organelles, attracted increasing interest in the field of cell death (Cristea and Degli Esposti, 2004; Degli Esposti, 2003). Since it seems increasingly evident that mitochondria play an important role in the regulation of programmed cell death via release of pro-apoptotic agents and/or disruption of cellular energy metabolism (Olson and Kornbluth, 2001; Mignotte and Vayssiere, 1998), it may well be that, by changing mitochondrial function, a pathology caused by coal-derived organic compounds, thereby lipid abnormalities produce triggering pathogenic mechanism(s) responsible for the development of BEN.

The extent and characteristics of cellular membrane lipid alterations caused by LCAT inhibiting activity of organic compounds and their role in the etiology of BEN and associated urothelial cancer remains to be elucidated. The need to raise the question for defining healthy drinking water based on new criteria seems feasible. The question is whether we could use LCAT activity as a novel tool for assessing potential pathogenicity of any environmental factor(s), and whether the LCAT activity can be a part of "bio-effect assessment index" and therefore reflect general toxicity in an integrative manner (Broeg et al., 2005).

Our ongoing work is exploring the contribution of factors mentioned in this study to multifactorial etiology and occurrence of BEN as a global issue in order to determine the role of environment in human pathology. BEN and BEN-like diseases (chronic renal failure and renal/upper urinary tract cancer) linked to toxic organic compounds derived from coal in drinking water supplies may be widespread, offering a most interesting venue of research for the field of environmental medicine and medical geology in general.

Acknowledgements

The authors thank Vuk Maksimovic Ph.D. from the Center for Multidisciplinary Studies in Belgrade, and Tijana Cvetic biologist, from Faculty of Biology Belgrade for excellent assistance in performing laboratory measurements, and Dr. Florin Margineanu from the County Hospital Drobeta Turnu Severin, Romania, for the fieldwork assistance. Part of this work was supported by grants from the US Geological Survey (Reston, VA, USA), and NATO (CLG grant # 980104).

References

Apostolov, K., Spasic, P., Bojanic, N., 1975. Evidence of a viral etiology in endemic (Balkan) nephropathy. Lancet 2, 1271–1273.

Atanasova, S.Y., von Ahsen, N., Toncheva, D.I., Dimitrov, T.G., Oellerich, M., Armstrong, V.W., 2005. Genetic polymorphisms of cytochrome P_{450} among patients with Balkan endemic nephropathy. Clin. Biochem. 38, 223–228.

Borysiewicz, L.K., Soutar, A.K., Evans, D.J., Thompson, G.R., Rees, A.J., 1982. Renal Failure in familial lecithin: cholesterol acyltransferase deficiency. Q. J. Med. 51 (204), 411–426.

Broeg, K., Westernhagen, H.V., Zander, S., Körting, W., Koehler, A., 2005. The "bioeffect assessment index" (BAI) A concept for the quantification of effects of marine pollution by an integrated biomarker approach. Mar. Pollut. Bull. 50, 495–503.

Cristea, I.M., Degli Esposti, M., 2004. Membrane lipids and cell death: an overview. Chem. Phys. Lipid. 129 (2), 133–160.

Danilović, V., Djurišić, M., Mokranjac, M., Stojimirovic, B., Zivojinovic, J., Stojakovic, P., 1957. Nephrites chroniques provoquees par l'intox-

ication au plomb par voie digestive (farine). Presse. Med. 65, 2039–2040.

Degli Esposti, M., 2003. The mitochondrial battlefield and membrane lipids during cell death signalling. Ital. J. Biochem. 52 (1), 43–50.

Feder, G.L., Radovanovic, Z., Finkelman, R.B., 1991. Relationship between weathered coal deposits and the etiology of Balkan endemic nephropathy. Kidney Int. 40 (Suppl 34), S9–S11.

Feder, G.L., Tatu, C.A., Orem, W.H., Paunescu, V., Dumitrascu, V., Szilagyi, D.N., Finkelman, R.B., Margineanu, F., Schneider, F., 2002. Weathered coal deposits and Balkan endemic nephropathy. Facta Univ., Ser. Med. Biol. 9 (1), 34–38.

Ferluga, D., Hvala, A., Vizjak, A., Trnacević, S., Halilbasić, A., 1991. Renal function, protein excretion, and pathology of Balkan endemic nephropathy III. Light and electron microscopic studies. Kidney Int. 40 (Suppl 34), S57–S67.

Idzior-Walus, B., Sieradzki, J., Kostner, G., Małecki, M.T., Klupa, T., Wesołowska, T., Rostworowski, W., Hartwich, J., Waluś, M., Kieć, A.D., Naruszewicz, M., 2006. Familial lecithin-cholesterol acyltransferase deficiency: biochemical characteristics and molecular analysis of a new LCAT mutation in a Polish family. Atherosclerosis 185 (2), 413–420.

Ivic, M., 1970. The problem of etiology of endemic nephropathy. Acta Fac. Med. Naiss. 1, 29–37.

Krogh, P., Hald, B., Plestina, R., Ceovic, S., 1977. Balkan (endemic) nephropathy and foodborne ochratoxin A: preliminary results of survey of food stuffs. Acta Pathol. Microbiol. Scand. B. Microbiol. 85, 238–240.

Lee, A.G., 2005. How lipids and proteins interact in a membrane: a molecular approach. Mol. Biosyst. 1 (3), 203–212.

Maksimovic, Z.J., 1991. Selenium deficiency and Balkan endemic nephropathy. Kidney Int. 40 (Suppl 34), S12–S14.

Mall, S., Sharma, R.P., East, J.M., Lee, A.G., 1998. Lipid–protein interactions in the membrane: studies with model peptides. Faraday Discuss. 111, 127–136, Discussion 137–15.

Mall, S., Broadbridge, R., Sharma, R.P., Lee, A.G., East, J.M., 2000. Effects of aromatic residues at the ends of transmembrane alpha-helices on helix interactions with lipid bilayers. Biochemistry 39 (8), 2071–2078.

Mignotte, B., Vayssiere, J.L., 1998. Mitochondria and apoptosis. Eur. J. Biochem. 252 (1), 1–15.

Norum, K.R., Gjone, E., 1967. Familial plasma lecithin: cholesterol acyltransferase deficiency. Biochemical study of a new inborn error of metabolism. Scand. J. Clin. Lab. Invest. 20, 231–243.

Olson, M., Kornbluth, S., 2001. Mitochondria in apoptosis and human disease. Curr. Mol. Med. 1 (1), 91–122.

Orem, W.H., Tatu, C.A., Feder, G.L., Finkelman, R.B., Lerch, H.E., Maharaj, S.V.M., Szilagyi, D.N., Dumitrascu, V., Paunescu, V., Margineanu, F., 2002. Environment, geochemistry and the etiology of Balkan endemic nephropathy: lessons from Romania. Facta Univ., Ser. Med. Biol. 9 (1), 39–48.

Orem, W.H., Tatu, C.A., Lerch, H.E., Maharaj, S.V.M., Pavlovic, N.M., Paunescu, V., Dumitrascu, V., 2004. Identification and environmental significance of the organic compounds in water supplies associated with a Balkan endemic nephropathy region in Romania. J. Environ. Health Res. 3 (2), 53–61.

Pavlovic, N.M., Varghese, Z., Persaud, J.W., Stefanović, V., Strahinjić, S., Savić, V., Moorehead, J.F., 1991. Partial lecithin-cholesterol acyltransferase (LCAT) deficiency in Balkan endemic nephropathy. Kidney Int. 40 (Suppl 34), S102–S104.

Stefanović, V., Cosyns, J.P., 2004. Balkan Nephropathy. In: Davison, A.M., Cameron, J.S., Grunfeld, J.P., Ponticelli, C., Van Ypersele, C., Ritz, E., Winearls, C.G. (Eds.), Oxford Textbook of Clinical Nephrology. Oxford University Press, Oxford, pp. 1095–1102.

Stefanovic, V., Toncheva, D., Atanasova, S., Polenakovic, M., 2006. Etiology of Balkan endemic nephropathy and associated urothelial cancer. Am. J. Nephrol. 26, 1–11.

Tanchev, Y., Dorossiev, D., 1991. The first clinical description of Balkan endemic nephropathy (1956) and its validity 35 years later. IARC Sci. Publ. 115, 21–28.

Tatu, C.A., Orem, W.H., Maharaj, S.V.M., Paunescu, V., Dumitrascu, V., 2003. Organic compounds derived from Pliocene lignite and the etiology of Balkan endemic nephropathy. In: Skinner, H.C.-S., Berger, A.R. (Eds.), Geology and Health: Closing the Gap. Oxford University Press, New York, pp. 159–162.

Toncheva, D.I., Gerov, T.D., Tzoneva, M.T., Bouchakliev, Z.P., 1991. Spontaneous and induced chromosome aberrations in Balkan endemic nephropathy. Kidney Int. 40 (Suppl 34), S97–S101.

Toncheva, D., Dimitrov, T., Stojanova, S., 1998. Etiology of Balkan endemic nephropathy: a multifactorial disease? Eur. J. Epidemiol. 14, 389–394.