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Metal-Ligand Homeostasis in Epidermic Cells of Chernobyl Accident Liquidators

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Abstract

The present article is an attempt to reveal the connection (Pearson) between potassium (K) and sodium (Na), K and zinc (Zn) levels on the basis of analytical determination of elemental content in human scalp hair (atomic emission spectrometry in 954 Chernobyl accident liquidators and 947 healthy persons). The negative K-Zn correlation and also the increase in epidermic cells K and Na and reduction of calcium (Ca) and Zn can indirectly point, in the authors opinion, to the participation of membrane ATPas (P-type) in the origin of metal-ligand homeostasis shifts and serve as oxidative and nitrosative stress discriminators.

Introduction

Analysis of dynamic characteristics of metal-ligand homeostasis (MLH) in different biosubstrates is an intriguing, virtually not studied question of modern elementology. Such analysis, of course, cannot be confined (though this information itself is undoubtedly valuable) to only quantitative estimation of the content of one or another metal in a biosubstrate. Data on the metal-ligand complexes themselves, first on metalloproteins, which are the main participants of intra- and extracellular MLH events, are not less actual.

It is known that existence of metals in the organism in "free" form (as ions) is virtually "forbidden" even for such essential metals as copper (Cu), zinc (Zn), magnesium (Mg) etc., to say nothing of cadmium (Cd) and mercury (Hg), which are yet considered in literature as "toxic" [1]. As it was shown in recent experiments on a model of yeast cells, copper concentration in their plasma was less than 10^{-18} M, i.e. less than one copper atom per cell [2].

At the same time, there are apparently no difference in principle between toxic effect of Zn^{2+} or Cu^{2+} on a cell and that of Cd^{2+} or Hg^{2+}. Most probably, the matter is some faults in binding metals by specialized protein molecules (metallothioneins, metallochaperones) and/or ineffective removal of these ions from cells by assistance of membrane ATPases.

The major role in binding (detoxification) of metals in the organism belongs to metallothioneins (MT), low-molecular proteins (6-7 kDa), where 20 of 60-68 amino-acid residues are cysteine. Since 1957, when a Cd, Zn-containing MT was found first [3], new data on MT participation in MLH appeared.

It was found that MT besides detoxification of heavy metals and/or metals with variable valence (Fe, Cu) protect cell structures from damaging effect of oxygen/nitrogen radicals [4,5], influence activity of nuclear transcription factor NF-kB, participate in regeneration of liver and nervous cells [6,7], cause corrigent influence on humoral and cell immunity [8,9].

In cells, MT synthesis is induced (besides metals themselves and oxygen/nitrogen radicals) by glucocorticoids, anti-inflammatory cytokines (TNFα, IL-1), α-interferon [10-13].

There are four MT classes. Two of them, MT-1 and MT-2, are expressed in almost all mammalian tissues. They play a key role in homeostasis of Zn^{2+}, Cu^{2+}, Cd^{2+} and Hg^{2+}. The other two, MT-3 and MT-4, are tissue-specific for neural (CNS) and epithelial tissue, respectively [14]. Zn^{2+} ions incorporate MT molecule relatively easy and are claimed to be equally easy displaced by excess Cd^{2+} under certain conditions.

The metal/thiols ratio in MT is not random. There is about 1 Cu atom per 2 cysteine residues in Cu-containing MT (Cu-MT), and 1 atom of Cd or Zn per 3 cysteine residues in Cd/Zn-containing MT (Cd/Zn-MT). In Cu-MT, each copper atom is trigonally surrounded by sulphur (S) atoms, while the corresponding complex in Cd/Zn-MT has tetrahedral structure.

Structural regularity of the metalloprotein (MP) molecules allows existence of linear bounds between metals included in MP. These bonds can serve like "markers" of MP, and can be detected by correlation analysis (Pearson) of metal concentrations in a certain biosubstrate. We guess that such approach could be efficient for studying dynamic changes of MLH in normal and various pathological states. Using this method, one could trace MLH changes in such a biosubstrate as epidermis under conditions of oxidative and nitrosative stress, e.g. in Chernobyl disaster liquidators. Moreover, the subject for direct determination of metal concentrations could be not the epidermal cells themselves, but their derivatives (hair). Though, in this case, we should assume that metal concentrations in cells correspond to those in hair. This assumption looks reasonable and can be verified in further observations. Advantages of hair use for non-invasive and retrospective observation on shifts in MLH of epidermal cells are obvious and would be especially appropriate for mass (population) investigations.

The deepness of retrospection in such observations can be easily estimated on the basis of average speed of human hair growth ca. 0.2 mm/day: a sample of hair ca. 3 cm long reflects events, happened in epidermis during five recent months.

At the same time, the choice of biosubstrate for elemental analysis (hair) requires carefulness when interpreting the investigation results.

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It is known that epithelium (with its derivatives), which takes part in assimilation of chemical elements and their removal from the organism, is an area, where regulatory mechanisms of MLH cannot help manifesting themselves. Therefore an increase in relative amount of metals (or other chemical elements) in hair, if not being due to external causes, can be explained by:

1) normal working of MLH regulatory system (e.g. accelerated excretion of a metal in response of its excess intake from food);
2) a fault of regulatory mechanisms promoting metal retention in the organism, that leads to development of metal-deficient states.

In its turn, at insufficient intake of one or another metal from outside (or from chelate complexes inside the organism), proper work of the homeostasis regulatory system is directed to retention of the metal inside (minimization of losses), that can be manifested in a decrease of relative amount of the metal in such biosubstrate as hair.

Among oscillations in concentration of chemical elements (in a rather wide range) registered in spectrometric analysis of hair, how to distinguish those inherent in normal homeostatic regulation from pathological shifts, signs of elemental imbalance? Where are distinctive criterions of these shifts?

These questions are yet to be answered. However already today one should admit that it is hardly reasonable to extrapolate data of hair analysis upon whole organism, i.e. to diagnose "total" elemental imbalance on the basis of just quantitative determination of hair mineral content ("more - less").

Such extrapolation is probably applicable only in case of distinct (general) deficiency or excess of certain elements, when rough disturbances of mineral content can be found in most tissues.

That is why a significant advance (in theoretical and especially practical sense) would be the very possibility to use results of hair spectrometry for retrospective estimation of MLH events in epidermal cells. In other words, in case of quite predictable identity of mineral content of epidermis and its derivatives (hair), the latter could be a reliable and affordable source of information about mineral status of epidermal cells.

In this work we made an attempt to reveal pair associations between concentration of sodium (Na) and potassium (K), as well as between K and Zn as an indirect confirmation of participation of membrane ATPases (P-type) in MLH changes, observed on the background of oxidative and nitrosative stress.

For this purpose we used data on atomic-emission spectrometry of hair from Chernobyl disaster liquidators and healthy persons.

Materials and Methods

Hair mineral content of 947 healthy persons, 2-86 years old (238 men, 709 women) and 954 Chernobyl accident liquidators, 37-82 years old, living in Moscow, Russia (741 men, 213 women), was analysed by atomic emission spectrometry with inductively coupled plasma (ICP-AES) on an Optima 2000 DV (Perkin Elmer Inc., USA) instrument in ANO "Centre for Biotic Medicine" (Moscow, Russia).

In addition, in order to reveal possible gender and age dependent differences, hair mineral content of 402 healthy residents of Riga, Latvia, 2-86 years old (322 women, 80 men) was also investigated by ICP-AES. All the persons were divided in 3 groups by age: Group 1 – 2-32 years old (n=154), Group 2 – 33-44 years old (n=127), Group 3 – 45-86 years old (n=121). Comparative analysis of the spectrometry data was made for different genders and for two opposite age groups: Group 1 and Group 3.

The hypothesis of normal data distribution was tested using Jarque-Bera [15] and Kolmogorov-Smirnov [16] tests. In statistical calculations of the spectrometry data we did not use ordinary statistical methods (t-test), because the normality testing disproved the hypothesis of normal data distribution with high probability for all chemical elements except zinc (Zn) [17]. Therefore, alternative approaches (bootstrap method) were applied, which do not require normal distribution of *a priori* data [18]. Correlation analysis was made using standard computer application packages Microsoft Excel and Matlab.

Results

Comparative analysis of gender and age dependent differences in mineral hair content of healthy persons has given the results presented in (Tables 1,2).

Interval estimation of average hair content of such elements as calcium (Ca), magnesium (Mg), manganese (Mn), phosphorus (P), tin (Sn) was considerably different in healthy men comparing to women. Mean values (M, μg/g) and limits of the confidential intervals (bootstrapping) for men are: Ca=749.1 [521.1-1125.4]; Mg=73.4 [59.9-89.6]; Mn=0.73[0.55-0.97]; P=154.3 [146.3-161.8]; Sn=0.18[0.14-0.21]; V=0.12[0.09-0.15]; for women: Ca=1537.5[1410.9-1666.4]; Mg=188.9 [169.6-209.1]; Mn=1.61 [1.33-1.96]; P=139.2 [134.7-143.9]; Sn=0.85[0.52-1.34]; V=0.07 [0.06-0.08]. Age dependent differences between two marginal age groups (Group 1 and Group 3) were found only for Mg: in Group 1 Mg=141.9 [120.9-166.1] μg/g; in Group 3 Mg =203.4 [168.9-243.4] μg/g.

The results indicate necessity of being careful, as mentioned above, when generalizing estimation of mineral status by hair spectrometry on the whole organism. For instance, the fact of higher Ca content in hair of women comparing to men do not testify good state of Ca homeostasis in women's bone tissue, where 99% of this mineral is situated.

Comparative analysis of mean concentrations of 23 chemical elements in hair of healthy persons and Chernobyl disaster liquidators was made using interval estimation of the mean [18]. The results are presented in (Table 3).

Sex	Ca (μg/g) [M]	Mg (μg/g) [M]	Mn (μg/g) [M]	P (μg/g) [M]	Sn (μg/g) [M]	V (μg/g) [M]
Men (n=80)	521,1<**749,1**<1125,4	59,9<**73,4**<89,6	0,55<**0,73**<0,97	146,3<**154,3**<161,8	0,14<**0,18**<0,21	0,09<**0,12**<0,15
Women (n=322)	1410,9<**1537,5**<1666,4	169,6<**188,9**<209,1	1,33<**1,61**<1,96	134,7<**139,2**<143,9	0,52<**0,85**<1,34	0,06<**0,07**<0,08

Note: hereinafter in the tables the bold font designates values of average (M), usual - borders of confidential intervals (bootstrap-method).

Table 1: Sex dependent differences in hair mineral content of healthy persons.

Age groups	Mg [M] мкг/г
Group 1 (n=154) men – 32 (20,8%); women – 122 (79,2%)	120,9<**141,9**<166,1
Group 3 (n=121) men – 26 (21,5%); women – 95 (78,5%)	168,9<**203,4**<243,4

Table 2: Age dependent differences in hair mineral content of healthy persons.

Healthy persons (n=947) M (μg/g)	**Chernobyl disaster liquidators (n=954)** M (μg/g)
Al=8.1<**8.77**<9.5	*__Al__=19.3<**20.1**<20.9 ↑
As=0.07<**0.09**<0.13	*__As__=0.38<**0.40**<0.43 ↑
Be=0.007<**0.008**<0.01	**Be**=0.008<**0.01**<0.02
Ca=1176.8<**1249**<1318.9	*__Ca__=623.4<**654.8**<685.2 ↓
Cd=0.04<**0.05**<0.06	*__Cd__=0.23<**0.25**<0.29 ↑
Co=0.04<**0.05**<0.06	*__Co__=0.14<**0.15**<0.16 ↑
Cr=0.48<**0.51**<0.54	*__Cr__=0.85<**0.9**<0.92 ↑
Cu=19.06<**20.7**<22.3	*__Cu__=10.6<**10.99**<11.4 ↓
Fe=19.3<**21.07**<23.1	**Fe**=22.4<**23.7**<25.07 ↑
K=277.4<**317.7**<361.1	*__K__=365.8<**394.8**<422.4 ↑
Li=0.03<**0.04**<0.05	*__Li__=0.053<**0.06**<0.062 ↑
Mg=125.5<**134.3**<143.2	*__Mg__=43.9<**46.8**<49.9 ↓
Mn=1.02<**1.17**<1.3	*__Mn__=0.74<**0.8**<0.86 ↓
Na=427.9<**480.9**<542.9	*__Na__=757.5<**822.3**<892.4 ↑
Ni=0.53<**0.62**<0.73	*__Ni__=0.41<**0.46**<0.51 ↓
P=144.3<**146.8**<149.5	*__P__=150.8<**153**<155.4 ↑
Pb=1.04<**1.1**<1.27	*__Pb__=1.5<**1.8**<2.2 ↑
Se=0.62<**0.91**<1.3	*__Se__=1.46<**1.55**<1.65 ↑
Si=44.7<**48.5**<52.7	*__Si__=18.4<**19.9**<21.6 ↓
Sn=0.39<**0.51**<0.7	*__Sn__=0.91<**0.96**<1.0 ↑
V=0.06<**0.072**<0.077	*__V__=0.10<**0.11**<0.12 ↑
Ti=0.84<**1.17**<1.66	*__Ti__=0.49<**0.54**<0.59 ↓
Zn=181.5<**185.2**<189.3	*__Zn__=162.5<**165.8**<169.0 ↓

Note: *– significant difference

Table 3: Interval estimation of mean content of chemical elements in hair of Chernobyl disaster liquidators and healthy persons.

As follows from (Table 3), content of virtually all investigated elements in hair of Chernobyl disaster liquidators significantly differs from control. It is notable that content of essential metals (Cu, Zn, Mg etc.) in hair of liquidators is lower than in control group, but content of so-called "toxic" metals (Cd, Pb, Sn) is higher. What is behind these shifts? Could one consider them as distinct faults of metal-ligand homeostasis, or they are just a peculiar compensatory response of the organism under conditions of oxidative/nitrosative stress, aimed at saving vital elements inside and removing less important or potentially toxic ones?

To try answering these questions, we have postulated the following issues, based on published data and our own observations.

1. The main distinctive feature of biochemical processes in Chernobyl liquidators is high activity of oxygen/nitrogen radicals, or chronic oxidative/nitrosative stress, which directly concerns events of metal-ligand homeostasis observed inside and/or outside cells.
2. Key role in metal-ligand homeostasis belongs to metal-proteins (MT, chaperons, metal-enzymes).
3. If assume that ratio of metals (and, consequently, their protein ligands too) in hair is equal to that in epidermal cells (and this assumption can be confirmed by further investigations), therefore a real possibility appears to judge about events of metal-ligand homeostasis in epidermis on the basis of changes in mineral content of hair.
4. Linear correlations in pairs "metal-metal" or "metal-protein" can serve as additional characteristics of their intracellular interactions and/or as an indirect evidence of structural bonds between metals (e.g. in MT molecule).

Some technical peculiarities of detection of the linear associations between metals in hair spectrometry results should be explained [19].

It was established that a reduction of the subgroup size (to n = 100) caused a multiple increase in the number of detected linear associations as compared with the whole group (n = 1000). It points to the fact that most of these correlations are casual and dependent on the subgroup size. This circumstance determines the following rules of the correlation analysis procedure.

To reveal the maximum number of significant correlations, entire assemblies of spectrometry data (in our observations n=947 and n=954) was subjected to repeated "shuffle" ("shaking"). The "shuffle" was made in accordance with specially designed formats (standards). For this purpose, in the entire assembly we found the ratio of concentrations in each possible pair of chemical elements for each observation. It was essential that the sum of numerators was more than the sum of denominators in each set of ratios for a given pair.

The set of such pairs, as determined by number of possible paired combinations of *m* (where *m* is the number of analysed minerals), is a set of formats for the 'shuffle' of the entire assembly. If m = 23 (as in this case), the number of formats is 253.

In the present study we used the following 20 formats only: Mg/Pb, Al/As, Cu/As, As/Cd, K/As, Zn/Li, Ca/Al, K/Mg, Fe/Cu, Fe/Cd, Si/Cr, Cr/Co, Si/Ni, Si/Se, Na/Mn, P/Ni, Si/Co, P/Si, Na/Se, Ni/Cr.

In each obtained format, the 'ranking' (or 'formatting') of individual data was performed according to value of elements ratio in the given pair in descending order (from high to low). The optimum size of sample for studying correlations is approx. 100 persons. In the same order (from high to low) groups for the correlation analysis were designed. For example, if the entire assembly is ca.1000 persons, than Group 1 includes #1-#100, Group 2 - #101-#200, Group 3 - #201-#300, etc.

The correlations between chemical elements, on which the shuffle was formatted (and only within the given particular format), were not allowed for, because, by experience, this method of formatting leads to artificial overstating of Pearson correlation coefficient (*r*).

After calculating *r* in all groups and both entire assemblies (healthy persons and disaster liquidators), a new combined assembly was created involving only groups with *r*>0.2. Within this new assembly, formed for a given pair, the spectrometry results were selected further depending on *r* values.

For this purpose, for each case a so-called 'occurrence coefficient' (OC) was found, which was equal to the number of repetitions of a given observation in the entire assembly. At no repetitions OC=0, one repetition corresponds to OC=1, two repetitions - OC=2 etc. For the investigated relations, the near-to-zero *r* values were found among those cases, where repetitions were absent (OC=0) or rarest. At the same time, the maximum number of repetitions was accompanied by maximum *r* values. Such an approach allowed relatively easy separation of persons with absence or presence of a sought-for linear association

between concentrations of chemical elements among the whole totality of cases.

Alongside with the measurements of pair correlations between metals, we were interested in the associations between the concentrations of these metals in hair and the *r* values. Of special interest were possible deviations of the results obtained in the Chernobyl disaster liquidators from the norm. Such deviations could claim to be the distinctive markers of the chronic oxidative/nitrosative stress provided that the destructive action of oxygen/nitrogen reactive species extends to metal-ligand homeostasis of epidermal cells.

When performing the correlation analysis, we were first interested in associations between concentrations of Zn, K and Na. It should be noted that Zn is a part of MT molecule while a linear correlation between K and Na concentrations in hair, according to our preliminary data [19], is very constant, characterized by relatively high r values (0.6-0.7), and does not depend on the sample size. At the same time, the K-Na association depends on the biosubstrate type: it is present in hair and absent in plasma. It suggests that this correlation reflects, directly or indirectly, the fine-tuned operation of membrane Na,K-ATPases, which are constantly present in the cell and ensure the transmembrane transport of metals.

It was interesting to find out whether there were any correlations between K and Zn and what kind of correlations could be observed in the Chernobyl disaster liquidators and healthy persons.

The obtained results have not just confirmed the existence of the K-Zn correlation but also revealed the differences in its manifestation in the compared groups. Thus, the negative linear correlation between K and Zn (r = -0.43; p<0.05) was only manifest in 18.1% of practically healthy persons; in 36.3% it was negligible (r = -0.23; p<0.05), and in 45.6% it was altogether absent (r = 0.05).

Whereas the K-Zn correlation was negative and significant (r - from -0.41 to -0.62; p<0.05) in the absolute majority of the Chernobyl disaster liquidators (88%), and in 12% of the liquidators it was not revealed at all (r = - 0.03). Besides, the K-Zn correlation at the highest $|r|$ (in both the liquidators and the healthy subjects) was accompanied by a significantly higher level of K and Na and lower level of Zn than in subjects showing no K-Zn correlation (See Table 4).

It is indicative that the |r| size at K-Zn correlation varies with the increase of OC (Table 5,6), just like the average concentration values of not only K, Na and Zn but some other metals as well.

The negative correlation between K and Zn means that a decrease of intracellular zinc concentration must result in a proportional increase of intracellular potassium concentration. This fact should be discussed at greater length. Here, however, we can only confine ourselves to assuming that the most probable cause of such relations between K and Zn is an increase in production of nitric oxide (NO), which selectively releases Zn from MT molecules. Besides, NO (or its derivatives and

Groups	Correlation (r) and incidence of K-Zn association at the given r	Potassium (K) µg/g [M]	Sodium (Na) µg/g [M]	Zinc (Zn) µg/g [M]
Healthy subjects	**r = - 0,05** [45,6%] (n=432)	92,2<**125,3**<165,8	183,8 <**209** <243,2	194,3<**200,1**<205,7
	r = - 0,43 [18,1%] (p<0,05) (n=171)	729,0<**894**<1084,5	996,2<**1233,9**<1474,1	143,5<**150,7**<158,1
Chernobyl disaster liquidators	**r = - 0,03** [12,0%] (n=115)	102,5<**150,5**<208,3	199,4 <**261,8**< 328,0	172,3<**183,5**<194,5
	r = - 0,62 [21,5%] (p<0,05) (n=205)	502,9<**578,1**<660,5	982,7<**1131,3** <1286,4	153,5<**159,3**<165,4

Note: The Table gives the extreme (max and min) r values only

Table 4: K-Zn correlation and K, Zn content in hair of Chernobyl disaster liquidators and healthy persons.

Number	OC	n	r	Cu µg/g [M]	K µg/g [M]	Na µg/g [M]	Zn µg/g [M]	Ca µg/g [M]	Ca µg/g [M]	Mg µg/g [M]	Mg µg/g [M]
1-253	**4-10**	**253**	**-0,01**	**21,0**	**127,0**	**197,8**	**204,3**	**915,3**	**1737,9**	**93,9**	**171,0**
254-432	**11**	**179**	**-0,17**	**23,1**	**122,9**	**224,8**	**194,2**	**938,5**	**1463,8**	**92,3**	**151,6**
433-619	**12**	**187**	**-0,20**	**21,5**	**210,8**	**356,8**	**189,4**	**719,5**	**1287,0**	**76,3**	**141,1**
620-776	**13**	**157**	**-0,24**	**21,5**	**346,8**	**557,0**	**176,8**	**900,2**	**1234,8**	**107,0**	**145,1**
777-872	**14**	**96**	**-0,31**	**16,8**	**522,9**	**943,2**	**164,0**	**1040,1**	**970,8**	**116,0**	**109,9**
873-947	**15-17**	**75**	**-0,44**	**15,0**	**1368,9**	**1605,9**	**133,6**	**637,0**	**915,2**	**72,9**	**138,6**

Note: $Ca_м$ and $Mg_м$ - Ca and Mg concentrations in men's hair; Ca_f and Mg_f - in women's hair

Table 5: K-Zn correlation and concentration of metals in epidermic cells of healthy subjects depending on occurrence coefficient (OC).

Number	OC	n	r	Cu µg/g [M]	K µg/g [M]	Na µg/g [M]	Zn µg/g [M]	Ca µg/g [M]	Ca µg/g [M]	Mg µg/g [M]	Mg µg/g [M]
1-115	**6-11**	**115**	**-0,03**	**10,9**	**150,5**	**261,8**	**183,5**	**706,5**	**1418,8**	**47,1**	**119,6**
116-229	**12**	**154**	**-0,31**	**10,8**	**224,9**	**477,2**	**177,4**	**592,9**	**1081,7**	**38,2**	**89,1**
270-503	**13**	**234**	**-0,43**	**11,2**	**394,9**	**833,2**	**158,6**	**595,5**	**910,5**	**40,3**	**64,6**
504-749	**14**	**246**	**-0,43**	**10,9**	**464,8**	**1036,3**	**162,4**	**531,2**	**710,8**	**35,5**	**55,9**
750-954	**15-17**	**205**	**-0,62**	**11,0**	**578,1**	**1131,3**	**159,3**	**537,7**	**611,3**	**40,5**	**44,6**

Note: $Ca_м$ and $Mg_м$ - Ca and Mg concentrations in men's hair; Ca_f and Mg_f - in women's hair

Table 6: K-Zn correlation and concentration of metals in the epidermic cells of Chernobyl disaster liquidators depending on occurrence coefficient (OC).

first of all – peroxynitrite) apparently initiates transmembrane metal transport processes (Zn – from the cell, K – into the cell), where an active role is played by transport proteins (metallochaperones) and membrane ATPases [20].

Assuming that the linear association between Na and K is due to the coordinated work of membrane ATPases, there emerges a reasonable interest in comparative analysis of K–Zn correlation in Chernobyl disaster liquidators and healthy subjects because both Na,K-ATPase and the membrane pump for Zn belong to the same type (P-type) of ATPases, although they represent different subgroups of those.

In our view, the negative nature of association between Na and K concentrations in both assemblies (in the presence of K-Zn association) can testify an increase in activity of P-type ATPases regarding differently-directed transmembrane transport of K^+ and Zn^{2+} under the conditions of oxidative/nitrosative stress.

In this connection, it would be appropriate to refer to the results of the redox status research in Chernobyl disaster liquidators received earlier [21] (See Table 7).

As Table 6 shows, the analysis of the redox status of Chernobyl disaster liquidators evidences an appreciable and stretched in time prooxidant shift (chronic oxidative stress) which cannot be liquidated completely even after antioxidant therapy.

At the same time, accepting the assumption that the negative K-Zn association can indirectly point to an increased intracellular production of oxygen and nitrogen radicals, one must explain the fact that a part of healthy subjects (18.1%, see Table 4) in this respect varies little from disaster liquidators. It is also unclear why in both investigated groups, regardless of gender (Table 5 and 6), the Ca concentration in epidermic cells falls while $|r_{K\text{-}Zn}|$ increases?

The close relation between NO and intracellular calcium homeostasis is well known: via activation of soluble guanylyl cyclase (sGC), cyclic guanosine monophosphate (cGMP), cGMP-dependent protein kinase (PKG-I) and Ca^{2+}ATPase [22]. One of the final effects of NO-dependent induction of this signalling path (at least for muscular cells) is increased excretion of Ca from the cell.

Our comparative analysis of Ca concentration values in people with and without detected negative K-Zn correlation separately for men and women (let us remind that hair Ca content according to our data was dependent on gender) gave the following results.

Regardless of gender, in groups with $r_{K\text{-}Zn}$= -0,43 (healthy persons) and $r_{K\text{-}Zn}$= -0.62 (disaster liquidators) the hair Ca content was significantly lower than in the group with $r_{K\text{-}Zn}$= -0.05 (healthy persons) and $r_{K\text{-}Zn}$= -0.03(disaster liquidators).

Thus, in healthy men, the mean Ca content in the group with $r_{K\text{-}Zn}$ = -0.05 was 934.7 [745-1137.5] μg/g, while in the group with $r_{K\text{-}Zn}$ = -0.43 it was just 856.9 [633.9-1107.1] (in square brackets – interval estimation of the mean by the bootstrap method). In healthy women with $r_{K\text{-}Zn}$ = -0.05 this parameter was equal to 1627.8 [1486.8-1776.5] μg/g while in those with $r_{K\text{-}Zn}$ = -0.43 – just to 946.8 [787.1-1128.5] μg/g. In the group of Chernobyl disaster liquidators with coefficient $r_{K\text{-}Zn}$ = -0.03, equal for men and women, the Ca level in men was 706.5 [622.9-803.4] μg/g, in women – 1418.8 [1059.5-1815.8] μg/g. while in those with $r_{K\text{-}Zn}$ = -0.62 this parameter in male liquidators was equal to 537,7 [506,1-570,4] μg/g; in female liquidators it was 611,3 [508,1-793,3] μg/g (See Table 8).

The obtained results agree with our suggestion that the negative association between K and Zn together with significant decrease of Ca level can indicate activation of intracellular radical reactions with NO participation.

One can try to reveal interrelation between Ca, K and Zn concentrations without correlation analysis. For this, in both entire assemblies (separately for men and women) one needs to compare mean levels of K, Zn at patently low level of Ca (lower than the lower limit of interval bootstrap estimation of the mean) with mean K, Zn in the rest of persons within the subsample. The results of such comparison are shown in (Tables 9,10).

As (Tables 9,10) show, the low Ca level (groups I) was accompanied by increased K concentration and decreased Zn concentration (as compared with Groups II), with confidence being proved by interval estimation of the mean (bootstrapping) in most cases.

Analyses/Units	Years 1998 – 1999	Year 2007
Lipid peroxides and hydroperoxides [LOO·, LOOH] (conv.units)	210,0 ± 30,7 [normal value < 80]	121,7 ± 10,84 [normal value < 80]
Blood plasma oxydizability (conv. units)	450, 0 ± 44,8 [normal value < 200]	440,6 ±51,82 [normal value < 200]
Lipid peroxidation processes ratio (conv. units)	9, 94 ± 1,01 [normal value < 4,0]	6,67 ± 0,71 [normal value < 4,0]
Selenium content in blood plasma (μg/L)	56,1 ± 3,3 (min.17,0 μg/L) [normal value. 80 - 120]	79,8 ± 3,94 (min.43,0μg/L) [normal value 80 - 120]
Glutathione Peroxidase in blood plasma (IU/L)	380,0 ± 19,4 [normal value 450 - 600]	398,0 ± 21,[normal value 450 - 600]
Glutathione Peroxidase in blood (IU/gHb)	26,1 ± 1,98 [normal value 35 - 50]	38,25 ± 1,84 [normal value 35 - 50]

Table 7: Redox status of Chernobyl disaster liquidators.

Groups	Correlation (r)	Ca (μg/g) [M]	
		men	women
Healthy subjects	**r = - 0,05** (n = 432)	745,0 <**934,7**< 1137,5	1486,8<**1627,8**<1776,5
	r = - 0,43 (p<0,05) (n = 171)	639,9< **856,9**< 1107,1	787,1 <**946,8**<1128,5
Chernobyl disaster liquidators	**r = - 0,03** (n = 115)	622,9 **<706,5 <** 803,4	1059,5<**1418,8**<1815,8
	r = - 0,62 (p<0,05) (n = 205)	506,1**<537,7<** 570,4	508,1**<611,3<** 739,3

Note: The Table gives the extreme (max and min) r values only

Table 8: K-Zn correlation and Ca concentration in epidermic cells.

Discussion

Undoubtedly, any conclusions that could be drawn on the basis of this investigation are largely provisional. However, the long-felt requirement in additional (apart from quantitative analysis) information about the events really happening with metal-ligand complexes seems inarguable. These events, named metal-ligand homeostasis, are unlikely to be correctly interpreted without understanding the role of ligands in realization of the known biological effects which are traditionally attributed to metals only.

Analysis of linear correlations between chemical elements (on the basis of spectrometry results) is to help in getting such information about MT, which are the most prevalent protein ligands for cadmium, copper and zinc. In this connection it seems expedient (together with determination of metals) to make quantitative estimation of MT or their apoforms, thioneins, in one or another biosubstrate (which can be the subject of further investigations).

It was found that a MT molecule includes two domains, α and β, which have considerable, differences [23]. One of these differences is unequal terminal amino acid residues in metal-binding clusters. In the α domain, designed mostly for 'toxic' metals (particularly for Cd), the terminal residues are carboxyls (carboxyl-terminal domain), in the β domain – amino groups (amino-terminal domain). This β domain chiefly binds essential metals (Zn, Cu), and it is the one from which nitroxide selectively releases Zn^{2+} through nitrosylation of thiols (formation of S-nitrosothiols), leaving Cd-containing α domain intact [24].

Other examples of functional interrelation between MT and NO are also known. It was found that they have some common transmitters (TNFα, IL-1, lipopolysaccharides) for induction of their intracellular synthesis, and the MT molecule can serve as a trap for aggressive nitrogen radicals. In addition, according to some researchers, Zn^{2+} released from MT can inhibit inducible NO-synthase (iNOS) and thus prevent NO hyperproduction in cells [24].

Zn^{2+} release largely depends on the redox status of the cell where prooxidant shifts (e.g. accumulation of oxidized glutathione – GSSG) facilitate Zn^{2+} release from MT molecules while reduced glutathione (GSH) without presence of GSSG inhibits this process [25,26]. Thus, Zn bond with β domain becomes unstable under conditions of both oxidative and nitrosative stress [27,28].

This circumstance directly concerns Chernobyl disaster liquidators, whose redox status was found [21] to demonstrate distinct prooxidant shifts: 3-5 fold increase of plasma chemiluminescence above norm, a significant decrease in activity of erythrocytic Se-dependent glutathione peroxidase (GSH-Px-I).

Nitrosylation of thiols by nitroxide in β domains can release not only Zn^{2+} but also Cu^{2+} from Cu-containing MT (Cu-MT). In this case, if Cu^{2+} binds to apoform of Cu,Zn-superoxide-dismutase (apo-ZnSOD), then Cu-MT can play the role of metallochaperone for one of the key antioxidant enzymes in the cell [29].

After breaking from bonds of MT by means of nitrogen or oxygen radicals, essential metals (Zn, Cu) have a choice: either to remain in the epidermal cell in order to help it overcome oxidative/nitrosative stress (e.g. as a part of antioxidant enzymes and/or as a suppressor of NO hyperproduction), or to leave the cell. The latter choice looks apparently less beneficial for epidermis and its derivatives. However, this is the choice which allows the organism to save essential metals, whose necessity obviously rises at activation of radical processes.

An absolutely different situation exists for 'toxic' metals, particularly Cd, in epidermis and its derivatives. Being efficiently secured from the release-effect of nitrogen/oxygen radicals in MT's α domain, these metals not only can remain in the cell but, according to our observations (See Table 3), can also accumulate in the epidermal derivative (hair). What does such accumulation mean for the whole organism?

It means that all the body surface (in a man – ca.$2m^2$), almost completely covered by hair (except for palms and soles), becomes the area where the organism removes excess of heavy and/or 'toxic' metals by pushing them into permanently desquamating epidermis and growing hair. Such a reaction seems efficient and evolutionary reasoned, and it can be surely claimed a compensatory/adaptive one not only against heavy metal poisonings, but also in case of chronic disturbances of the organism's redox status – prooxidant shift, which happens in Chernobyl disaster liquidators.

This idea is further confirmed by the data on the significant increase of Zn, Cu level in blood of disaster liquidators as compared to norm [21]. It is notable that the considerable increase of Cu concentration in blood of liquidators was accompanied by normal level of ceruloplasmin. Unfortunately, the aforesaid authors did not determine the MT concentration in plasma; therefore, it is difficult to interpret the data.

Metal	**Healthy subjects** (n=947)			
	Women (n=709)		**Men** (n=238)	
	Group I (n=437)	Group II (n=272)	Group I (n=149)	Group II (n=89)
Ca (μg/g)	675.7<**707.5**<739	2494.6<**2615.7**<2746.6	318.5<**335.4**<351.3	888.1<**1096.5**<1408.2
K (μg/g)	268.6<**336.3**<412.6	171.6<**226.2**<293.2	290.5<**380.2**<475.2	191.1<**324.7**<494.7
Zn (μg/g)	171.3<**176.2**<181.1	196.4<**205.6**<215.1	154.5<**162**<168.8	190.2<**205.3**<220.3

Table 9: Comparative analysis of hair K, Zn content in groups of healthy subjects with different Ca level.

Metal	**Chernobyl disaster liquidators** (n=954)			
	Women (n=213)		**Men** (n=741)	
	Group I (n=134)	Group II (n=79)	Group I (n=453)	Group II (n=288)
Ca (μg/g)	477.6<**505**<739	1457.6<**1634.7**<1852.2	394.8<**403.1**<411.9	814.7<**851.9**<893.3
K (μg/g)	285.6<**357.2**<439.3	159.9<**216**<281	424<**468.8**<512.6	291.9<**346.3**<404.1
Zn (μg/g)	156.7<**164.2**<171.6	170.9<**183.5**<197.8	148.7<**152.6**<156.8	176.3<**182.6**<189.4

Note to Tables 9, 10: Group I – persons whose individual Ca levels do not exceed the lower limit of interval bootstrap estimation of the mean; Group II – the rest of the given subsample. Bold font – mean values, regular font – limits of confidence intervals

Table 10: Comparative analysis of hair K, Zn content in groups of Chernobyl disaster liquidators with different Ca level

Particular mechanisms of metal transfer through cell membrane are insufficiently studied. A key role in this process belongs to rather large set of ATPases, which consists of five subsets (I, II, III, IV and V). The P-type of these enzymes (firstly P_{Ib} and P_{II}) is most interesting for us because the P-type ATPases provide transmembrane transport of such ions as H^+, Na^+, K^+, Cu^+, Zn^{2+}, Ca^{2+}, Mg^{2+}, Cd^{2+} etc [30,31]. Energy necessary for such transport, which runs against concentration gradient, comes from ATP hydrolysis. It is assumed that nitrosylation of S-containing proteins in the structure of K_{ATP} channels under conditions of nitrosative stress leads to activation of these channels [20].

It seems no mere chance to us that the existence of interrelation between K^+ and Zn^{2+} ion counter-flows (which can be seen in the detected negative correlation K - Zn in Chernobyl liquidators and partly in healthy subjects) is accompanied by a significant decrease of Ca concentration in epidermal cells. In our opinion, such a combination (being a kind of discriminator) can point to activation of redox processes involving nitrogen/oxygen radicals in epidermis.

Unfortunately, the authors had no data about the doses of radioactive elements (J^{131}, Cs^{137}, Sr^{90}) received by the Chernobyl accident liquidators. Therefore, it was not deemed possible to evaluate the different effects of these radioactive atoms on the change of metal-ligand homeostasis.

Conclusions

1. The epidermis derivative (hair) represents a convenient object for noninvasive and retrospective supervision of events in metal-ligand homeostasis epidermic cells, suitable for mass (population) investigations. However, when conducting a quantitative analysis of hair's mineral structure, one should avoid generalizing estimations by virtue of special, still understudied role of epithelium in receipt and deducing of metals. Moreover, one cannot exclude that in most cases changes of metal concentration in epidermis can be of a re-distribution nature, mismatching their true content in the organism.

2. The correlation analysis of spectrometry data with the subsequent selection of significant correlations opens new opportunities for studying metal-ligand homeostasis and its changes connected with the redox status in organism (in particular, with oxidative and nitrosative stress).

3. The results of the present work allow offer the following parameters as kind of discriminators of an oxidizing and nitrosative stress: a) increase in epidermis concentration values K and Na alongside with reduction Ca and Zn, b) the presence of significant negative K-Zn correlations according to hair spectrometry analyses.

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