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A new hypothesis for the etiology of Kaschin-Beck disease

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Abstract_ Kaschin-Beck disease (KBD) could be exogeneous free radical induced endemic osteoarthropathy under conditions of lacking necessary antioxidant defences.especially selenium-cored defence in Chinese eco-environment. Experimental evidences have been provided for the internal relations among previously suggested pathologic factors, i.e., selenium deficiency, organic matters of drinking waterand mycotoxins from contaminated grains, based on the facts that the former is a preventory factor while the others are exogenous free radical carriers. ESR spectrum shows that these exogenous free radical carriers have a property of semi-quinone compounds and are toxic in in-vitro cartilage cell culture and animal tests in inducing lipid peroxidation process.
Keywords: humic acid; mycotoxin; free –radical; Kaschin –Beck disease.

PROGRESS AND QUESTIONS

The history and research development of KBD has been reviewed by L. Sokoloff (1989), in which the pathologic factors can be grouped into three, i. e. , selenium deficiency, organic matters of soil and water (OM) and mycotoxins (MT) elaborated by rotted grains. Geochemical surveys in 1960s has revealed a selenium deficient belt in China, co-distributing with KBD, Keshan disease (KSD) and animal white muscle disease, from which, therefore, KBD was attributed to selenium deficiency. A nation wide preventory practice of supplementing selenium-selenite has been carried out since 1970s and has been claimed to be effective in most cases. Meanwhile, preventory practices of changing food sources and changing water sources or ameliorating water qualities to eliminate toxic factors have also been carried out in some KBD regions and have been claimed to be effective. These three pathogenic concepts are scientifically tenable, but are lacking nation wide suitability, cause and effect explanation and scientific evidences of etiologic mechanism. For example, Fig. 1 gives the statistic results of selenium contents in human hair and blood of normal, KBD and KSD patients, and it can be seen that KBD or KSD is characterized by selenium deficiency. But, as shown in Fig. 1, this fact can not explain why there are people in-land and abroad living in selenium deficient state without developing into KBD cases. On the other hand, there are people living in KBD regions with rather higher selenium content in hair and blood developing in KBD cases (Mo, 1989). Content of organic matters in drinking water of KBD regions were found comparably higher than neighboring non-KBD regions, but could not be higher than some regions crossing the country. Mycotoxins of *Fusarium*

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has certainly been found in northern part of China, but it can not be detected in central or south of China with KBD occurrence (Bai, 1990). Besides, there are KBD regions in arid or semi-arid zones with less chances of fungi contamination of grain and there are non-KBD regions in humic zones with more potentiality of fungi contamination. These facts and the fact of lacking necessary pathologic evidences lead to the present dispute in the discussion of the cause of KBD and make us reconsider the role of each pathogenic factor and, more important it may be, the relations between proposed KBD pathogenic factors.

In past years, a comprehensive research on the causes of KBD has been conducted, where three pathologic factors, i. e., organic matters of water and mycotoxins of grains and selenium deficiency both in KBD eco-environmental and biological senses are taken into consideration. Based on the results, a new hypothesis is proposed in which both fulvic acid (FA) from drinking water and mycotoxins from rotted grains are seen as exogenous free radical carriers, in causing KBD through lipid peroxidation process, and selenium is regarded as a protectory factor

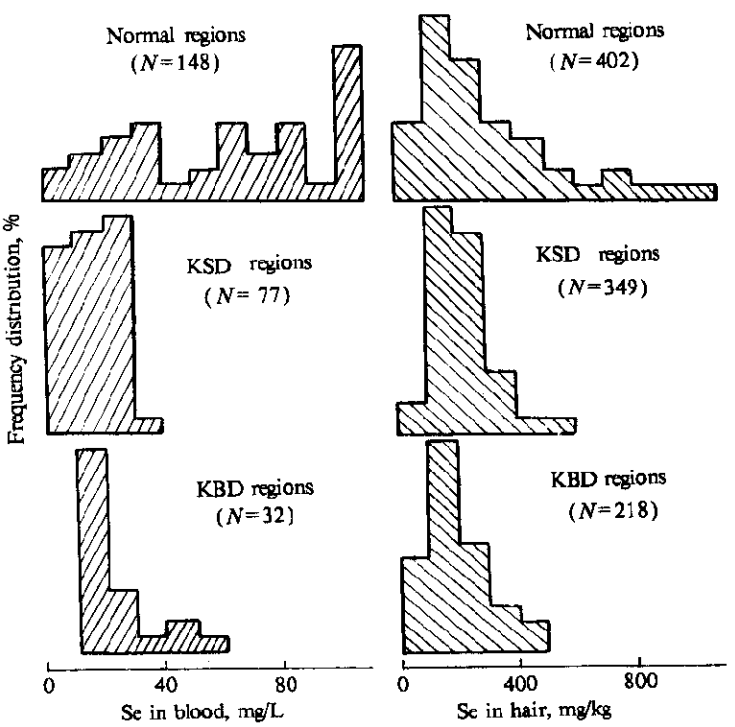

Fig. 1 Frequency distribution of selenium content in blood and hair from normal, KSD and KBD regions (data collected from Chinese journals up to 1987)

of eliminating free radicals in biological media. KBD is developed under the situation of an unbalanced toxic factors to protectory factor (s).

IMPORTANCE OF VARIOUS FACTORS IN KBD ECO-ENVIRONMENT

In a first view of the importance of organic matters of water, mycotoxin of grain and selenium in KBD eco-environment, a typical KBD region in central China is selected and the results are given in Table 1. In this region. KBD villages are characterized by the generally higher content of organic matters in drinking water and of mycotoxin such as Alternaric methyl-ether, in maize, and lower level of selenium of human beings. Comparing the parameters of the health island (Liujia) with those of the KBD villages, one can conclude on the equal importance of three pathogenic factors, non of which can give a better correlation with KBD incidence. It needs to mention that, instead of *Fusarium* as reported by Yang (1981), the dominant species of fungi in the region is *Alternaria* (Bai, 1990).

Table 1 Pathogenic survey on organic matters of soil and water, mycotoxins and Se nutrient state in 5 KBD villages and one health island of Tianshui, Gansu Province

Sampling site[1]	TOC of water, mg/L	Fungi mycotoxin[2]		Se nutrient state			KBD incidence
		D. A, %	AME, μg/kg	in hair, mg/kg	in blood, mg/L	GPX, EU/ml	by X-ray[3], %
MU-JIAO	27.0	60	181	0.161	0.043	11	55.1
GAO-JIA	9.2	71	–	0.172	0.055	18	66.7
REN-JIA	21.0	60	651	0.170	0.046	19	19.0
HE JIA	20.5	62	381	–	0.047	–	92.3
LIU-JIA	8.3	7	97	0.203	0.071	36	0.0

1) Abbreviations represent village's name

2) D. A. means detection rate of *Alternaria* in wheat, AME represents alternaric-methyl-ether content in wheat, no *Fusarium* has been detected in the region

3) KBD incidence is represented by X-ray detection rate of hands

4) Table is adapted from data in the summary report (1991)

From a further comparison of these three parameters on nation wide scale, i. e., five provinces from north to south (Table 2), one can recognize the significant differences of the parameters between KBD and neighboring non-KBD regions. But it is less meaningful to compare quantitatively among provinces and to indicate the importance of one parameter out of three. Also, the differences can still be found in the historical and degenerative KBD regions where the KBD incidence has approached zero.

Table 2 Pathogenic survey on organic matters of soil and water, mycotoxins and selenium in 6 typical KBD regions with respect to neighboring non-KBD regions

Sampling site[1]	Organic matter		Detection rate of fungi,[2]	Selenium content			KBD incidence by X-ray[3],
	in water,	in soil,		in soil,	in water,	in maize,	
	mg/L	%	%	mg/kg	μg/L	mg/kg	%
HLJ-SZ	1.20	1.02	–	0.058	0.054	0.004	28.2
	(0.37)	(0.21)	–	–	–	–	
HLJ-SL	1.61	0.97	20	0.097	0.060	(0.004)	30.2
	(–)	(0.28)	–	–	–	–	
JL-FS	1.14	0.78	38	0.085	0.055	0.004	2.4
	(0.38)	(–)	–	(0.095)	(0.089)	(0.012)	
SD-Q Z[4]	0.38	1.12	14 – 97	0.035	0.201	0.007	0.0
	(0.13)	(0.31)	(21 – 67)	(0.049)	(0.145)	(0.003)	
GS-TS	0.39	0.24	10 – 100	0.044	0.129	0.003	62.1
	(0.36)	(0.14)	(20 – 33)	(0.048)	(0.466)	(0.005)	
SC-AB	0.69	1.12	–	0.031	0.099	–	16.7
	(0.34)	(0.44)	–	(0.071)	–	–	

1) abbreviation: HLJ, JL, SD, GS and SC represents Heilongjiang, Jilin, Shandong, Gansu and Sichuan provinces, respectively.

2) Detection rate of fungi in maize, mainly *Penicillia*, *Chaetomium* and *Fusarium*.

3) KBD incidence is represented by X – ray detection rate of hands.

4) Degenerated KBD village and neighboring non-KBD village.

5) Table is adapted from data in summary report (1991), data in parentheses indicate those from neighboring non-KBD village (s).

The finding of *Alternaria* in central China instead of *Fusarium* in the northern part in Table 1 indicates that different metabolic products may exist in different regions. For organic matters, we notices that while FA either from KBD or non-KBD region has a similar structure, the significantly differentiated small mol. wt. organic compounds in drinking water of KBD and neighboring non-KBD villages may vary from region to region (this issue). It implies, therefore, that the role of different organic components of water and different mycotoxins of grains in the development of KBD should be realized through a common mechanism and might be in a mutually exclusive manner, otherwise we cannot explain the large regional and structural variation of these factors and cannot explain the similarity of these factors between KBD and non-KBD regions without the partitioning of selenium.

FREE RADICALS IN KBD ECO-ENVIRONMENT AND PATHWAY INTO BIOLOGY

The medicochemical survey in KBD regions indicates an abnormal free radical signal intensity in water (this issue) and in grains (Table 3) with respect to that in non-KBD regions. Approximately a dozen times higher in water and several times higher in grain of free radical signal is presented by a single peak in the ESR spectra with a g values of 2.003 – 2.004, suggesting that it could be originated from semi-quinone structure of organic components in both water and grain. This free radical signal could not be due to metal ions, because the ESR spectrum was determined at room temperature and the peak width and g value differs significantly from those of metal ions. It is easy to explain the higher free radical signal in water of KBD regions, because it is well known that fulvic acid, the main component of organic matters in surficial water, contains quinone and semi-quinone structure. But it would be more interesting to notice the correlation between free radical concentration, measured as peak height, and content of fulvic acid in drinking waters of KBD regions (Zhang, 1990) and the coincidence of higher free radical signal occurred both in water and grain of KBD regions. The origins of free radical in grain has not been reported, but it is certainly caused by fungi contamination in this study. As shown in Table 4 the fungi *Fusarium* contaminated maize has a much higher free radical signal and a very similar g value to those obtained in water and grain of KBD regions.

Table 3 Free radical in maize of a KBD region (SX-YS) with respect to that of Beijing (BJ)

Sampling site	KBD incidence, %	Concentration, 1×10^{15} spin/g	g value	ΔH, G[2]
BJ	0	38	2.0029	8.0
YP-BM[1]	73	84	2.0038	8.4
YP-WF	73	84	2.0033	8.5
CL-JL	94	94	2.0038	8.4
CL-MJ	92	112	–	9.0
YP-QJ	95	85	2.0032	9.5

1) Abbreviation: SX-YS to Yongshou County, Shannxi Province, others are village's name

2) ESR parameter refers to Peng, 1989

It has been well documented that the quinone and semi-quinone structure evolves into oxy-free radical when pH and E_H of the system changed and that the oxy-free radical can induce the production of both of hydroxy-free radical and peroxides. The elimination of latter depends heavily

Table 4 ESR characteristics of maize contaminated by fungi *Fusarium*

Parameter	Normal maize	Maize contaiminated by *Fusarium*			
		F. Oxy. 032	F. Oxy. 033	F. Moni.	F. Subgluti
FR Conc., 1×10^{n}	0.38	2.97	2.95	6.58	5.92
ΔH, G	8.0	4.9	6.8	4.5	5.0
g value	2.0031	2.0034	2.0038	2.0029	2.0034

1) Abbreviation: FR, and ΔH, G represent free radical, half peak width and F. Oxy, F. Moni and F. Subgluti., represent oxysporum, moniliform and subglutinans of *Fusarium*

2) ESR parameter refers to Peng, 1989

on activity of glutathione peroxidase and other antioxitants in biological media. The finding of the very similar behavior of organic matters of water and mycotoxins of grain in terms of exogenous free radical carriers bridged a connection between two main pathologic factors of KBD, which involves also the participation of selenium deficiency through its property of free radical scavenger.

Exogenous factor such as FA taken via drinking water can be accumulated and the free radical signal in bone increased, as verified by animal tests (this issue). In one of such tests, Kuming rats were injected abdominally with various concentrations of FA from coal (0--150 mg/kg/d) for 130 days, significantly higher ESR signals were observed in FA-injected groups with respect to the control group and positively correlated with FA concentration. Besides, FA has been characterized from the HCl-NaOH extracts of bone by different spectra

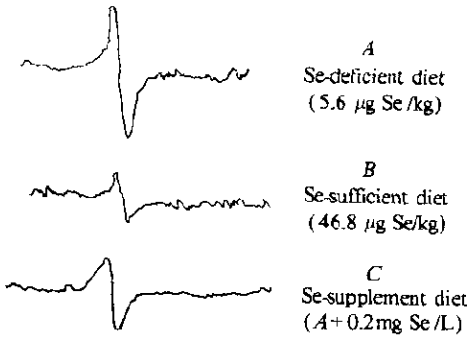

Fig. 2 Influence of selenium intake on the free radical reaction of FA in bone of the rat (FA from KBD region was prepared in water (200 mg/L). ESR conditions are: PW = 10 mW, SW = 50 G, MD = 8.378 g, TC = 40.96 M sec, ST = 167.77 s, MF = 9.75 GHz, Cf = 3474 G, RC = 2×10^{3})

(Yang , 1990). In another test , FA from KBD regions was dissolved in the drinking water of the Wistar rats (200mg/L)for 4 weeks and the intensity of free radical signal was found to be correlated with selenium content in diet and selenite supplemented , as shown in Fig .2. The animal tests provides the experimental evidences of metabolic pathway and action mechanism of exogenous FA in the bone metabolism .

We still do not know the actual forms of FA, especially MT in bone or cartilage of human being, but from discussions above, we can expect at least one of their actions, i. e., their stress on the oxidative action in biological media.

TOXIC EFFECTS OF ORGANIC MATTERS AND MYCOTOXINS AND THE ROLES OF SELENIUM

The effect of FA or MT in adrenalin autooxidation system has been examined by addition of both FA and MT from KBD regions to the system (Peng, 1991). The optical intensity generated by superoxide anion of the adrenalin autooxidation process is increased and can be reduced by addition of selenite or superoxide dismutase (SOD). In the animal test, antagonistic effect between FA and selenium (Peng, 1987) and between Fusarium mycotoxin and selenium (Tang, 1987) has been reported previously. The toxic effects of FA or FM could also be observed in a human embryonic cartilage cell system (HEC), in which addition of either FA or FM to the culture results in the cell swelling and accumulation of a large quantity of degenerative products, cells were slender or body broken, poor density with blank area, and multivesicular bodies and lipid bodies appeared when compared to the control's. Such characteristics of damage is more or less resembles the cartilage necrosis of KBD.

The damage of cell by FA or FM was thought to be due to lipid peroxidation (LPO)process , because an enhancement of a degree of lipid peroxidation in HEC was observed when both FA and FM were added and the level of LPO could be reduced by adding selenite , or SOD . Also, in case of addition of selenite , an increase of the activity of glutathione peroxidase (GPx) could be observed (Peng , 1991), implied that the role of selenium in preventing the damage of FA and FM was being realized through synthesis of GPx. The hypothesis of lipid peroxidational damage of FA could be verified by comparing the LPO induced by acetyl phenyl hydrazine (APH), the known LPO inducer , FA from coal and FA from KBD region in Kuming rats, as shown in Table 5. It can be seen from the table that FA from KBD region behaves very similarly to APH , comfirming that FA acts as a exogenous carrier in inducing the peroxidation process.

To simulate the toxic effect of FA, model compounds of the predecessors and decomposition products of FA were used in a chicken embryonic cartilage (CEC) cell culture system. The results were shown in Fig. 3. Based on the morphology of CEC and the degree of lipid peroxidation (as content of shiff's base), the model compounds can be grouped and arranged in an order of quinonic > phenolic = FA > phenolic acid and organic acid > xanthonic functional

groups. This is an indication of that the toxic effect of FA could be most probably due to the oxy or hydroxy functional groups in the structure. This conclusion has been verified in an animal test, where the liver LPO of the rats decreased and activity of GPx increased when hydroxy groups of FA were blocked before feeding the rats (this issue).

Table 5 Influences of FA and APH on the lipid peroxidation process of the rat (Kuming strain, N=6)

Condition	Hb, %	Reticulocyte, %	Rbc-MDA, O.D	Liver-MDA, O.D	Liver GPx Eu/g
Control	15.2±1.0	2.55±1.3	0.00±0.00	0.21±0.07	77.0±6.0
FA-coal	15.1±1.2	2.7 ±1.0	0.00±0.00	0.19+0.09	–
APH	10.3±2.2	64.6±8.3	92.7±21.4	0.60±0.16	66.0±8.8
APH+FA-coal	13.3±0.7	42.5±8.1	71.0±9.11	0.38±0.16	84.3±4.6
FA-KBD	14.1±1.0	3.0 ±0.7	9.20±0.79	0.27±0.08	61.7±2.6

1) Abbreviation: Hb, Rbc, MDA represent hemoglobin, red blood cell and carbonyl malondialdehyde, respectively, others see the paper.

2) FA from coal (FA-coal) and from KBD region (FA-KBD) were injected abdominally (50mg/kg/d) for 10 days.

3) APH was injected on day 1 (200mg/kg), 4 and 7 (100mg/kg).

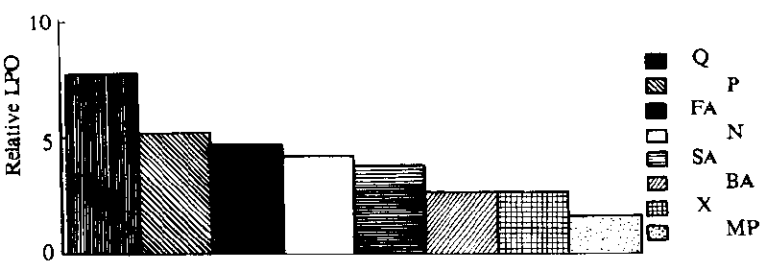

Fig. 3 Influence of FA and its model compounds on lipid peroxidation of Chick embryo cartilage cell (measured as fluorescence, calculated as ratio to the control group)

Q: average of 2-hydroxy 1,4 quinone and 3-hydroxy 1,6-quinone

P: average of 1,4-diphenol, 1,3-diphenol and 1,3,5-triphenol

FA: FA from KBD region

N: α-naphthol

SA: Salicylic acid

BA: Benzic acid

X: Xanthenone

MP: average of 4-methyl-phenol and 3,4-dimethyl-phenol

By comparing among hydroxy superoxide free radical generated from $FeSO_4$-EDTA, superoxide free radical generated from xanthine oxidase-hypoxanthine and FA from KBD region

in CEC, very similar toxic consequences have been reported, in terms of cell morphology and abnormal collegen metabolism (Wang, 1991). Medicochemical evidences together with all the pathological features of KBD, such as cartilage necrosis, early ossification, bone deformation and dwarfism, described the ethiology of KBD through an exogenous factor, i. e., FA and FM, induced cell damage (Wang, 1991).

MOLECULAR BIOLOGICAL BASES OF KBD ETIOLOGY

Exogenous free radicals, such as FA and MT, can attack chondrocyte, causing differentiation, and can attack collegens in extracellular matrix, causing the hydroxyapatite crystalization from inhibite to promote (Wang, 1991). In the experiment with chick embro, injecting of FA from KBD region results in a decrease of 21.7% in the weights of femurs, a slight lower ratio of protein to DNA in epiphysis and metaphysis, and a lower proportion of α 2(I) to α 1(I) and α 1(II) in cartilage collagen with respect to the control, as shown in Table 6. The results imply an impaired ossification induced by FA. Furthermore, molecular defect of type II collagen and another abnormal fraction of collagen (PN α (II)) have been reported in the extract from cartilage of KBD patients (Yang, 1991) and a small concentration of pro α 1(II) collagen could be detected in the epiphysis of chick embryos by immunoblot technique. Therefore, the role of FA in KBD etiology could also be through its influence on the conversion of PN-collagen II to collagen II in articular cartilage. Another criteria of KBD patient is the higher excretion of hydroxyproline in urine (Zhang, 1984) and the higher ratio of hydroxyproline to proline in the cartilage of KBD patients (Yang, 1991). Unfortunately, we cannot obtain the similar results in the chick embryo experiment, as indicated in Table 6, probably because the strict condition of selenium deficiency has not been performed in this experiment.

Table 6 Effect of FA from KBD region on the development of chick embryos

Parameter	Control N=30	FA group N=28
Body weight, g	20.4	18.3
Femur weight, mg	60.0	47.0
Mineral content, %	50.0	43.0
Protein/DNA in EP	25.5±0.1	24.0±0.1
Protein/DNA in MD	26.0±0.1	19.8±0.5
Hyp/pro in EP	0.516±0.020	0.472±0.005
Hyp/pro in MD	0.621±0.009	0.446±0.003

1) EP and MD represent epiphysis and metaphysis, hyp and pro represent hydroxyproline and proline

2) FA was injected into the eggs on 9th day after starting the incubation, treated on 16th day

CONCLUSION REMARKS AND PROSPECTS

From the discussions above and more experimental evidences not illustrated in this paper (also see this issue), it is indicated that both FA of water and mycotoxins of grain are the pathogenic causes of KBD. Both FA and mycotoxin can be regarded as exogenous free radical carrier, which induce lipid peroxidation process through their oxy and hydroxy functional groups under oxygenic conditions. This exogenous factor induced cartilage cell damage could cause an abnormal extracellular matrix and consequentially abnormal biomineralization, or impaired conversion of pre α collagen to collagen. The roles of selenium in the etiology of KBD would be the protector characteristics of GPx against lipid peroxidation process. On this basis, three concepts, i. e., organic matters of water, mycotoxins of grain and selenium deficiency, on the causes of KBD could be generalized through the suggested hypothesis.

Accordingly, the hypothesis can be easily expressed in a three terms mathematical function, where both organic matters (OM) and mycotoxin (MT) are positively correlated with KBD incidence while selenium, in a proper manner, such as activity of GPx, is negatively correlated with it, i. e.,

$$\text{KBD incidence, \%} = F(OM) + F(MT) - F(Se).$$

In this expression, F (OM), F (MT) and F (Se) are functions of various eco-environmental and biological parameters, such as temperature, humidity, soil types, plantation and humification and so on for F (OM) and F (MT), and content and speciation of selenium in eco-environment , bioavailability, antagonistic and coordinative effects of other elements in food chain, and even the size of metabolic pool of selenium in KBD patients for F(Se).

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