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The roles of organic degradation products and selenium in the pathogenesis of Kaschin–Beck disease

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Abstract — An investigation on the organic degradation products and selenium in the pathogenesis of Kaschin-Beck disease was carried out. The results demonstrated that the organic degradation products existed in food and drinking water were the pathogens of the KBD; their leading pathological process of cells would be "membrane injury" due to peroxide. As GSH-Px is a selenium contained enzyme, therefore insufficient selenium would be one of the most important conditions to cause KBD.

Keywords: Kaschin-Beck disease; humic acids; selenium; antioxidase; lipid peroxide.

Kaschin-Beck disease (KBD), as a serious and wide spread endemic osteoarthropathy in China, mainly occurs in remote districts where living condition is poor and economy is backward. Up to now, there has not been an as certained cause about it. Recently the evidences found by the experts in China have proven that: (1) This disease often onsets at deficient Se areas of China, and can be prevented and cured by enriching Se in food and water (Lee, 1979; Teng, 1985). (2) The level of humic acids which contaminates drinking water is closely correlated with the state of the illness (Monographic Research Group of Ecoenvironment, 1984; Wu, 1987). (3) KBD is a kind of "membrane defect" disease (Lee, 1984) and so on.

Undoubtedly, it is quite beneficial to investigate the intemal relations among those three aspects as mentioned above and the actions of each in KBD pathology so as to probe its cause and pathogenesis. Thus, our study consisted of three parts.

STUDY ON CULTURED CHONDROCYTES

Chondrocytes in long bones of limbs from aborted human embryo of 3–4 months, after digested and dispersed, was diluted with growth-promoting media (Ham F12) into 40×10^4 cells ml^{-1} and then cultured in bottles for 40 h; and then those cultured bottles containing eugonic chondrocytes were randomly grouped by using an inverted microscope. FA (a fraction of humic acids) was extracted from the soil of KBD region, Jilin Province.

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Each experiment was conducted by synchronous observations and comparisons between control and experimental groups. The results were as follows: (1) Cultured for 8 days in presense of 25 ppm FA, chondrocytes had an obviously impaired growth — thin, small, and defect with many fat droplets in cytoplasm; meanwhile the activities of antioxidase ——GSH-Px (Hafeman' s method) and the capacity of synthesizing matrix mucopolysaccharides (total glucosaminoglycans, TGAG, including sulfate glucosaminoglycan, SGAG, and hyaluronic acid, HA; Gold's method) were markably decreased; (2) for the experiments in the presence of both Se (Na_2SeO_3, 0.1 ppm) and FA, the cells showed intact, eugonic and unimpaired, the GSH-Px activity rose, and synthesis of mucopolysaccharide was kept normal (Table 1).

Table 1 The impaired effects of FA and protective effects of selenium on chondrocytes, $M \pm SE$

Indices	Groups			
	Control	FA	Se	Se+FA
Number of cells, 10^6/bottle	108.9±15.7	98.9±13.5*	116.3±13.9	110.8±18.0***
GSH-Px, U/4×10^6 cells	23.5±15.0	20.3±5.6	22.7±4.7	29.2±6.0***
TGAG, μg/bottle	57.6±5.6	48.0±5.4**	56.7±6.2	56.9±6.0***
SGAG, μg/bottle	22.2±3.2	17.9±3.6	21.9±4.2	20.9±3.5***
HA, μg/bottle	31.2±6.2	25.0±5.7	29.6±8.5	31.4±5.6***

*$p<0.05$; **$p<0.01$, compared with control group; ***$p<0.05$, compared with FA group

The results shown in Table 1 are consistent with the previous studies (Wang, 1989a; 1989b), namely, in KBD area this disease was mainly caused by longterm intake of drinking water contaminated by humic substances; and the preventive effect of selenium on KBD was realized by preventing the activity decrease of GSH-Px in cells impaired by humic acids.

ANIMAL EXPERIMENTAL OBSERVATION

Juvenil Wistar rats, weighted 65±5 g, with the equal numbers of male and female, were separated into three groups, eight each. For the KBD group, the rats were fed on corn and wheat (1:4) from another KBD area (Tianshui of Gansu Province), sesame seeds (2%) and NaCl (1%), and drank distilled water with 200 ppm of HA (extracted from the soil of Tianshui); the non-KBD group rats on diet (with the same ingredients) and water containing HA from a non-KBD region (Beizhen County, Liaoning Province) served as a control group. For the added Se group, the rats were fed on Tianshui food and drinking water containing 200 ppm of Tianshui HA plus 0.1 ppm of Se. After feeding for five weeks, all the animals were decollated to get blood sample and heart muscle, liver, gastrocnemius muscle, which were assayed for the activites of GSH-Px and SOD (pyrogallol autooxidize method) and

the level of LPO (TBA method), respectively. The statistic results of seven assays were as follows:

1. The GSH-Px activity in KBD group was far lower than that in non-KBD group in blood and tissues of liver, muscle, of which the muscle was found to have the most marked decrease (about 83.7%) in the enzyme activity while heart muscle remaining no change in the enzyme activity. However, these reductions disappeared when 0.1 ppm of Se was added into the drinking water (Tabel 2).

Table 2 The effects of diet and HA water from KBD area on GSH-Px activity in animals and Se action, $M \pm SE$

Sample	Non-KBD group	KBD group	p_1	Se-added group	p_2
Blood, u/ml	54.2±8.3	21.4±4.0	<0.01	42.5±4.5	<0.01
Heart muscle, u/g	720.5±135.4	832.9±76.4	>0.05	884.0±113.9	>0.05
Liver, u/g	884.6±77.5	667.4±102.6	<0.01	1130.7±88.1	<0.01
Muscle, u/g	243.6±64.7	39.5±23.3	<0.01	187.2±90.2	<0.01

p_1 compared with non-KBD group; p_2 compared with KBD group

2. On the contrary to GSH-Px, the SOD activity of KBD group rats was raised obviously ($p<0.01$) both in blood and muscle, and in the latter case the activity increased by 60%. But a little amount of Na_2SeO_3 mixed with water could not affect the efficacy of SOD activity (Table 3).

Table 3 Effects of diet and HA water from KBD region on SOD activity in rats and Se action, $M \pm SE$

Sample	Non-KBD group	KBD group	p_1	Se-added group	p_2
Blood, u/ml	289.9±78.7	408.7±28.2	<0.01	361.9±36.8	>0.05
Heart muscle, u/g	281.7±35.1	296.6±33.6	>0.05	269.4±28.9	>0.05
Liver, u/g	1149.8±84.4	969.8±173.4	>0.05	975.8±12.2	>0.05
Muscle, u/g	70.3±19.4	112.8±32.6	<0.01	106.1±16.8	>0.05

3. As compared with those of non-KBD group rats, the concentrations of LPO in blood and tissues of KBD group rats were distinctly elevated ($p<0.01$), especially by about three times in heart and muscle. These changes could not be observed when 0.1 ppm of Se was added in drinking water, that was the same as non-KBD group rats (Table 4).

Table 4 Effects of diet and HA water from KBD area on LPO level of rats and Se action, $M \pm SE$

Sample	Non-KBD group	KBD group	P_1	Se-added group	P_2
Blood, nmol/ml	2.77±0.90	6.86±0.74	<0.01	2.20±0.61	<0.01
Heart muscle, nmol/g	210.2±37.2	773.4±120.2	<0.01	320.3±47.5	<0.01
Liver, nmol/g	181.5±102.8	369.3±79.5	<0.01	242.5±52.9	<0.01
Muscle, nmol/g	274.7±47.5	804.8±139.6	<0.01	284.9±52.7	<0.01

The findings above indicated that damaged chondrocytes and decrease of GSH-Px activity by humic acids could be prevented by a right amount of Se. This was again proven by the animal experiment. The results also pointed out that there was a similar efficacy of HA and FA, and the food from KBD region could serve as the same pathogens as humic substances.

Both SOD and GSH-Px were important antioxidases; in these experiments SOD activity was raised, which could not be a key link of pathogenesis, but a reflection of forming more free radicals in the tissues of organism. The key point of the illness would be the decrease of GSH-Px activity by organic degradation products (humic acids and degenerative grain).

REPLICATION OF ANIMAL PATHOLOGICAL MODEL

A number of female Wistar rats were mated with male rats for 10 days under a good feeding condition, and were then separately fed. Three of them were found pregnant: control group was fed with Beizhen grain and distilled water, non-KBD group fed with Beizhen grain and water containing 100 ppm of HA and KBD group fed with Tianshui grain and water with 100 ppm of HA. The durations from mating to giving birth were 21, 24, 22 days, respectively. During experiment, both mother-and baby-rats in every group looked lively as usual, with smooth and gloss fur, without any abnormal manifestations. However, baby-rats in different groups showed a different growth: the non-KBD group grew most quickly while KBD group the slowest. Their weights, measured after 6 weeks from given birth, presented significant differences(Table 5).

Table 5 Comparison of body weights of baby rats, g

Groups	N	$M \pm SD$	Mean of deviation	t	p
Control	8	72.5±2.7			
Non-KBD	5	91.6±9.4	19.1	5.52	<0.001
KBD	6	47.7±3.3	43.9	11.96	<0.001

The cut sections of heart, liver, spleen, kidney, gastrocnemius muscle and the proximal end of tibia in baby rats after decollated were made and stained. Examination outcomes showed an obvious pathologic change only in tibia proximal end of KBD group. The findings of six cases were: (1) The thickness of articular cartilage became thin, reduced by 15% as compared with the non-KBD group; one case presented a slight depressing on its surface; moreover, the nuclei of chondrocytes in the depths in four cases nearly disappeared. (2) The thickness of the growth plate of epiphyseal cartilage had notably thinned to approximately 40% of that of non-KBD group; and the number of the cells in proliferous layer reduced too; toluidine blue(TB) chromoscopy indicated a decline of acidic mucopolysaccharide synthesized; moreover, the deletion of nuclei and cytoplasm in the hypertrophic cells of depth layer could be universally found (remarkable in four cases), the shadows formed by remaining cellular membrane were also easily seen. (3) Ossification band was thinned, to about only a quarter of the thickness of that in non-KBD group, meanwhile the bone trabeculae was seldom seen (Tabel 6).

Table 6 Comparison of thickness of articular cartilage and growth plate cartilage of baby rats among the groups, 0.01 mm

Group	Number of cases	Articular c. $M \pm SD$	p	Growth plate c. $M \pm SD$	p	Ossification band $M \pm SD$	p
Control	8	20.9±3.8	>0.05	59.4±8.0	<0.05	179.4±20.4	>0.05
Non-KBD	5	21.4±3.0		69.6±7.2		167.5±23.8	
KBD	6	18.2±1.3	<0.01	29.5±4.4	<0.01	43.3±12.1	<0.01

As mentioned above, those baby rats, given birth and breast by such female rats fed with grain and water containing HA from KBD region, showed slow in growth, light in weight and decline of acidic mucopoly saccharide, degeneration of hypertrophic chondrocytes and some bone disturbance changes like the growth plate of their limb bones becoming thinned, and sparseness of bone trabeculae, all of which was quite similar to KBD in pathological observations. Thus, this kind of pathologically duplicated pattern for KBD again proved that the cause of KBD lies in the degenerated grain and drinking water contaminated by humic acids.

DISSCUSION

As an organic pollutant in drinking water, humic substances from the soil surrounding wells is a mixture of degradation products from animals and plants remains having been

decomposed by microorganisms. As a soluble part of the mixture, FAand HA were at least 80% of all organic matters in drinking water.

Up to now, there has been no report about the direct relations between humic substances and pathogens except for KBD. Some epidemiological surveys support KBD ctiological theory of intoxication due to organic matters in water, and suggest that this disease results from water polluted by humic acids (monografic research group, 1984; Wu, 1987; Wang, 1989a; 1989b). In our previous reports(Wang, 1985; 1986) it had been proven that much lower concentration (6.25 ppm) of HA or FA could lead cultured chondrocytes to be damaged, being very much nearer to the limit concentration (5 ppm) of them at KBD area. In KBD areas or invasion regions, the impairments of humic substances to chondrocytes were more serious than in non-KBD areas or non-attack regions. Following the improvement of drinking water (by digging deeper wells), humic acids decreased as a result, the incidence of KBD went down notably and thus the injury effects of this matter on chondrocytes decreased. Therefore, it may be safely said that the humic acids-degradation products of natural organic substances as contaminants in drinking water from wells may be the cause of the disease at KBD area.

On the other hand, degenerated grain due to fungus and other microorganisms also can release a series of degradation products which are similar to those in humic acids. It has been reported that the grain infected by Alternaria during harvest and storage under moist circumstances can produce some toxic substances (Seitz, 1984). Moreover, the dominant bacteria which infect grains at KBD region of Tianshui exactly were Alternaria (Bai, 1990). Yang (1989) has pointed out that in the grains contaminated by Fusarium oxysporum at KBD area the concentration of volatile basic nitrogen has a positive, unignorable correlation with the state of the disease. It follows that grains infected by microorganisms can yield many toxic substances, which may be some organic degradation products and perform the same function to cause KBD as humic acid does.

In this study, it has been demonstrated the actions and mutual relations of such three aspects as organic degradation products in grain and water, insufficiency of Se and "membrane defect" due to peroxidation injury in onset of KBD. First of all, it has been found that humic acids from KBD region can result in the damage to cultured chondrocytes, the decline in mucopolysaccaride synthesis in cellular matrix and the drop of GSH-Px activities. In addition, the GSH-Px activities in blood and tissues of rats fed with grains and water containing humic acids from KBD regions decrease notably while LPO level remarkably increases, which indicates that the organic degradation products in grain and drinking water in KBD areas are the cause of KBD. This damage effect of those substances to cell and tissues makes GSH-Px activity lower, i.e., reducing it's capacity of antioxidation so as to cause LPO level greatly increased inside cells, and resulting in peroxidation "membrane injury".

GSH-Px is a well known Se containing enzyme. Deficiency of Se in grain from KBD areas (8.4±1.1μg/kg in KBD area feed, 46.8±1.5μg/kg in non-KBD area feed) can undoubtedly affect GSH-Px activity in cells and tissues. Yet, an onset area of KBD would not be necessarily an area lack in Se. For example, in the world there are many areas with serious deficiency of Se, where quite a few kinds of injuries due to lack this element were found but not KBD. Many experiments on Se have never shown the pathological changes in cartilages and bones. In this study, in animal blood and tissues of KBD group the activity of GSH-Px went down but that of SOD was not affected by the presence of Se. This suggested that lack of Se was not the necessary factor for KBD onset; probably it influenced the effect of organic degradation products to cause KBD through changing the activity of GSH-Px inside cells. Therefore, insufficiency of Se may be an important condition for KBD attack, but not the pathogenic factor for it.

Secondly, not only a pathological process of chondral ossification disturbance like KBD was duplicated in long bones of growing baby rats fed with grain and humic acids from KBD areas, but also more serious damage of the muscle was found than that of the other tissues, which was consistent with the bone growing disturbance and disformation as well as myospasm or myophagism of KBD cases.

Se content determined in different tissues of seven normal rats showed that the level of the element in costal cartilage (21±22 ppb, hard to assay) was much lower than that in muscle (510±140 ppb), liver (1020±340 ppb) and blood (271.4±67.1 ppb). As a result, it needs to be proven through further studies whether or not the different impairments to respective tissues owing to organic degradation products have something to do with the Se concentration or its sufficient supply to every tissue or keeping GSH-Px activity of cells.

Up to now, scholars have ever believed that KBD is not an infectious disease. Nevertheless, the formation of organic degradation products is much influenced by several environmental factors such as microorganisms, weather and rainwater. In addition, there are so many and so complex pathogenic factors that there are great differences in the attack of KBD in different areas. Therefore, although organic degradation products which have been proven to be the cause of KBD, are responsible for the specificity of KBD onset, but their intensity to this disease depends upon the areas where they are. So, only by investigating and analyzing concretely the respective action of each conditional factor on the cause of KBD in a certain region we can gain a correct knowledge about it so as to carry out the effective prophylaxis and treatment for this disease.

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