Distribution of arsenic species and pathological characteristics of tissues of the mice fed with arsenic-supplemented food simulating rice

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ABSTRACT — The exposure and harm of arsenic have attracted wide attention. Rice is an arsenic-rich crop. The purpose of this study was to learn the distribution of arsenic species and the pathological changes in tissues of mice exposed to arsenic-supplemented food simulating rice. Test groups of mice were orally exposed with prepared arsenic feeds supplemented with four arsenic species (arsenite iAsIII, arsenate iAsV, monomethylarsonate MMA, and dimethylarsinate DMA) at three doses (total As concentration: 0.91, 9.1 and 30 μg/g), which simulated the arsenic species ratio in rice. After 112 days, the concentrations of the arsenic species in the spleen, thymus, heart, skin and hair were detected, and histopathology of the spleen, heart and skin was observed. Each arsenic species was detected and their total concentration increased in a dose-dependent manner with a few exceptions. One interesting phenomenon is that ratio of the organic arsenic to inorganic arsenic also increased in a dose-dependent manner. For the other, the order of tissues from high to low arsenic concentration was the same in the medium- and high-dose groups. The histopathological sections of the spleen, heart and skin showed dose-dependent debilitating alterations in tissue architecture. Hyperplasia, hyaline degeneration and sclerosis of fibrous connective tissue occurred in the spleen. Myocardial cell atrophy and interstitial edema occurred in the heart. Hyperpigmentation, hyperkeratosis and atypia of basal cells occurred in the skin. In summary, the long-term intake of high arsenic rice has a health risk. Further studies are needed to assess it.

Key words: Arsenic, Rice, Food exposure, Tissue-distribution of arsenic species, Lesions

INTRODUCTION

Metalloid elemental arsenic exists in the earth’s crust ubiquitously with the average concentration of approximately 5 mg/kg (Wu et al., 2016). It can be released to the soil and water biosphere in the toxic inorganic species through the action of weathering and geotherm metamorphism (O’Shea et al., 2015). Then, the toxic inorganic arsenic will be absorbed into cells by the phosphorus transporter or aquaporin (Maciaszczyk-Dziubinska et al., 2012). In an organism, the toxic inorganic arsenic can be metabolized into various other species by reduction or methylation. The methylation mainly occurs in animals and microorganisms, which possess the critical transforming enzymes, arsenic (+3 oxidation state) methyltransferase (AS3MT) and arsenic methyltransferase (ArsM), respectively. An interesting phenomenon is that dimethylated compounds are the final arsenic metabolites in most mammals, while fungi (Li et al., 2018; Verma et al., 2018) and bacteria (Chen et al., 2013) can produce the trimethylated arsenicals by arsenic methyltransferases. Higher plants can’t methylate inorganic arsenic, and
no methylase gene has been found in them (Hirano, 2020). However, plants can absorb organic arsenic from the soil, which is transformed from inorganic arsenic by rhizosphere microorganisms (Li et al., 2018).

There are many species of arsenic in organisms, which are directly absorbed from the environment or transformed in vivo. Arsenate (iAsV), arsenite (iAsIII), monomethylarsenate (MMA) and dimethylarsinate (DMA) are the most common species. The others include arsenic glutathione complexes, arsenosugars and arsenolipids, i.e. (Chávez-Capilla et al., 2016). In general, the toxicity of trivalent arsenic species, for example iAsIII, MMAIII and DMAIII, is more toxic than that of pentvalent arsenicals, for example iAsV, MMAV and DMAV, because trivalent arsenic species prefer to react with sulfhydryl groups of some enzymes (Moe et al., 2016). The arsenic in MMA and DMA is generally pentavalent without special notes, because MMA and DMA with trivalent arsenic exist only in organisms and are unstable.

Arsenite exposure is known to cause many adverse health effects in humans and animals, such as dermal lesions, and pulmonary, hepatic, renal, respiratory, cardiovascular, gastrointestinal, haematological, immunologic, developmental, neurological, reproductive, genotoxic, mutagenic, and carcinogenic damage (Mandal and Suzuki, 2002). The conventional wisdom is that the primary source of arsenic contact is drinking water (Renu et al., 2018). Also, arsenic in drinking water is mostly inorganic arsenic released from arsenic ore. Accordingly the arsenic toxicity study focused mainly on the exposure to inorganic arsenic (iAsV and iAsIII) in drinking water or liquid added inorganic arsenic into (Li et al., 2020; Chi et al., 2019; Lewchalermvong et al., 2018). However, rice, the staple food for most people, contains the highest concentrations of arsenic among food crops (Bakhat et al., 2017), because rice can enrich arsenic. And, eating rice has been considered to be the main route of arsenic exposure in many countries (Saifullah et al., 2018), except for countries that consume a lot of seafoods, such as Japan (Hata et al., 2007). Furthermore, rice matrix can prolong the residence time of arsenic and improve the biological accessibility of arsenic. Furthermore, the organic arsenic in rice may produce harmful effects or transform into more toxic species after entering the body (Yin et al., 2019; Chávez-Capilla et al., 2016). Thus, it is necessary to study the health hazard of arsenic exposure via rice. The total arsenic concentrations in rice grains generally range from 0.023 to 0.989 mg/kg in different countries (Althobiti et al., 2018; Prasanna and Rasool, 2018). The main arsenic species in rice include inorganic arsenic iAsIII and iAsV, organic arsenic DMA and MMA generally, with the different relative amounts depending on the country or region (Prasanna and Rasool, 2018; Guillod-Magnin et al., 2018). One study showed that the arsenic accumulation in tissues (liver, kidney, pancreas, testis and aorta) did not change significantly after intragastric administration of 240–1860 mg/kg refined rice or brown rice extract for 28 days (Lewchalermvong et al., 2018). In another study, it has been observed that the increase in the bladder, hair, lung, kidney, liver, blood, from high to low, accompanied with the oxidative stress, when the mice were treated with the arsenic-contaminated rice for 100 days (Souza et al., 2017). In our latest study, we also found the high accumulation of arsenic in the kidney, intestine and liver accompanied by pathological damage. There is obvious difference in the arsenic species distribution in faeces, urine, kidney, intestine and liver after mice were exposed to four arsenic species according to the arsenic composition in rice for 8 and 16 weeks (Wang et al., 2020b). However, the research is inadequate on the distribution of arsenic species and pathological changes in the small tissues, such as the spleen, thymus and heart of mammals exposed to arsenic through food, because it needs higher technology level to operate on them.

It is known that absorbed arsenic can accumulate in hair for the binding of arsenite to sulfhydryl groups in keratin (Hughes, 2006). Accordingly, the arsenic concentration in hair is always used as the indicator of chronic exposure to arsenic. High-level arsenic was found in the hair samples of people living in an environment with high arsenic concentrations in the drinking water (Hinwood et al., 2003), and hair of the mice from a habitat with high arsenic concentrations in nature (Erry et al., 2005). Skin is quite susceptible to arsenic exposure (Rahman et al., 2009). Arsenic has been detected in human skin in a high-arsenic area (Samanta et al., 2004), and also in the skin of mice exposed to arsenic in drinking water (Kenyon et al., 2008) and intravenously (Kato et al., 2017; Lindgren et al., 1982).

In order to understand the distribution of arsenic in vivo, this study determined the concentration of five kinds of arsenic species in the spleen, thymus, heart, skin and hair, by the efficient extract method and the detection method (HPLC-ICP-MS) with high sensitivity and specificity after the mice were exposed orally to food supplemented with four arsenic species simulating the arsenic species and composition in rice. Meanwhile, the pathological changes in the spleen, skin and heart were also observed in biopsies.
MATERIALS AND METHODS

The experimental process is shown in Fig. 1. The specific methods are described as follows.

**Design of arsenic-supplemented feed**

To avoid the result interference caused by innutrition or eating disorder in mice, the control group was fed with the standard feed including meat meal, cardamom, bran and various nutrient elements, which was prepared according to GB 14924.3-2010. The raw materials were dehydrated, crushed and made into granular feed, and then sterilised by irradiation. For its ubiquitous existence in the upper crust, arsenic is inevitably present in feeds. The background arsenic concentration in the feed was shown to be 0.4 μg/g dry weight (water content less than 10%) (Table S1), much lower than the maximum limit of 2 μg/g for animal feed (Adamse et al., 2017). In order to achieve the biological accessibility of arsenic similar to rice (about 80%) (Chávez-Capilla et al., 2016), the mixed aqueous solution of arsenic species was directly mixed into the raw material powder of mice feed, and then the granular feed was prepared. The mixed aqueous solution of arsenic species was designed according to the species and composition of arsenic in rice (Lewchalermvong et al., 2018; Nookabkaew et al., 2013; Ma et al., 2017; Huang et al., 2015; Wang et al., 2017), with iAsV (sodium arsenate), iAsIII (sodium arsenite), MMA (sodium monomethyl arsenate) and DMA (sodium dimethylarsenate) at 7.3%, 72.7%, 1.0% and 19.0% respectively. The simulation dose \( S, \) total concentration of arsenic \( (C_{tAs}) = 0.91 \mu g/g \) was calculated through the equivalent dose conversion between mice and humans after the daily arsenic intake from the rice was estimated, of which the details are described in our previous paper (Wang et al., 2020b). Then ten times of the simulation dose was taken as the middle-dose group \( (M, C_{tAs} = 9.1 \mu g/g) \). And 30 μg/g was taken as the high-dose group \( (H, C_{tAs} = 30 \mu g/g) \), in which the concentration of iAsIII is 1/3 of the LD50 of iAsIII for mice exposed orally (ATSDR, 2005). The concentration of the arsenic species and total arsenic in the prepared feed is shown in Table S1. The arsenic concentration in the drinking water was below the detection limit.

**Animal experiment design**

Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Pearl Laboratory Animal Sci. Co., Ltd. (Fig. S1). The sensitivity of mice to arsenic is related to gender, which may be due to the higher expression of estrogen receptor gene in female mice than in male mice, or related to gender-specific differences in bile acid composition after arsenic exposure (Che et al., 2019; Chi et al., 2016; Org et al., 2016). To avoid the influence of gender, male mice were selected as experimental objects.

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**Fig. 1.** Experimental flow chart. The experimental mice were fed with the arsenic-supplemented food simulating the arsenic species and composition of rice for 112 days. Then the arsenic species and pathology of the separated tissues were analyzed.

| Raw material powder | Mixed with arsenical solution | Feed for group C | Feed for group S: \( C_{tAs} = 0.91 \mu g/g \) | Feed for group M: \( C_{tAs} = 9.1 \mu g/g \) | Feed for group H: \( C_{tAs} = 30 \mu g/g \) |
|---------------------|-------------------------------|-----------------|---------------------------------|---------------------------------|---------------------------------|
|                     | \( C_{tAs} = 7.3\%\text{AS}^V + 72.7\%\text{AS}^{III} + 1.0\%\text{MMA} + 19.0\%\text{DMA} \) |                  |                                 |                                 |                                 |
| Feed the mice for 112 days (n=6) |
| Separate the tissues |
| Spleen, thymus, heart, skin, hair |
| Arsenic species determination |
| Histopathology analysis |
Twenty-four male C57BL/6 mice were randomly divided into four groups, with six mice in each group. One group was given control diet (group C), the other three groups were treated with arsenic-supplemented feed for 112 days. During the whole experiment, the mice could drink and eat freely. After 112 days, the mice were anesthetized with ether and sterilized in 75% ethanol. Then the mice were dissected on an ultra-clean platform. The spleen, thymus, heart, skin and hair of the mice were collected and weighed. All collected samples were transferred to −80°C for subsequent tests. Three tissues were collected and weighed. All collected samples were transferred to −80°C for subsequent tests. Three tissues were combined for arsenic species analysis and the other three tissues were for histopathological analysis in each group.

Histopathological examination of mice

The obtained mouse tissues were fixed with 4% paraformaldehyde for 16~24 hr, then embedded in paraffin and sectioned with a thickness of 4 μm. After dehydrating and hydration, the sections were stained with hematoxylin for 15 min, differentiated by 1% hydrochloric acid alcohol for 10 sec, eosin staining for 20 sec, dehydrated by gradient alcohol, transparent xylene, and sealed with neutral gum. Histopathological changes of mice were observed under a microscope.

Concentration determination of arsenic species in mice tissues

The extraction method with the highest extraction efficiency and with the lowest transformation possibility in arsenic species (Wolle and Conklin, 2018) was used. In brief, the ground tissues were extracted with 0.15 mol/L HNO₃ for 2.5 hr at 90°C. Five arsenic species (iAs(III), iAs(V), MMA, DMA and AsB) were separated and determined by HPLC-ICP-MS (Agilent 1260 Infinity II, Agilent 7800, Santa Clara, CA, USA). The Agilent 1260 Infinity II was equipped with an IonPac Ag 19 protective column (4 × 50 mm) and IonPacAS 19 separation column (4 × 250 mm). The external standard method was used for quantitative analysis. The standard substance was prepared by iAs(III) (0.233 mol/g), iAs(V) (1.011 mol/g), MMA (0.355 mol/g), DMA (0.706 mol/g) and AsB (0.518 mol/g). The chromatographic conditions were as follows: the mobile phase was 25 mmol/L ammonium carbonate, pH = 9.5, the flow rate was 1.0 mL/min, and the column temperature was 25°C. The calibration points were 0, 2.5, 5, 10, 50 and 100 μg/L, respectively. Each sample was measured three times in parallel.

The analytical performances of the arsenic species determination with the external standard method are shown in Table S2. In order to reflect the recovery of the arsenic determination from tissues indirectly, the recovery tests using feces and CRMs (Green Chinese onion, GBW10049, Rice, GBW100358, Pork liver, GBW10051, Yellow-finn tuna, GWB08573, and lobster hepatopancreas, NRC TORT-3) were done, and the data are shown in Table S3 and Table S4.

RESULTS

Total concentration of five arsenic species

The total concentration of five arsenic species increased in a dose-dependent manner in every tissue, except skin (Fig. 2 and Table S5). AsB, another arsenic species which might be from biotransformation except a small amount from feed background in mice (Wang et al., 2020a, 2020b), was also analysed together with the four species supplemented to feed since it was detected in every tissue. As shown in Fig. 2, arsenic accumulation showed tissue favouritism, for the total concentration of five arsenic species presenting different levels when the tissues were exposed to the same dose. For another, the total concentrations of five arsenic species in the spleen, thymus and heart are not particularly higher (Fig. 2A), while the concentrations of skin and hair are significantly higher than that of blood (Fig. 2B).

Concentration of arsenic species

The concentration of every arsenic species increased with the increase of exposure dose, except iAs(V) in thymus, skin, iAs(III) in skin and AsB in the spleen (Fig. 2 and Table S5). And the trend of DMA was the most obvious. It may be related to that DMA is usually the downstream or end product of methylation metabolism of inorganic arsenic in most mammals (Waters et al., 2004).

The chromatograms showed an interesting trend in that the relative peak height of iAs(III) and iAs(V) in each tissue decreased with the increase of exposure dose. In contrast, the relative peak height of DMA and MMA increased with the increase of exposure dose (Fig. 3). For this reason, the ratio of the organic arsenic to inorganic arsenic was calculated, and it also increased in a dose-dependent manner (Fig. 4A).

Another interesting phenomenon is that the order of tissues according to the ratio of the organic arsenic to inorganic arsenic is the same in the middle- and high-dose groups. The values from high to low are the heart, thymus, blood, spleen, hair and skin (Fig. 4B). This repeatability between the middle- and high-dose groups can increase the credibility of the experimental results obtained from the smaller sample sizes.
Organ coefficients of the spleen, thymus and heart in mice

After 112 days of exposure to arsenic in food, the mice and separated tissues were weighed (the original data are shown in Table S6 in supplementary material), and the organ coefficients were calculated. As can be seen in Table 1, there was no significant difference \((P > 0.5)\) in the organ coefficients of the spleens and hearts among the four groups, while the organ coefficients of thymuses showed the difference \((P < 0.05)\) among the four groups. The organ coefficient of thymus in group S was smaller than that in group C and group H statistically, so only the specific reasons need to be further studied.

Histopathology changes in the heart, spleen and skin

Pathological analysis of the spleen (Fig. 5) showed, compared with the control group, with the increase of arsenic dose, the structure of splenic corpuscle and lymph node in white pulp (red arrows) was disordered, the proportion of red pulp (yellow arrows) was decreased, and a large amount of hemosiderin was found in lymphocytes (purple arrows). With the increase of arsenic dose, the fibrosis degree of spleen tissues was aggravated (orange arrows), the fibrosis and sclerosis degree of splenic tissues in group H was more serious in the interstitial tissue, as well as hyperplasia and hyaline degeneration of small vessel wall (sky blue arrows), which resulted in thickening of vessel wall and narrowing of its lumen.

Pathological analysis of the heart (Fig. 6) showed, compared with the control group, with the increase of arsenic dose, myocardial cells were atrophied (sky blue arrows), lipofuscin granules (green arrow) were observed in the cytoplasm of groups H and H, and haemorrhage (yellow arrows) was seen in intercellular space. Myocardial interstitial edema (purple arrows) and cytoplasmic vacuolation (blue arrows) were observed in groups S and H. Severe myocardial atrophy and increased intercellular space (black arrows) were observed in some areas of groups M and H.

Pathological analysis of the skin (Fig. 7) showed, compared with the control group, with the increase of arsenic dose, the number of keratinocytes decreased (red arrows) and the keratinocytes gradually break and fall off (green arrows), resulting in the thinning of the cuticle. Also, a large number of melanin particles (pigmentation) were deposited in the basal layer of the skin (black arrows). Hyperkeratosis, obvious desquamation (green arrows) and pigmentation (black arrows) of skin were observed in groups M and H, as well as the spinous layer cells gradually atrophied and disappeared (yellow arrows). In group H, the skin was atrophied and thinned, the disorders of basal layer cells (larger and dark stained nucleus) show its atypia (orange arrows) and the tendency of squamous cell canceration, the edematous stroma and collagen fibre rupture (purple arrows) in dermal skin, the number of skin appendages (sebaceous glands, sweat glands and hair follicles) decreased, and the structure was disordered (blue arrows) too.

**DISCUSSION**

Arsenic pollution has become a global health threat, and the research on arsenic toxicity has become a hot spot. In previous studies, the main arsenic species were
Fig. 3. Chromatograms of arsenic species in the spleen, thymus, heart, skin and hair of mice exposed to arsenic in food for 112 days. After 112 days of arsenic exposure, tissues were separated from the euthanized mice. Then the tissues were ground and extracted. Subsequently, the five arsenic species were detected by HPLC-ICP-MS and analysed.
inorganic arsenic, and the exposure routes were mainly drinking water (Li et al., 2020; Chi et al., 2019; Lewchalermvong et al., 2018). However, organic arsenic absorbed is also an important source of toxicity. Furthermore, rice, an important staple food that can enrich arsenic (Bakhat et al., 2017), has become the main way of arsenic exposure in many countries (Saifullah et al., 2018), and its health hazards need to be evaluated. Rice contains not only inorganic arsenic, but also organic arsenic. The toxicological effect of arsenic exposure in food is probably different from that in drinking water. Therefore, the study of arsenic exposure in rice is more conducive to assess the health risk of arsenic pollution in the environment. In this study, after 112 days of feeding, the tissue distribution of arsenic in the spleen, thymus, heart, skin and hair was detected, and the pathological changes of these tissues were analysed.

Our data showed that the five arsenic species and their total amount increased in a dose-dependent manner with a few exceptions, and there was tissue preference for arsenic retaining. In addition, a series of degenerative structural changes were observed at the pathological level, as Figs. 5–7 shown. And the degree of damage was also dose-dependent, which is consistent with the increase of arsenic concentration in tissues.

The spleen and thymus are important immune organs. Acute arsenic exposure to sodium arsenite in drinking water can increase the concentration of arsenic (including iAs, MMA and DMA) in the thymus and spleen of mice (Duan et al., 2015), and causes oxidative stress and inflammatory reaction (Duan et al., 2015, 2017; Jamal et al., 2020). In this study, long-term exposure to four species of arsenic supplemented to food resulted in a significant dose-dependent increase of five arsenic species of spleen and thymus in the middle- and high-dose groups (Fig. 2A and Table S5). The difference is that the proportion of organic arsenic in the thymus is higher than that in blood, while that in the spleen is lower than that in blood in the middle- and high-dose groups (Fig. 4B).

The physiological lesion of spleen was analysed. As shown in Fig. 5, there was a large number of hemosiderin-containing cells in the spleen and this might be due to the arsenic-induced inflammation and autophagy. The mRNA of key inflammatory mediators in the spleen, including Tgf-beta, Tnf-alpha, Il-12, Il-1beta and Il-6 were significantly increased in arsenic-treated mice (Duan et al., 2017). Also, arsenic could further up-regulate Hsp90, eventually resulting in stabilisation of an impor-

| Table 1. Body weight and organ coefficients\* of mice exposed to arsenic in food for 112 days. |
|-----------------------------------------------|
| Group C | Group S | Group M | Group H | $P^*$ |
|---------|---------|---------|---------|------|
| Spleen  | 0.27 ± 0.02 | 0.33 ± 0.08 | 0.33 ± 0.03 | 0.27 ± 0.04 | 0.327 |
| Thymus  | 0.14 ± 0.02 | 0.09 ± 0.02 | 0.14 ± 0.02 | 0.18 ± 0.05 | 0.042 |
| Heart   | 0.62 ± 0.11 | 0.61 ± 0.06 | 0.69 ± 0.01 | 0.57 ± 0.06 | 0.167 |

\*: (g/g BW)×100. \*: $P$ values of the four groups were obtained from ANOVA difference analysis.
tant autophagy initiating factor, Beclin-1 (Jamal et al., 2020). Red blood cells escaping from blood vessels with inflammation are ingested by macrophages and degraded by lysosomes, which makes Fe^{3+} from erythrocyte hemoglobin combine with protein to form ferritin particles. Some ferritin particles aggregate into large brown-yellow refractive particles under a light microscope, which is the haemosiderin shown in Fig. 5. The repair of inflammatory and necrotic substances by organs and tissues will result in tissue fibrosis, as shown in Fig. 5.

Cardiotoxicity is one of the most important hazards of arsenic exposure. However, previous studies mainly focused on the cardiotoxicity of liquid arsenite or sodium arsenite in rats. Arsenite in water exposure orally sig-
nificantly increased the tissue arsenic deposition, micronuclei frequency (Hemmati et al., 2018), myocardial arsenic content, serum cardiac marker enzyme activities (Panneerselvam et al., 2020), and pathological damage (Liang et al., 2020) in the rat. In this study, exposure to four species of arsenic supplemented to food also increased the content of arsenic in the heart in a dose-dependent manner (Fig. 2). Also, a very interesting phenomenon was also observed, that is the highest proportion of organic arsenic in the heart among the tissues detected (Fig. 4B). The high proportion of organic arsenic may have resulted from the tissue favouritism for arsenic accumulation (Kato et al., 2017; Kenyon et al., 2008). The main lesions of arsenic exposure includ-

Fig. 6. Pathological changes of tissues in mice fed with arsenic-supplemented food for 112 days. After 112 days of arsenic exposure, the separated heart tissues were fixed with 4% paraformaldehyde solution, then were used to prepare paraffin sections. The sections were stained with hematoxylin and eosin and then observed under a microscope. Sky blue arrow, atrophied myocardial cells. Green arrow, lipofuscin granules. Yellow arrow, haemorrhage. Purple arrow, interstitial edema. Blue arrow, cytoplasmic vacuolation. Black arrow, increased intercellular space by myocardial cell atrophy.
ed the atrophy of myocardial cells and the appearance of obvious intercellular spaces, in which the unequal amount of blood exposure or a large amount of edema fluid could be seen (Fig. 6). These pathological changes should be related to oxidative stress, inflammatory and apoptosis induced by arsenic (sodium arsenite) in cardiac tissue. In rats treated with sodium arsenite orally (Oyagbemi et al., 2017) and intraperitoneally in water (Bharti et al., 2012), the amount or activity of some enzymes related to oxidative stress increased in the cardiac tissues. In another study, the expression of some inflammatory cytokines and Bax/Bcl-2 increased in...
mRNA level in rats exposed sodium arsenite (Prasanna and Rasool, 2014). However, the molecular events after several arsenic exposures, including organic arsenic to inorganic arsenic in food, might be different from only sodium arsenic exposure in drinking water, which need further study.

Arsenic was enriched in hair and skin, in which the arsenic concentration was much higher than that in the blood (Fig. 2). That the trivalent arsenic is prone to binding with the sulphydryl group in keratin irreversibly may be one of the reasons for the accumulation in hair and skin (Hughes, 2006). Therefore, hair and skin are the important destinations for arsenic in other tissues, except for excretion through urine and faeces. It is worth noting that the skin not only had a high total arsenic concentration, next only to hair (Fig. 2B), but also had the highest proportion of inorganic arsenic among all the detected tissues (Fig. 4B). Furthermore, inorganic arsenic in the skin is mainly trivalent inorganic arsenic (Fig. 2B), which is considered to have the highest toxicity in several tested species. This should be used to explain the fact that the skin lesions are the most common and initial clinical symptoms of arsenicosis (Rahman et al., 2009).

Skin is an important target organ of endemic arsenicosis, and its clinical diagnosis mainly depends on skin triad (hyperkeratosis on the palms and soles, cutaneous hyperpigmentation and hypo-pigmentation). There are already a lot of studies on the pathogenesis of skin lesions after arsenic exposure through drinking water, but few on food exposure. In our data (Fig. 7), low dose exposure will lead to pigment deposition and hyperkeratosis, which are the common and typical symptoms. With the increase of dose, the cells in the basal layer of animal skin showed heteromorphism, which indicated an increased risk of squamous cell carcinoma. Arsenic can change the normal metabolism of melanin in the process of melanin synthesis and transportation, resulting in abnormal pigmentation (Isokpehi et al., 2012). The mechanism of skin keratinisation and carcinogenesis is generally considered to be related to the arsenic toxicities, such as inhibiting differentiation (Perez et al., 2003), promoting proliferation (Huang et al., 2013) and causing oxidative stress injury (Lee et al., 2013) of keratinocytes.

According to current pathological methods, it is difficult to quantify the pathological changes from the perspective of histopathology, which was usually judged by morphological observation and description. Specifically speaking, with the increase of arsenic dosage, the fibrosis degree of spleen tissue in mice was significantly aggravated, the myocardial cells of the heart began to atrophy and myocardial interstitial edema, which reflected the irreversible damage of myocardial cells, and the basal layer cells in skin appeared precancerous lesions as well, these are all very serious injuries.

An interesting phenomenon is that the ratio of organic arsenic to inorganic arsenic increases with the exposure dose (Figs. 2 and 4A). This phenomenon may be explained from two aspects, the methylation by the intestinal microorganisms and by the methylase in tissues. The number of arsenic-related genes in microorganisms isolated from a high-arsenic environment is four times that from other environments (Li et al., 2014). Also, the intestinal microbial community in mice will change after long-term arsenic exposure (Wang et al., 2020b). Thus, it is reasonable to speculate that the higher the exposure dose, the more arsenic transformation-related microorganisms (genes), including arsenic methylated microorganisms (genes), the higher the proportion of arsenic methylation in the intestinal tract. Accordingly, the proportion of organic arsenic in blood increased with the exposure dose, and the proportion of organic arsenic in tissues transported from blood also increased with the exposure dose. Whether methylase in various tissues can be induced by high-dose arsenic is not clear enough. The arsenite methyltransferase activity wasn’t induced by iAsV in drinking water in mice (Healy et al., 1998), while the mRNA-expression level of As3mt changed after iAsS exposure of some mice strains (Stýblo et al., 2019). Folic acid supplementation could rapidly and significantly increase methylation of inorganic arsenic to DMAs in human (Bozack et al., 2019), which reflects that arsenic methylase or its activity can be induced by some factors.

In conclusion, exposure to food supplemented with the arsenic species and composition simulating rice increased arsenic concentration in the spleen, thymus, heart, skin, and hair of mice, and caused a series of visible degenerative pathological damage. Therefore, it can be inferred that long-term intake of high arsenic rice has a health risk. More systematic studies are needed to evaluate it.

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Conflict of interest---- The authors declare that there is no conflict of interest.
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