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Assessment of anti-inflammatory effects of Japanese Kampo medicine and functional foods

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ABSTRACT

Traditional Japanese drugs called Kampo medicine are widely used in Japan. Each Kampo medicine consists of several crude drugs, most of which are derived from medicinal plants. Clinical administration has empirically evaluated the effects of Kampo medicine. In contrast, functional foods are prepared from foods and edible plants (e.g., herbs, vegetables, and fruits). Due to the relatively low content of pharmacologically active constituents in functional foods, their effectiveness has not been well evaluated and thus should be better investigated. Kampo medicine and functional foods have beneficial effects for humans, and many of them exhibit anti-inflammatory effects. Here, we discuss the principles and methods to assess the anti-inflammatory effects of functional foods and Kampo medicine.

To investigate pharmacological effects of functional foods and Kampo medicines, their constituents should be isolated to identify their chemical structures. Cell-based studies are commonly performed to evaluate anti-inflammatory effects of the constituents in Kampo

medicine and functional foods. Primary cultured rat hepatocytes are used and produce pro-inflammatory mediators, including nitric oxide. When an extract from a Kampo medicine, functional food, or a respective constituent is added to the medium, pro-inflammatory mediator production decreases, and the anti-inflammatory activity is estimated. Animal experiments have been performed using disease models, such as the endotoxemia model for animals, to which bacterial endotoxin is administered. Administering an effective functional food or Kampo medicine improves the survival of the model animals. The action of the anti-inflammatory effects of functional foods and Kampo medicines can be investigated by the above-mentioned methods. The studies using cells and animals will provide a basis for the safe and effective use of functional foods and Kampo medicine in humans to treat diseases or improve health conditions.

Keywords: herbal drug, Kampo medicine, nitric oxide, inflammation, hepatocytes.

COMPARISON OF KAMPO MEDICINE AND FUNCTIONAL FOODS

Traditional Japanese medicine called Kampo medicine is widely used in Japan. Kampo medicine has the roots in ancient Chinese medicine, which was directly incorporated from China in the 7th century by diplomatic delegations, such as Kenzuishi and Kentoshi. In the 18th century (late Edo period), Chinese medicine was uniquely modified and adopted to Japanese people (i.e., Japanized), and Japanese Kampo medicine was developed. Although there was a decline of Kampo medicine due to modernization by Western medicine during the late 19th century (Meiji period), education about Kampo medicine is currently included in Japanese curricula in most schools of medicine. Therefore, medical doctors in Japan prescribe the Kampo formulae to treat diseases (e.g., influenza, cold, and postmenopausal syndrome) and predisease, which is presymptomatic disease or an unhealthy condition without clear symptoms.

Each Kampo medicine consists of several “crude drugs” most of which are derived from medicinal plants. Therefore, a Kampo medicine is also designated a Kampo formula. During Japanization in the 18th century, the formulas showing adverse effects have been eliminated. The specifications and standards of general Kampo formulas and crude drugs are strictly defined by the *Japanese Pharmacopoeia*, which describes 34 Kampo formulas and 157 constituent crude drugs [1]. When other Kampo formulas that are not included in the *Japanese Pharmacopoeia* are added, 148 total Kampo formulas are used in Japan at present. Crude drugs of Kampo medicine are extracted by hot water and dried *in vacuo*, and the resultant powder is granulated and packaged in small sachets as Kampo extract products.

Edible plants, including herbs, fruits, and vegetables, can be used as "functional foods," which have certain function(s). The definition of functional foods is different depending countries and laws. For example, the Functional Food Center defines functional foods as "natural or processed foods that contain biologically active compounds; which, in defined, effective non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, for the prevention, management, or treatment of chronic disease or its symptoms" [2].

Pharmacologically active constituents are more highly abundant in the crude drugs of Kampo medicine than in foods and functional foods. In Japan, the usage of herbs and plants is classified by pharmacological activity into two classes (primarily medical use and nonmedical use) by the Ministry of Health, Labor and Welfare, Japan [3]. Licorice (roots and stolons of *Glycyrrhiza uralensis* L.) is used as a constituent crude drug of Kampo medicine and a sweetener for foods. A part of one plant species is used for the crude drugs of Kampo medicine, and the other parts are used for foods. For example, the peel of Satsuma mandarin (*Citrus unshiu)* is used as a crude drug, whereas the inside is eaten as a fruit. The features of Kampo medicine are compared with those of functional foods in Table 1.

Table 1. Comparison of Kampo medicine and functional foods

	Kampo medicine	Functional foods
Components	Several crude drugs that are selected as a formula	Foods, processed foods, or constituents derived from these foods
Action	Combination of empirically known effects of crude drugs	Mild pharmacological activity
Total pharmacological potency	High	Lower than drugs
Pharmacological potency of constituents	High (principal constituents); low to medium (other constituents)	Low to medium (many cases)*
Constituent content	High	Low
Purpose of use	To treat diseases and predisease	To improve health conditions

* See details in the text.

When comparing pharmacological potencies of Kampo medicines, those of functional foods are lower. When a functional food possesses high potency that is comparable with a drug, it may be classified to a drug. Therefore, pharmacologically active (bioactive) constituents of functional foods generally show low to medium potency, whereas pharmacologically active constituents (i.e., principal constituents) in Kampo medicine show high potency. A highly

potent constituent is sometimes present in a functional food, although its content is generally very low. Because the pharmacological effect of a constituent is determined by its pharmacological potency and content, the total potency of the functional food is not high in these cases.

VERIFICATION OF PHARMACOLOGICAL EFFECTS ON HUMANS

Functional foods and Japanese Kampo medicine must have beneficial effects on humans. The effects of Kampo medicine have been empirically evaluated by clinical administration for a long time. Due to a low abundance of pharmacologically active constituents, the pharmacological effectiveness of all functional foods has not been well evaluated. Therefore, the effects of functional foods on humans should be better investigated to increase the numbers of people who take functional foods to improve their health conditions. A safety test and toxicity assessment of functional foods are essential. Furthermore, a randomized, double-blind study with a placebo control is preferable to analyze the effects of the supplementation of a functional food.

Several levels are used to qualify functional foods and Kampo medicine (Figure 1). Significant evidence of pharmacological activity at the cell and animal levels is essential for human use of functional foods, although functional foods are prepared from foods or edible plants. To develop a new drug from functional foods or Kampo medicines, the order from cells to animals to humans is strict. A constituent in functional foods or Kampo medicine that is pharmacologically effective can serve as a lead compound for the synthesis of a new drug with maximum effect.

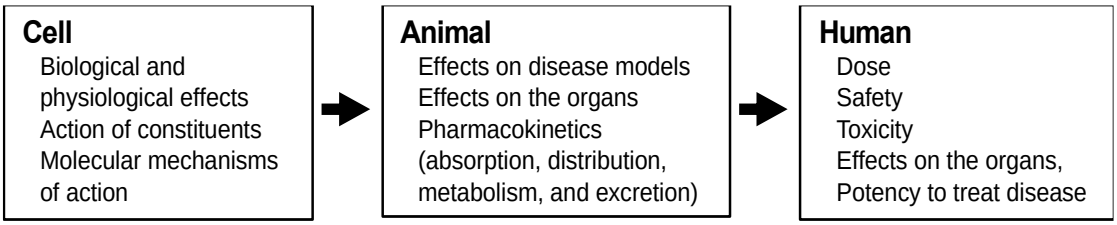

Figure 1. Qualification and development of drugs and functional foods. The points to be examined at each level are indicated in the boxes.

ISOLATION OF PHARMACOLOGICALLY ACTIVE CONSTITUENTS FROM CRUDE DRUGS AND FUNCTIONAL FOODS

Purification of their constituents are essential to clarify pharmacological effects because each crude drug from Kampo medicine or a functional food consists of many constituents. For a variety of pharmacological analyses, the constituents should be isolated from crude drugs of Kampo medicine or functional foods to identify their chemical structures. Isolating and identifying principal constituents from functional foods is more difficult than isolating crude

drugs from Kampo formulas due to the low content and low pharmacological activity of the constituents in functional foods.

Extracts are prepared from functional foods or crude drugs of Kampo medicine using hot water or an organic solvent (e.g., methanol and ethanol). Hot-water extraction (decoction) is often used to prepare Kampo medicine, and hydrophilic constituents are more recovered by this method than methanol extraction. Next, the extract is often fractionated by hydrophobicity into three crude fractions, including ethyl acetate-soluble, *n*-butanol-soluble, and water-soluble fractions [4]. The resultant fractions are further purified by various methods, such as silica gel chromatography, thin-layer chromatography (TLC), or high-performance liquid chromatography (HPLC), to identify the effective constituents. Their chemical structures are determined by nuclear magnetic resonance (NMR) and mass spectra analyses [for example, 5,6].

Anti-inflammatory effects were examined by monitoring the production of a pro-inflammatory mediator. Therefore, measurement of the levels of pro-inflammatory mediators will help isolation and identification of pharmacologically active constituents in both Kampo medicines and functional foods. For example, adenosine was identified as a hepatoprotective constituent in standardized extract of cultured *Lentinula edodes* mycelia (ECLM, AHCC®) [7].

EVALUATION AND DISCUSSION OF ANTI-INFLAMMATORY EFFECTS USING CELL-BASED ASSAYS

Once the constituents are purified from crude drugs of Kampo medicine or functional foods and identified, they are ready to be examined. Cell-based studies are common for a variety of functional food and Kampo medicine analyses. For example, primary cultured rat hepatocytes are used as a liver injury model. In response to the pro-inflammatory cytokine interleukin (IL)-1β, pro-inflammatory mediators, such as nitric oxide (NO), pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α) and IL-6, and chemokines are produced [8,9]. These pro-inflammatory mediators in the conditioned medium and the cells can be detected by enzyme-linked immunosorbent assay (ELISA) and western blot analysis [for example, 9]. The levels of their mRNAs are analyzed by northern blot analysis and reverse transcription-polymerase chain reaction (RT-PCR).

When a functional food, Kampo medicine, or a constituent is added to the medium, the production of these pro-inflammatory mediators is decreased in a dose-dependent manner. A half-maximal inhibitory concentration (IC_{50}) is calculated unless cytotoxicity is observed. The IC_{50} values are used to compare anti-inflammatory potencies. Macrophages, including macrophage lines (*e.g.*, RAW264.7), are often used to evaluate anti-inflammatory effects in response to the bacterial endotoxin lipopolysaccharide (LPS) [9,10]. Macrophages produce pro-inflammatory mediators, including NO, prostaglandins, and pro-inflammatory cytokines. Differences in IC_{50} values for the suppression of NO production have been found between

macrophage lines and rat hepatocytes [9]. Therefore, the anti-inflammatory activity of functional foods and Kampo medicine can be easily estimated using these systems.

The effects on the expression of many genes encoding inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX2; i.e., prostaglandin-endoperoxide synthase 2, which produces prostaglandin H_2), pro-inflammatory cytokines, and chemokines can be analyzed in cells at the molecular level by a variety of methods. These methods include those such as western blot analysis, ELISA, northern blot analysis, and RT-PCR. Transcriptional regulation by transcription factors and post-translational regulation, such as phosphorylation, can be also analyzed to examine the effects or functional foods and Kampo medicine. Functional foods were also investigated using animal and human studies (Table 2). These approaches may clarify the molecular mechanisms of action of these drugs and the pathophysiology of various diseases.

Table 2. Anti-inflammatory effects of functional foods

Constituent	Origin	Verification*	References
Standardized extract of cultured *Lentinula edodes* mycelia (ECLM, AHCC®)**	*Lentinula edodes* (mushroom)	H, A, C	Matsui et al. [11], Nakatake et al. [12], Matsui et al. [13]
Chlorogenic acid	Flowers and buds of *Lonicera japonica*, coffee, etc.	H, A, C	Ohno et al. [4], Farah et al. [14], Xu et al. [15]
Curcumin	Rhizome of *Curcuma longa* (turmeric)	H, A, C	Rahmani et al. [16], Nakatake et al. [17]
Standardized extract of *Asparagus officinalis* stem (EAS, ETAS®50)**	Stems of *Asparagus officinalis* (asparagus)	H, A, C	Ito et al. [18,19], Nishizawa et al. [20]
Standardized oligomerized-polyphenol from *Litchi chinensis* fruit extract (OPLFE, Oligonol®)**	Fruit of *Litchi chinensis* (lychee)	H, A, C	Ogasawara et al. [21], Nishizawa et al. [22], Yamanishi et al. [23]
Perilla extract	Leaves of *Perilla frutescens* (green perilla)	H, A, C	Ueda & Yamazaki [24], Ueda et al. [25], Baba et al. [26], Nakajima et al. [27]
pyroGlu-Leu (pEL)	Wheat gluten hydrolysate	H, A, C	Kiyono et al. [28], Oishi et al. [29], Sato et al. [30]

* H, human trial, or used as a food or a Kampo medicine; A, animal experiment; C, cell-based assay. ** AHCC®, ETAS®50, and Oligonol® are trademarks of Amino Up Co., Ltd., Sapporo, Japan.

The anti-inflammatory effects of many Kampo formulas (e.g., Inchinkoto, Ninjinyoeito, and Saireito) and their constituents (e.g., flavonoids, alkaloids, and phenylpropanoids) have been analyzed, as shown in Table 3. Please note that some of the constituents also included in foods and functional foods (e.g., chlorogenic acid). In Japan, there is legal classification, which

discriminates crude drugs (i.e., primarily medicinal use) and others (e.g., foods) [3], whereas functional foods are differently defined the Japanese laws.

Table 3. Anti-inflammatory effects of crude Kampo medicine drugs and their constituents

Constituent	Origin	Use*	References
Inchinkoto	Three crude drugs (Kampo formula)	K	Arai et al. [31], Matsuura et al. [32]
Ninjinyoeito	Twelve crude drugs (Kampo formula)	K	Cyong et al. [33], Tanaka et al. [34]
Saireito	Twelve crude drugs (Kampo formula)	K	Watanabe et al. [35], Miki et al. [36]
Gomisin N	Fruit of *Schisandra chinensis*	K	Oh et al. [10], Takimoto et al. [37]
Isoliquiritin	Roots and stolons of *Glycyrrhiza uralensis* (licorice)	F, K	Tanemoto et al. [6], Asl & Hosseinzadeh [38]
Limonin	Bark of *Phellodendron amurense*	K	Fujii et al.[5], Leu et al. [39]
Nobiletin	Peel of *Citrus unshiu* (Satsuma mandarin)	F, K	Nogata et al. [40], Yoshigai et al. [41]
Shisoflavanone A	Leaves of *Perilla frutescens* (green perilla)	F, K	Ueda & Yamazaki [24], Ueda et al. [25], Baba et al. [26], Nakajima et al. [27]

K, used for Kampo medicine. F, used as foods or functional foods.

EVALUATION AND DISCUSSION OF ANTI-INFLAMMATORY EFFECTS USING ANIMAL MODELS

Animal experiments using disease models are often performed to evaluate anti-inflammatory effects of not only drugs, but also Kampo medicines and functional foods. Sepsis is characterized by systemic inflammatory responses induced by infection [42]. Initially, bacterial endotoxin (lipopolysaccharide, LPS) causes endotoxemia, which triggers systemic inflammation, followed by multiple organ failure (i.e., sepsis). Therefore, endotoxemia/sepsis model animals are widely used to evaluate anti-inflammatory effects (Table 4). LPS and D-galactosamine is administered to rats or mice to evoke endotoxemia and sepsis with liver failure [43,44]. Although LPS alone causes endotoxemia, it does not lead to sepsis in all animals [45]. D-Galactosamine (i.e., an inhibitor of RNA synthesis) causes a liver injury (hepatitis) and provokes sepsis when administered with LPS [46]. The levels of pro-inflammatory mediators can be monitored in these model animals.

Table 4. Examples of endotoxemia and sepsis models in mice and rats

Method	Description	Reference
D-Galactosamine and LPS administration	D-Galactosamine and LPS are simultaneously administered; endotoxemia and sepsis are associated with D-galactosamine-induced liver failure	Tanaka et al. [43], Miki et al. [44]
Partial hepatectomy and LPS administration	After partial (70%) hepatectomy, LPS is administered; endotoxemia and sepsis are associated with liver failure by partial hepatectomy	Nakatake et al. [12], Tsuji et al. [47]
Cecal ligation and puncture (CLP)	After ligation, the caecum is punctured to release the contents (feces) into the peritoneal cavity and evoke peritonitis	Wichterman et al. [49], Dejager et al. [50]

Alternatively, a partial hepatectomy followed by LPS administration is used as another endotoxemia model with liver failure [47]. In these experimental models, endotoxemia initiates after injection of LPS. Administration of an effective functional food or Kampo medicine improves the survival of endotoxemia model animals. For example, a standardized extract of cultured *Lentinula edodes* mycelia (AHCC®) had protective effects on endotoxemia model rats with liver failure after hepatectomy [12]. The mRNAs encoding pro-inflammatory mediators in the partial hepatectomy/LPS model rats expressed more than those in the D-galactosamine/LPS model rats [48].

The cecal ligation and puncture (CLP) model is also used to mimic human sepsis [49,50]. This sepsis model leads to the growth of intestinal bacteria and the subsequent release of LPS from dead bacteria in parallel during peritonitis caused by the bacteria. The released LPS leads to endotoxemia, and then sepsis occurs with multiple organ failure. Because the onset and severity of sepsis may change by each animal, the reproducibility of this model is not very high.

CONCLUSION

The principles and methods to assess anti-inflammatory effects of Kampo medicine and functional foods are reviewed. The same methods can be applied to assess the pharmacological effects of both functional foods and Kampo medicine at the cell and animal levels. These studies will provide evidence for the mechanisms of action of functional foods and Kampo medicines. Furthermore, accumulating data will become the basis for the development of safe and effective functional foods and will improve health conditions and prevent disease.

List of Abbreviations: NO, nitric oxide; IL, interleukin; IC_{50}, half-maximal inhibitory concentration; TNF-α, tumor necrosis factor α.; LPS, lipopolysaccharide.

Competing Interests: None declared.

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