Contents of Lecithin and Choline in Crude Drugs[1)]

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The determination of lecithin and choline in crude drugs was established by a combination of high performance liquid chromatography (HPLC) with electrochemical detector (ECD) and enzyme reaction. Lecithin in crude drugs extracted with a mixture of chloroform–methanol (2 : 1) at room temperature was hydrolyzed by phospholipase D. The hydrolyzate was injected to HPLC, and choline was separated from impurities by reverse phase column. The choline was converted to betaine and hydrogen peroxide by passing through column packed with immobilized choline oxidase. This hydrogen peroxide was detected by ECD. The peak area of hydrogen peroxide derived from lecithin was proportional to the concentration of lecithin from 0.10 to 1.52 μg/ml. Choline in crude drugs was extracted with ethanol under reflux and determined under the same HPLC conditions as lecithin. The peak area of hydrogen peroxide derived from choline was proportional to the concentration of choline from 0.01 to 0.45 μg/ml. The contents of lecithin and choline in 31 kinds of crude drugs were determined by these established methods. The results showed that Cervi Parvum Cornu, Kokurozin, Foenigraeci Semen and Psoraleae Semen contained more lecithin than other crude drugs, while Angelicae Radix, Foenigraeci Semen, Psoraleae Semen, and especially Hippocampus were found to contain more choline than other crude drugs.

Keywords crude drugs; lecithin; choline; electrochemical detection HPLC; phospholipase D; choline oxidase

Lecithin is present in brains, nerves, egg yolk and seeds of plants. Since this substance controls the increase of total cholesterol value and total lipid value in the blood serum, it is used in medical treatment of hyperlipemia and other conditions.[2)] Choline is present in various kinds of animal and plant tissue, especially in brains and bile, under combined or liberated situations. Choline is related to various important physiological actions, such as the regulation of osmotic pressure of cell membrane, anti-lipotropic factor action as one of vitamin B complex, the regulation of blood pressure and nerve transmission as acetylcholine. The existence of choline in Ginseng Radix,[3)] Foenigraeci Semen[4)] and Angelicae Radix[5)] has been reported, but there are no reports of lecithin. As part of an analysis of constituents of crude drugs, the high performance liquid chromatography (HPLC) method was used with an electrochemical detector (ECD) after treatment with an enzyme. Lecithin and choline contents were determined in 31 kinds of crude drugs which were relatively easily available to our laboratory: 5 kinds of animal crude drugs and 26 kinds of plant crude drugs originated from seed (3 kinds), sclerotium (1), herb (3), fruit (3), bark (3), rhizome (2), tuber (2) and root (9).

Experimental

Materials All crude drugs used were provided by Tenjin Li Sheng Pharmaceutical Factory, Tenjin, China. Coatsome MC-2020 (L-α-phosphatidylcholine dilauroyl, average molecular weight: 623, purity: 99.7%) used for lecithin reference standard for assay was a gift from Nippon Oil & Fats Co., Ltd., Tokyo, Japan. Choline hydrochloride (special grade) used for choline reference standard for assay was obtained from Nakarai Tesque, Inc., Kyoto, Japan. Phospholipase D (type I, from cabbage) was obtained from Sigma Chemical Company, St. Louis, MO, U.S.A. All other chemicals were of special grade.

Apparatus and HPLC Conditions HPLC apparatus consisted of a pump (LC-9A, Shimadzu, Kyoto, Japan), an injector (7125, Rheodyne, Cotati, CA, U.S.A.), a separation column (Asahipak ODP-50, 5 μm, 150 × 6 mm i.d., Asahi Chemical Industry Co., Ltd., Kanagawa, Japan), an immobilized enzyme column (ACH-EC, 5 × 4 mm i.d., Irica Instruments Inc., Kyoto, Japan), a column heater (CTO-6A, Shimadzu), an ECD with a platinum working electrode (Σ875, Irica Instruments Inc.) and an integrator (C-R5A, Shimadzu). Mobile phase was prepared with 30 g of disodium hydrogenphosphate·12H_2O, 0.37 g of disodium ethylenediaminetetraacetate and 1 g of sodium 1-octanesulfonate dissolved in 1000 ml of distilled water, adjusted to pH 8.3 with phosphoric acid, and filtered through a 0.2 μm membrane filter (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). HPLC separation and enzymatic reaction were performed at 37 °C. The ECD potential was set at +0.45V *vs.* Ag/AgCl. The flow rate was controlled at 0.6 ml/min. Sample injection volume was 20 μl.

Sample Preparation (1) Lecithin[6)] One gram of powder or a small piece of crude drug was extracted with 90 ml of a mixture of chloroform–methanol (2 : 1) for 24 h at room temperature. The extract was filtered and made 100 ml with a mixture of chloroform–methanol (2 : 1). Five milliliters of this solution or an appropriately diluted solution containing 0.16 to 2.43 nmol of lecithin was evaporated under reduced pressure. The residue was dissolved in 1 ml of chloroform and washed with 2 ml of water 3 times to eliminate the choline originating from the crude drug. After centrifugation at 4000 rpm for 5 min, the chloroform layer was evaporated under reduced pressure to give a lecithin extract. The extract was added with 0.4 ml of phospholipase D (5 unit/ml), 0.8 ml of 25 mM acetate buffer (pH 5.6), 0.8 ml of 10 mM calcium chloride and 1 ml of diethyl ether, and reacted by shaking vigorously at room temperature for 30 min. After centrifugation at 4000 rpm for 5 min, 1 ml of the aqueous layer was evaporated under reduced pressure. The residue was dissolved in 0.5 ml of water to give a sample solution for assay of lecithin.

(2) Choline One gram of powder or a small piece of crude drug was extracted with 90 ml of ethanol under reflux for 1 h. After cooling, the extract was filtered and made 100 ml with ethanol. Five milliliters of this solution was evaporated under reduced pressure. This residue was dissolved in 4 ml of water and washed with 2 ml of diethyl ether to remove the hydrophobic component. After centrifugation at 4000 rpm for 10 min, 2 ml of aqueous layer was evaporated under reduced pressure. The residue was dissolved in 1 ml or an appropriate volume of water to contain 0.09 to 3.68 nmol of choline per ml and give a sample solution for assay of choline.

Results and Discussion

Lecithin was hydrolyzed to phosphatidic acid and choline by phospholipase D. The hydrolyzate was injected to HPLC, and choline was separated from impurities

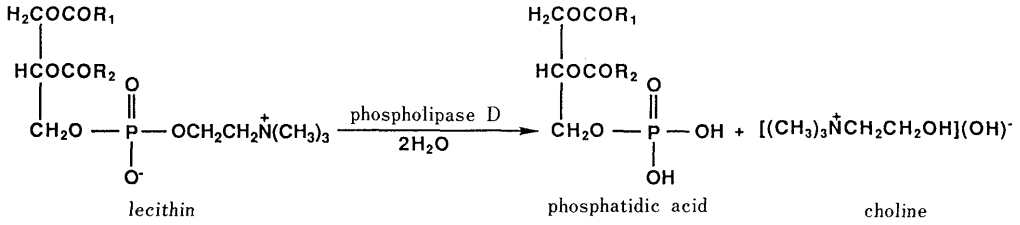

Fig. 1. Enzyme Reaction for Lecithin

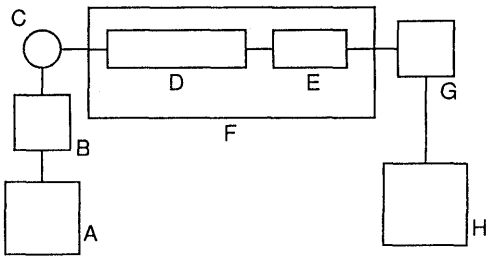

Fig. 2. Schematic of HPLC System

A, mobile phase; B, pump; C, injector; D, separation column; E, immobilized enzyme column; F, column heater; G, electrochemical detector; H, integrator.

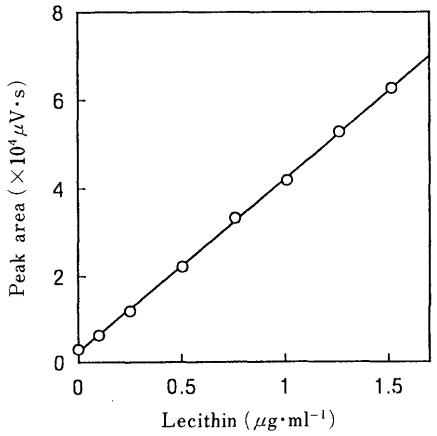

Fig. 3. Calibration Curve of Lecithin

$r=0.999$.

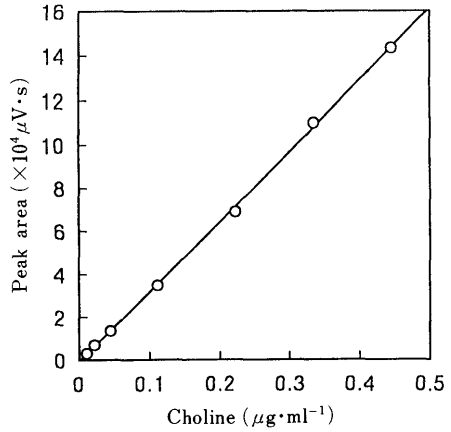

Fig. 4. Calibration Curve of Choline

$r=0.999$.

TABLE I. The Determination of Lecithin and Choline in Crude Drugs (μg/g)

Crude drug[a]		Lecithin[b]	Choline
Asini Gelatinum	(阿　膠)	1	7
Cervi Parvum Cornu	(鹿　茸)	3495	118
Hippocampus	(海　馬)	722	1711
Kokurozin[c]	(黒驢腎)	2670	224
Koukuzin[d]	(広狗腎)	393	306
Cuscutae Semen	(菟糸子)	298	286
Foenigraeci Semen	(胡蘆巴)	3096	720
Psoraleae Semen	(補骨脂)	2790	711
Poria	(茯　苓)	77	11
Cynomorii Herba	(鎖　陽)	69	267
Epimedii Herba	(淫羊藿)	53	134
Cistanchis Herba	(肉蓯蓉)	158	191
Corni Fructus	(山茱萸)	302	36
Lycii Fructus	(枸杞子)	63	66
Rubi Fructus	(覆盆子)	63	93
Cinnamonomi Cortex	(桂　皮)	3	5
Eucommiae Cortex	(杜　仲)	15	9
Moutan Radicis Cortex	(牡丹皮)	78	363
Atractylodis Rhizoma	(白　朮)	13	158
Curculiginis Rhizoma	(仙　茅)	286	38
Aconiti Tuber	(炮附子)	3	1
Ophiopogonis Tuber	(麦門冬)	4	24
Achyranthis Radix	(牛　膝)	33	231
Angelicae Radix	(当　帰)	112	989
Dipsaci Radix	(川続断)	7	296
Ginseng Radix	(人　参)	1118	31
Glycyrrhizae Radix	(甘　草)	44	136
Morindae Radix	(巴戟天)	3	155
Astragali Radix	(黄　耆)	35	15
Rehmanniae Radix	(熟地黄)	19	27
Scrophulariae Radix	(玄　参)	257	150

a) From Namba's writing (Colored Illustration of Wakan-Yaku),[4] except for Kokurozin and Koukuzin. *b*) As L-α-phosphatidylcholine dilauroyl. *c*) The external genitalia of male *Equus asinus* L. *d*) The external genitalia of male *Canis familiaris* L.

originating from the matrix of the crude drug and reaction mixture by separation column. The choline was oxidized by choline oxidase immobilized enzyme column to give betaine and hydrogen peroxide. Determination of lecithin and choline was made by measuring this hydrogen peroxide with ECD. These reaction and HPLC systems are shown in Figs. 1 and 2, respectively.

In this method, both lecithin and lysolecithin were determined, because phospholipase D from cabbage used this method acts on lysolecithin as well as lecithin.[7]

The relationship between concentration of lecithin or

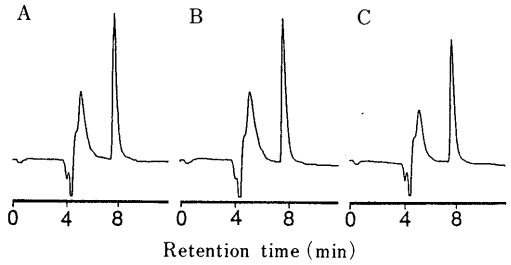

Fig. 5. Typical Chromatograms for the Determination of Lecithin in Crude Drugs

A, standard (0.75 μg/ml); B, Cervi Parvum Cornu (dilution rate: 250); C, Psoraleae Semen (dilution rate: 250).

choline and their peak area are shown in Figs. 3 and 4.

The peak area of hydrogen peroxide derived from lecithin and choline was proportional at a concentration from 0.10 to 1.52 μg/ml and 0.01 to 0.45 μg/ml, respectively. Results of the determination of lecithin and choline in the 31 kinds of crude drugs, with an average of 3 repetitions for 3 lots, are shown in Table I, and the typical chromatogram for the determination of lecithin is shown in Fig. 5.

The peak of hydrogen peroxide was observed at the retention time of about 8 min after the peak originating from the matrix in the chromatogram for the determination of both choline and lecithin.

Results showed that animal crude drugs except for Asini Gelatinum, especially Cervi Parvum Cornu and Kokurozin, and plant crude drugs originating from seeds of the leguminous plants Foenigraeci Semen and Psoraleae Semen contained more lecithin than other crude drugs. Though Cuscutae Semen, a convolvulaceous plant, is a crude drug originating from seed, it did not contain much lecithin. Plant crude drugs originating from Aconiti Tuber, Ophiopogonis Tuber, Dipsaci Radix and Morindae Radix, but not from Angelicae Radix, Ginseng Radix and Scrophulariae Radix, contained little lecithin. Angelicae Radix, Foenigraeci Semen and Psoraleae Semen, and especially Hippocampus were found to contain more choline than other crude drugs, while little choline was found in Asini Gelatinum, Cinnamonomi Cortex, Eucommiae Cortex, Aconiti Tuber and others.

Analysis of molecular species of lecithin in the 4 kinds of crude drugs containing a great deal of lecithin is now being done.

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References

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