い」と判定された事例を約3割程度認めた(図11)。

4) 考 察

「いわゆる健康食品」による肝障害に関しての一次調査，二次調査を通じて，健康食品によっても医薬品と同様に肝障害が惹起されることが示された。

今回のアンケート調査では，健康食品による肝障害は，比較的高齢の女性に多いということが明らかになった。また，併用薬が存在する頻度も高く，基礎に何らかの疾患を抱えていることが推測された。年齢，性別の差が発症のリスクに影響するかは明らかではないが，健康の保持に関心を持つ，あるいは健康に何らかの不安を持つこれらの層が健康食品を摂取する割合が高いことを反映しているものと思われる。飲酒歴やアレルギー症の既往についての割合は低く，これらを発症のリスクを予測する因子とすることは難しいと考えられた。肝障害のタイプは肝細胞障害型が多く，臨床病型は急性肝炎型が多かったが，これは他の報告とほぼ一致していた[13,15]。

肝障害事例の転帰は，二次調査においては，寛解，軽快が殆どを占め，肝障害が原因となった死亡例は認めなかった。また，治療においても，多くが原因食品の中止を含めた内科的治療で軽快しており，肝移植を要した例などは認めなかった。但し，二次調査では重症例の拾い上げが充分でなかった可能性があり，これについては今後の追加調査が必要と考えられる。

健康食品による肝障害の診断については，特に定められたものはないため，一般の薬物性肝障害の診断基準を用いることの妥当性を検討した。今回，二次調査回答例についてのICM基準，DDW-J基準案を用いてのスコアは総じて低く，関係なし・否定的(ICM基準)，可能性低い(DDW-J基準案)と判定されるものも多かった。原因としては，医薬品と異なり，服用開始時期・期間などが明確でない場合があること，他の肝障害の原因の除外が完全ではないこと，基礎に肝疾患を持つ人が服用している場合があり，これによりスコアが減じられる場合があることなどが挙げられた。健康食品の場合は，医薬品と異なり，処方箋なども存在しないため，服用量，服用形態などについても詳細に聴取する必要があると考えられる。また，診断に際して有用とされるDLSTの施行率は65%であったが，陽性率はそのうちの4割程度と決して高くはなかった。アレルギー性機序のものがそれほど多くなかったためとも考えられるが，DLSTの検査自体の信頼性，特に外注で検査された場合の問題点は以前より指摘されており，この点については今後検討されるべきと思われる。また，保険適用外検査であること，併用薬や複数の健康食品を摂取している場合があることなどが，検査の施行率に影響している可能性も考えられる。

上記は，既存の薬物性肝障害の診断基準を使うことの妥当性を否定するものではない。但し，以上のことを考慮すれば，診断基準および肝生検所見などを参考としながら，肝臓専門医が最終的な判断をすべきであると考えられる。

今回の調査でも，原因と考えられた健康食品は多岐に亘っており，受診まで至らない軽症例を含めれば，健康被害の実数はさらに増加するものと思われるが，これらの拾い上げの方法については，今後の課題になると考えられる。

「いわゆる健康食品」においては，製品の規格が厳密に決められていないことが，原因の究明を困難にしている。すなわち，主成分が同一の健康食品でも，その含有量やその他の含有成分は異なることがあり，主成分の量が肝障害の発生に寄与した可能性，あるいは主成分以外の含有成分が肝障害の原因である可能性については常に考慮が必要と考えられる。

実際，東京都の行った平成15年度 第1回 健康食品試買調査では80品目中9品目，平成16年度 第2回調査では79品目中2品目から，本来医薬品にしか使用できない成分が検出されたとされている[18]。先述の中国製やせ薬も，甲状腺末やニトロソフェンフルラミンが含有されており，これが肝障害の原因となっていた。また，健康食品の

原材料としてさまざまな動植物が使用されることより，環境中からの化学物質による汚染や，製造過程での化学物質の混入の可能性も考えられる。田端らによるアガリクスに含まれるガドニウム含有量に関しての報告は，この1例として挙げられる[19]。

また，今回の調査の原因として最も多く挙げられたウコンについては，多種類の製品が流通しており，やはり，肝障害の報告は製品ごとに異なる可能性もあることを考慮する必要がある。殆どは「秋ウコン」と呼ばれるものと考えられるが，同属の植物として「春ウコン」と呼ばれるキョウオウや「紫ウコン」と呼ばれるガジュツがある。これらの間で肝障害発症のリスクが同一であるという保証はなく，それを明らかにできなかった点は今回の調査の問題点と言える。また，ウコン数種の鉄含有量を調べた報告では，製品の中には牛レバーに匹敵する高い数値を示すものもあったとされ[20]，このような鉄含有量の多いウコン製品を長期間大量に内服すると，鉄過剰になることが予測される。C型慢性肝炎患者においては，肝臓への鉄蓄積により肝障害の増悪が見られることが明らかになっており，ウコンを内服することへの問題点を指摘する報告もある[14]。これについても今後検討が必要と考えられる。

以上のように，健康食品においては製品ごとの肝障害の発症頻度の検討なども必要と考えられるが，現状では困難と言わざるを得ない。多くの製品が流通していること，医薬品と異なり，一般への浸透の程度の把握が困難なことも，肝障害，健康被害の実態の把握を困難にしていると考えられる。

薬物性肝障害の原因の究明は，健康食品が原因である場合に限らず重大な医学的問題である。一般に肝毒性が報告されていない薬物による肝障害は，アレルギー性機序によって起こる場合，肝細胞内の薬物代謝酵素の特異性の違いが肝毒性の代謝物の増加の方向に作用する場合に大別される。健康食品による肝障害においても，同様の機序が関与していると考えられるが，それを明らかにするためにも，さらに詳細な個々の事例の調査が必要と考えられる。

おわりに

「いわゆる健康食品」によっても，肝障害などの健康被害が起こり得ることが明らかになった。健康食品による健康被害の拡大を防ぐためには，原因の究明を進めると共に，健康食品においても肝障害などが発生する場合があることを認識し，安易にこれに頼らぬよう，また，疾患の治療・予防に際しては，医師への相談を優先させるよう一般に啓蒙していくことが必要と考えられる。また，医師も健康食品の有効性や安全性については常に留意し，正確な情報を把握し，適切な指導が行えるようにする必要があると考える。下記に厚生労働省などの健康食品の安全性・有効性情報に関するホームページアドレスを記載するので参考にされたい。

厚生労働省(食品安全情報)
http://www.mhlw.go.jp/topics/bukyoku/iyaku/syoku-anzen/index.html
内閣府食品安全委員会　http://www.fsc.go.jp
独立行政法人国立健康・栄養研究所
http://hfnet.nih.go.jp/main.php
国立医薬品食品衛生研究所(食品に関する情報)
http://www.nihs.go.jp/hse/food-info/index.html
国民生活センター　http://www.kokusen.go.jp/
東京都健康局食品医薬品安全部(いわゆる健康食品ナビ)
http://www.fukushihoken.metro.tokyo.jp/anzen/supply/index.html

追　記

この調査研究は，平成15年度 厚生労働省科学研究費補助金 厚生労働省特別研究事業「いわゆる健康食品による健康被害事例のリスク分析のための研究」(主任研究者：愛知医科大学消化器内科 各務伸一)，平成16年度 厚生労働省科学研究費補助金 食品の安全性高度化事業「いわゆる健康食品の健康影響と健康被害に関する研究」(主任研究者：独立行政法人国立健康・栄養研究所食品機能研究部 斎藤衛郎)の一環として行われたも

のであること付記します。分担研究者，研究協力者，アンケートの多大なるご協力を頂いた施設の先生方に心より御礼申し上げます。一次調査には多数の施設からのご協力を頂きましたが，紙面の都合上，以下に，二次調査にご協力頂いた施設のみを列記させて頂きます。

【二次アンケート調査協力施設】

久留米大学病院，岩手医科大学，東京逓信病院，大阪大学医学部附属病院，兵庫医科大学，大阪労災病院，倉敷中央病院，熊本地域医療センター，赤磐医師会病院，獨協医科大学病院，自治医科大学，日立総合病院，東京慈恵会医科大学附属青戸病院，帝京大学，国際医療福祉大学・山王病院，新潟大学，長岡赤十字病院，富山県立中央病院，名古屋大学，中京病院，岐阜市民病院，県立岐阜病院，三重大学，京都府立医科大学，大阪市立大学，大阪鉄道病院，東大阪市立総合病院，市立吹田病院，市立貝塚病院，岡山大学，山陰労災病院，福岡市民病院，長崎大学，佐世保共済病院（以上）

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Ginkgo biloba extract modifies hypoglycemic action of tolbutamide via hepatic cytochrome P450 mediated mechanism in aged rats

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Abstract

We examined hepatic cytochrome P450 (CYP)-mediated interactions between *Ginkgo biloba* extract (GBE) and tolbutamide, an oral anti-diabetic agent, in aged and young rats. Tolbutamide was orally given to rats with or without GBE treatment, and time-dependent changes in blood glucose were monitored. The basal activity of six CYP subtypes in liver was lower in the aged rats than in the young rats, while the inductions of these enzymes by 5 day pretreatment of 0.1% GBE diet were more in the aged rats. Further, the pretreatment of GBE significantly attenuated the hypoglycemic action of tolbutamide in the aged rats, corresponding well to the enhanced activity of (S)-warfarin 7-hydroxylase, which is responsible for CYP2C9 subtype, a major isoform metabolizing tolbutamide. In contrast, the simultaneous administration of GBE with tolbutamide potentiated the hypoglycemic action of this drug. The in vitro experiments revealed that GBE competitively inhibited the metabolism of tolbutamide by (S)-warfarin 7-hydroxylase in the rat liver microsomes. In the young rats, the 5 day pretreatment with GBE significantly attenuated the hypoglycemic action of tolbutamide, but a simultaneous treatment had little influence on the tolbutamide effect. In conclusion, the present study has shown that the simultaneous and continuous intake of GBE significantly affects the hypoglycemic action of tolbutamide, possibly via a hepatic CYP enzyme-mediated mechanism, particularly in the aged rats. Therefore, it is

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anticipated that the intake of GBE as a dietary supplement with therapeutic drugs should be cautious, particularly in elderly people.

Keywords: Ginkgo biloba extract; Cytochrome P450; Tolbutamide; Blood glucose; CYP2C9

Introduction

Ginkgo biloba extract (GBE) is a popular herbal medicines, and the leaf extract of *Ginkgo biloba* contains some functional flavonoids and terpenoids (Kleijnen and Knipschild, 1992a; Gruenwald et al., 2000). In some European countries, standardized GBE has been clinically used mostly for cerebral insufficiency, dementia, memory impairment, tinnitus and intermittent claudication (Kleijnen and Knipschild, 1992b; Le Bars et al., 1997; Ernst, 1999; Pittler and Ernst, 2000), while it is marketed as a dietary supplement in the United States and Japan.

GBE exerts many pharmacological effects, such as free radical-scavenging action and improvement of microcirculatory action (McKenna et al., 2001; De Smet, 2002; Ernst, 2002). On the other hand, GBE has been reported to have side effects such as headache, gastric symptoms, diarrhea, and allergic skin reactions, although the occasion of these side effects is rare (Cohen and Bartlik, 1998; De Smet, 2002; Ernst, 2002). Recently, adverse reactions of herbal remedies and potential herb-drug interactions have received a great deal of attention. As a well-known example, St John's Wort has been reported to induce hepatic CYP3A4 activity, thereby leading to the attenuation of efficacy of therapeutic drugs such as cyclosporin, indinavir, and digoxin (Durr et al., 2000; Roby et al., 2000). In the case of GBE, the occurrence of bleeding has been reported in patients who took GBE and anticoagulant drugs such as aspirin, rofecoxib, or warfarin at the same time (Vaes and Chyka, 2000; Izzo and Ernst, 2001). It is proposed that the mechanism of this adverse interaction of GBE with these drugs is due to the inhibitory effect of platelet activating factors by ginkgolide B, which is one of the active constituents of GBE (Braquet and Hosford, 1991). However, other interactions between GBE and drugs have not been fully elucidated.

In previous studies, we found that feeding GBE to rats markedly increased the concentration of hepatic cytochrome P450 (CYP), the expression of various CYP mRNA, and the enzyme activities in a dose- and time-dependent manner (Umegaki et al., 2000; Umegaki et al., 2002; Shinozuka et al., 2002). Moreover, we reported that pre-treatment with GBE decreased the hypotensive action by nicardipine, a calcium channel blocker, which is extensively metabolized by CYP3A type (Shinozuka et al., 2002). These findings indicate that similar interactions of GBE with other drugs through the mediation of CYPs might occur.

Because of its reported beneficial effects, many elderly people take GBE (Cohen and Bartlik, 1998; De Smet, 2002; Ernst, 2002). Generally, elderly people tend to suffer from hypertension and diabetes and thereby take some other drugs simultaneously with GBE. In particular, GBE has been shown to improve peripheral blood flow (Iliff and Auer, 1983) and is expected to prevent periphery necrosis and retinopathy in severe diabetes (Lanthony and Cosson, 1988; Doly et al., 1992). It is also reported that GBE inhibits the increment of blood glucose concentration in glucose-loaded diabetic rats (Rapin et al., 1997). Thus, the simultaneous intake of GBE and anti-diabetic agents might occur, particularly in elderly people.

The present study was undertaken to examine the hepatic CYP-mediated interactions of GBE with tolbutamide in aged and young rats. Tolbutamide is one of sulfonylureas applied for non-insulin-

dependent, type 2 diabetes, and its metabolism is dependent mainly upon the CYP2C9 activity in the liver (Tal, 1993; Proks et al., 2002; Lee et al., 2003). The effect of GBE intake on the hypoglycemia of tolbutamide in rats was evaluated with a 5 day pretreatment and simultaneous treatment of GBE.

Methods

Materials

A standardized powder form of *Ginkgo biloba* extract (GBE) was supplied by Tama Seikagaku-Kogyo Co., Tokyo. The GBE contained 24.9% flavonoids and 10.6% total terpene, which consisted of 2.9% ginkgolide A, 1.4% ginkgolide B, 2.1% ginkgolide C, and 4.2% bilobalide, and less than 1 ppm of ginkgolic acid. Tolbutamide, resorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, testosterone, 6β-hydroxytestosterone, corticosterone, p-nitrophenol, 4-nitrocatechol, and 7-ethoxycoumarin were purchased from Sigma (St. Louis, MO, USA). (S)-Warfarin and 7-hydroxywarfarin were obtained from Ultrafine (Manchester, England). NADPH was obtained from Oriental Yeast (Tokyo, Japan). All other reagents were obtained from Wako Pure Chemical Ltd. (Osaka, Japan).

Animal experiment

Male Wistar aged (19 months old) and young (7 weeks old) rats obtained from Japan SLC (Shizuoka, Japan) were housed individually in stainless steel, wire-bottomed cages in a constant temperature room (23 ± 1 °C) under 12 hr light-dark cycle. Rats were divided into several groups with or without GBE treatment. GBE pretreated group was fed commercial rodent diet CE2 (Japan Clea, Tokyo) containing 0.1% GBE for 5 days. After the treatment, rats were orally administered tolbutamide (40 mg/kg). The simultaneous GBE treated group was given GBE (100 mg/kg body weight, p.o.) simultaneously with tolbutamide. After the tolbutamide administration, the blood was collected at predetermined time points and immediately blood glucose concentration was determined using a blood glucose detector Dexter Z II (Bayer Corp. Mishawaka, IN). After the treatment with or without GBE, the rat liver was removed and weighed, and a part of the liver was subjected to the enzyme assay.

All procedures were in accordance with National Institute of Health and Nutrition guidelines for the Care and Use of Laboratory Animals.

Analytical methods

Preparation of microsome and cytosolic fractions from the liver

The liver was rinsed with 0.9% NaCl solution, weighed and homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose. The homogenate was centrifuged at 10,000 × g at 4 °C for 30 min. The supernatant was further centrifuged at 105,000 × g at 4 °C for 60 min. The supernatant was used as the cytosolic fraction for the assay of glutathione S-transferase, the activity of which was determined by the method of Habig and Jakoby using 1-chloro-2,4-dinitrobenzene as a substrate (Habig and Jakoby, 1981). The pellet was washed once with 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose by

centrifugation at 105,000 × g at 4°C for 60 min, and the concentration and activities of CYP were analyzed.

Protein concentrations of microsome and cytosolic fractions were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

Analysis of CYP enzyme activities

Cytochrome P450 content was quantified by the method of Omura and Sato (Omura and Sato, 1964). The activities of various CYP enzymes were determined by HPLC methods as reported previously (Umegaki et al., 2002). The subtypes of CYP enzymes examined and the corresponding CYPs were methoxyresorufin O-demethylase, CYP1A2; ethoxyresorufin O-deethylase, CYP1A1; pentoxyresorufin O-dealkylase, CYP2B; p-nitrophenol hydroxylase, CYP2E1; testosterone 6β-hydroxylase, CYP3A; and (S)-warfarin 7-hydroxylase, CYP2C9.

The interaction between GBE and tolbutamide toward (S)-warfarin hydroxylase activity in vitro were evaluated by adding these substances at various concentrations (GBE; 1.0–30 μg/mL, tolbutamide; 0.1–30 μg/mL) to the enzyme assay system ((S)-warfarin concentration; 2–16 μM).

Statistical analysis

Statistical analysis of the data was carried out using ANOVA followed by a post hoc test of Fisher's PLSD. These statistical analyses were performed using a computer program (Stat View 5.0, ASA Institute Inc, Cary, NC, USA).

Results

Induction of hepatic drug-metabolizing enzymes by GBE

Effects of 5 days of feeding of 0.1% GBE diet on the liver weight and hepatic drug metabolizing enzymes are shown in Table 1. The calculated intake of GBE was about 14 mg per day in both groups and

Table 1
Effects of 5 day pretreatment with GBE on the liver weight, cytochrome P450 content, and glutathione S-transferase activity in aged and young rats

	Aged rats		Young rats	
	Control	0.1% GBE	Control	0.1% GBE
Body weight (g)	433 ± 26	450 ± 18	189 ± 7	190 ± 6
Liver weight (% /Body weight)	2.76 ± 0.09	3.28 ± 0.06[a]	4.14 ± 0.19	4.12 ± 0.29
Cytochrome P450 content (nmol/mg protein)	0.731 ± 0.08	1.692 ± 0.33[a]	0.921 ± 0.05	1.749 ± 0.22[b]
Glutathione S-transferase (nmol/mg protein/min)	565 ± 197	1054 ± 217[a]	667 ± 104	934 ± 236[b]

Male Wistar aged (19 months old) and young (7 weeks old) rats received commercial rodent diet CE2 with or without 0.1% GBE for 5 days. Each value is the mean ± SD from six rats. Significant differences from each control level are indicated by [a]$P<0.01$ and [b]$P<0.05$.

was 32 mg (per kg body weight in a day) in the aged rats and 72 mg in the young rats. The liver weight in the aged rats but not in the young rats was significantly increased to 1.2-fold by the GBE treatment.

Significant increases in the cytochrome P450 content and glutathione S-transferase activity were also detected by the GBE treatment, both in the aged rats (2.3- and 1.9-fold, respectively) and young rats (1.9- and 1.4-fold, respectively). Activities of various CYP are shown in Table 2. The basal levels of the activities of six CYP subtypes were clearly lower in the aged rats than in the young rats. The activity of (s)-warfarin 7-hydroxylase, which is responsible for the metabolism of tolbutamide, in the aged rats was about 41% of that in the young rats. Both in the aged and young rats, the GBE pretreatment significantly increased all CYP enzymes, especially in pentoxyresorufin O-dealkylase and (s)-warfarin 7-hydroxylase activity. The induction ratios in both groups were apparently higher in the aged rats; the ratios in the young and aged rats were 13- and 42-fold for pentoxyresorufin O-dealkylase, respectively, and 2.1- and 4.7-fold for (s)-warfarin 7-hydroxylase activity, respectively.

Effects of GBE on the efficacy of tolbutamide

The time-dependent changes in the blood glucose concentration by tolbutamide administration in the aged rats are shown in Fig. 1. The baseline concentration of blood glucose was approximately 100 mg/dl. In the control group, the blood glucose concentration decreased to the minimum level (the reduction: 32.2 ± 2.4 mg/dl, n = 6) at 3 hr after oral administration of tolbutamide (40 mg/kg) and remained at the lower level until for least 7 hr. In the 0.1% GBE diet (5 days) pretreated group, the hypoglycemic action of tolbutamide was considerably attenuated when compared with that in the control group without GBE, and the statistical significance was detected at 2–5 and 7 hr. This result indicated that the pretreatment of GBE reduced the pharmacological action of tolbutamide. On the other hand, a simultaneous treatment of tolbutamide with GBE at a single dose (100 mg/kg body weight) significantly lowered (at 4–6 hr) the blood glucose concentrations in the aged rats, compared with the treatment of tolbutamide alone in the control group (Fig. 1). This result indicated that the simultaneous treatment of tolbutamide and GBE potentiated the hypoglycemic efficacy of tolbutamide. In the young rats, the pretreatment (5 days) of 0.1% GBE diet significantly attenuated the hypoglycemic action of tolbutamide as in the aged rats,

Table 2
Inductions of various hepatic CYP activities by 5 day pretreatment with GBE in aged and young rats

	Activity (pmol/mg protein/min)			
	Aged rats		Young rats	
	Control	0.1% GBE	Control	0.1% GBE
Ethoxyresorufin O-deethylase (CYP1A1)	13.5 ± 0.9	37.9 ± 1.4[a]	24.7 ± 1.5[b]	44.2 ± 1.6[a]
Methoxyresorufin O-demethylase (CYP1A2)	15.5 ± 1.1	33.2 ± 2.0[a]	27.6 ± 2.4[b]	41.7 ± 1.9[a]
Pentoxyresorufin O-dealkylase (CYP2B)	8.2 ± 0.3	348.4 ± 39.6[a]	35.5 ± 6.4	476.7 ± 23.6[a]
(s)-Warfarin 7-hydroxylase (CYP2C9)	2.6 ± 0.4	12.1 ± 0.8[a]	6.3 ± 0.4[b]	13.5 ± 1.2[a]
p-Nitrophenol hydroxylase (CYP2E1)	1453 ± 99	3580 ± 215[a]	1717 ± 76	4064 ± 170[a]
Testosterone 6β-hydroxylase (CYP3A)	218 ± 15	564 ± 42[a]	295 ± 30	739 ± 20[a]

Male Wistar aged (19 months old) and young (7 weeks old) rats received commercial rodent diet CE2 with or without 0.1% GBE for 5 days. Each value is the mean ± SD from six rats. Significant difference from each control level is indicated by [a]$P<0.001$. Significant difference from control of aged rats is indicated by [b]$P<0.01$.

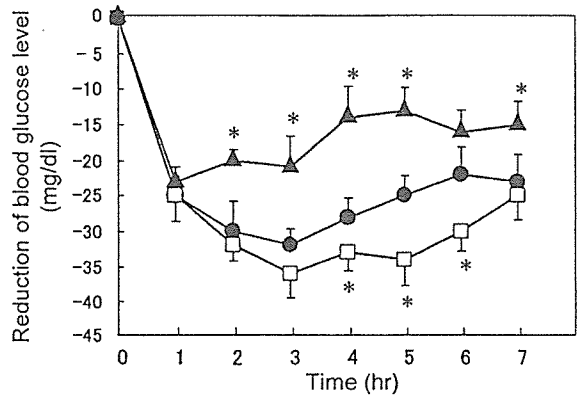

Fig. 1. Effects of simultaneous treatment and 5 day pretreatment with Ginkgo biloba extract (GBE) on the hypoglycemic effect of tolbutamide in aged rats. Aged rats (19 months old) were administered tolbutamide (40 mg/kg, p.o.) with or without GBE treatment. The GBE pretreated group was given feed containing 0.1% GBE for 5 days, and a simultaneous GBE treated group was given a single dose of GBE (100 mg/kg, p.o.) with tolbutamide. After the tolbutamide administration, blood was collected for the analysis of blood glucose concentrations. Each point represents the mean ± SD from six rats. ●, control group; ▲, GBE pretreated group; and □, a simultaneous GBE treated group. Significant difference from the control group is indicated by $*P<0.05$.

whereas the simultaneous GBE treatment at a single dose exhibited little significant interaction with tolbutamide (Fig. 2).

Competitive inhibition of GBE and tolbutamide on CYP2C9 activity in vitro

The direct interaction of tolbutamide and GBE toward (S)-warfarin 7-hydroxylase (CYP2C9) activity was examined using rat liver microsome in vitro. As shown in a Dixon plot, tolbutamide competitively

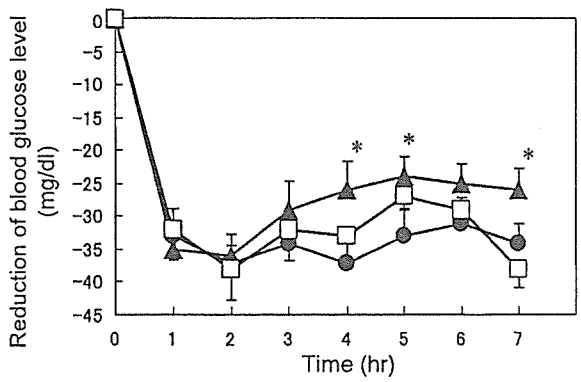

Fig. 2. Effects of simultaneous treatment and 5 day pretreatment with Ginkgo biloba extract (GBE) on the hypoglycemic effect of tolbutamide in young rats. Young rats (7 weeks old) were administered tolbutamide (40 mg/kg, p.o.) with or without GBE treatment. The GBE pretreated group was given feed containing 0.1% GBE for 5 days, and a simultaneous GBE treated group was given a single dose of GBE (100 mg/kg, p.o.) with tolbutamide. After tolbutamide administration, blood was collected for analysis of blood glucose concentrations. Each point represents the mean ± SD from six rats. ●, control group; ▲, GBE pretreated group; and □, a simultaneous GBE treated group. Significant difference from the control group is indicated by $*P<0.05$.

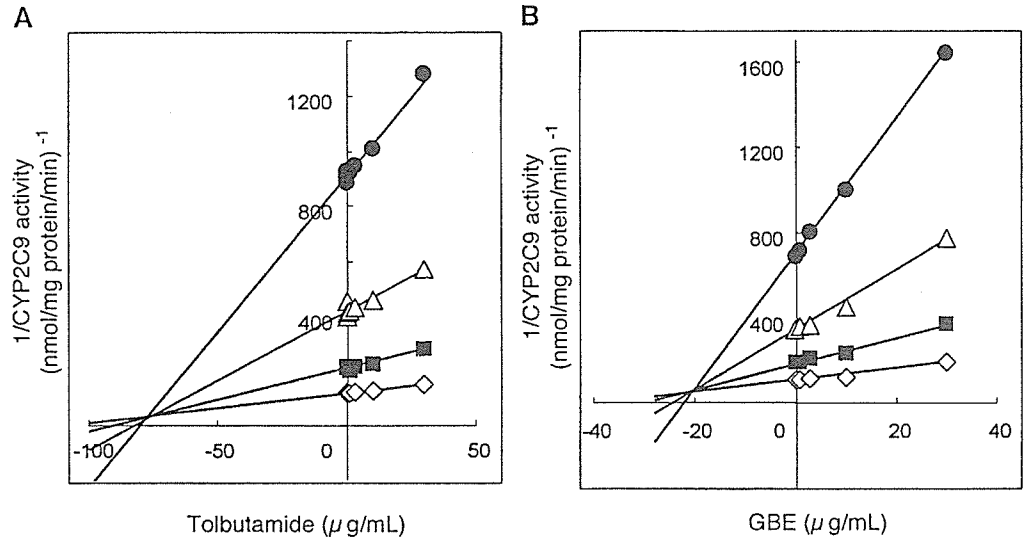

Fig. 3. Effects of tolbutamide (A) and Ginkgo biloba extract (B) on the (S)-warfarin 7-hydroxylase (CYP2C9) activity in rat liver microsome in vitro (Dixon plot). The (S)-warfarin 7-hydroxylase (CYP2C9) activities were measured in the presence of various concentrations of tolbutamide (0.1–30 μg/mL) or GBE (1.0–30 μg/mL) in rat liver microsome in vitro. Loaded (S)-warfarin concentrations were ●, 2 μM; △, 4 μM; ■, 8 μM and ◇, 16 μM. Each point represents the mean from five experiments.

inhibited the metabolism of (S)-warfarin by the (S)-warfarin 7-hydroxylase (Fig. 3A). Similarly, GBE decreased the (S)-warfarin 7-hydroxylase activity in vitro in a concentration-dependent manner, and showed competitive inhibition toward the enzyme activity (Fig. 3B). These results indicated that the hepatic metabolism of tolbutamide was competitively inhibited by GBE. The calculated Ki values were 19 μg/mL for GBE and 75 μg/mL for tolbutamide, respectively.

Discussion

Elderly people often suffer from functional impairments in their bodies and diabetes in these days. Considering these situations and the efficacy of GBE reported so far (Iliff and Auer, 1983; Lanthony and Cosson, 1988; Doly et al., 1992; Rapin et al., 1997), the simultaneous intake of GBE and anti-diabetic drug is most likely to occur in the elderly. For the safe use of both GBE and drugs, it is important to elucidate how GBE taken as a dietary supplement modifies the efficacy of therapeutic drugs. In the present study, we investigated the influence of GBE intake on the hypoglycemic efficacy of tolbutamide. We focused particularly on the elderly of advanced age and on the hepatic CYP-mediated interactions. The pharmacodynamic interaction was evaluated by inducing hypoglycemia with an excess administration of tolbutamide in aged (19-month-old) and young (7-week-old) rats.

As reported previously (Szabo et al., 1995; Umegaki et al., 2000), the intake of GBE did not change the basal blood glucose level in the aged and young rats with the pretreatment of GBE with diet (data not shown). However, the pretreatment and simultaneous treatment of GBE markedly influenced the hypoglycemic action of tolbutamide in the aged rats (Fig. 1). In the case of 5 days pretreatment of GBE, the hypoglycemic action of tolbutamide was attenuated both in the aged and young rats. As shown

in Table 2, the 5 day pretreatment of GBE markedly induced hepatic drug metabolizing enzymes, especially (S)-warfarin 7-hydroxylase, an enzyme corresponding to CYP2C9 which mainly metabolizes tolbutamide in the liver (Lee et al., 2003). Therefore, the induction by GBE pretreatment of hepatic CYP2C9 may reduce the plasma concentration of tolbutamide after oral administration, thereby leading to the attenuation of the hypoglycemic action of tolbutamide. In the present study, the plasma concentration of tolbutamide was not determined, due to the limited blood sample from the GBE-pretreated rats. However, the change in the blood glucose concentration was taken as the final outcome of interaction between GBE and tolbutamide, and the observed changes of CYP2C9 responsible for tolbutamide metabolism following the GBE pretreatment could well explain the mechanism of the pharmacodynamic interaction.

Kudolo (Kudolo, 2001) also reported that the ingestion of GBE by non-insulin-dependent diabetes mellitus subjects induced the hepatic metabolic clearance rate of insulin and hypoglycemic agents, suggesting an increase of blood glucose. In the previous paper (Shinozuka et al., 2002), we also showed that pretreatment of GBE significantly reduced the hypotensive action of nicardipine, which is metabolized by CYP3A2 isoform in rats. The mechanism of these interactions is similar to that in St John's Wort (Durr et al., 2000; Roby et al., 2000), a well-known inducer of CYP, indicating that the induction of CYP by pretreatment with GBE attenuates the efficacy of various drugs. Inasmuch as the beneficial effects of GBE in humans are expected after the continuous intake for more than 4 weeks (Gruenwald et al., 2000; Blumenthal, 1998), the induction of hepatic CYP would be taken into account for the interaction with drugs seen after a long-term intake of GBE as a dietary supplement.

In contrast to the GBE pretreatment, simultaneous treatment of tolbutamide with GBE as a single dose potentiated hypoglycemic action of tolbutamide in the aged rats (Fig. 1). In order to clarify the underlying mechanism of this interaction, a direct effect of GBE on the hepatic (S)-warfarin 7-hydroxylase (subtype of CYP2C9) activity was examined in vitro. As tolbutamide is mainly metabolized by (S)-warfarin 7-hydroxylase (Lee et al., 2003), tolbutamide competitively inhibited the hepatic metabolism of (S)-warfarin as shown in the Dixon plot analysis (Fig. 3A). Interestingly, GBE also showed the competitive inhibition toward the metabolism of (S)-warfarin (Fig. 3B), suggesting that GBE competitively inhibited the metabolism of tolbutamide by the CYP2C9 enzyme in the liver of rats. Thus, the pharmacodynamic interaction between GBE and tolbutamide after their single administration could be explained by the following mechanism. Following the simultaneous intake of GBE and tolbutamide, the hepatic metabolism of tolbutamide would be attenuated via the significant occupancy by GBE of the same active site on the CYP2C9 enzymes where this drug binds, resulting in an enhancement of hypoglycemic action of tolbutamide.

The induction of hepatic drug metabolizing enzymes and attenuation of hypoglycemic efficacy of tolbutamide were observed by the pretreatment of GBE both in the young rats and aged rats. However, the extent of these effects by GBE pretreatment was greater in the aged rats than in the young rats. This distinction may be explained by the difference between the two groups of rats in the induced level of CYP enzymes. In fact, the activities of pentoxyresorufin O-dealkylase and (S)-warfarin 7-hydroxylase were induced more (2 to 3-fold) by the GBE treatment in the aged rats than in the young rats (Table 2). This greater induction of CYP2B and CYP2C9 in the aged rats may be somehow related to the lower basal activities of these enzymes, which displayed about 23 and 41%, respectively, of the enzyme activity in the young rats. Interestingly, in the case of simultaneous treatment of GBE and tolbutamide, the potentiation of hypoglycemic action of tolbutamide was not detected in the young rats (Fig. 2). A clear explanation for the absence of enhancement of the pharmacological effect by tolbutamide in the young rats is lacking. Further

detailed experiments will be necessary to clarify pharmacokinetic and pharmacodynamic difference of tolbutamide between young and aged rats observed by the GBE treatment. In any event, it is evident that the aged rats may be more susceptible to the CYP-mediated interactions of GBE and tolbutamide when compared to the young rats. This finding may be applied for humans.

As a dietary supplement, 120 to 240 mg of GBE are generally taken in a day (Blumenthal, 1998; Gruenwald et al., 2000; Ernst, 2002), and this dose can be estimated as 2 to 4 mg/kg body weight. In this animal study, the intake of GBE was 30 ~ 100 mg/kg body weight per day both in dietary pretreatment and in a single dose treatment. In our previous study, a significant induction of (s)-warfarin 7-hydroxylase was detected even at the lower dose (10 mg/kg body weight) of GBE (Umegaki et al., 2002). Owing to the species difference in the susceptibility to GBE, it is still not clear whether the interaction of tolbutamide and GBE also occurs in humans. Although the extrapolation of pharmacokinetic and pharmacodynamic interaction of GBE and tolbutamide in the aged rats to the clinical situation should be interpreted with caution, it is anticipated, from our recent preliminary study with healthy volunteers, that the repeated oral intake of GBE may influence the pharmacokinetics and pharmacodynamics of tolbutamide in humans (unpublished observation).

In conclusion, the present study has shown that the intake of GBE significantly affects the efficacy of tolbutamide in the aged rats. Therefore, it is anticipated that the simultaneous and continuous intake of GBE as a dietary supplement with therapeutic drugs should be cautious, particularly in elderly people.

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EFFECTS OF SAW PALMETTO EXTRACT ON MICTURITION REFLEX OF RATS AND ITS AUTONOMIC RECEPTOR BINDING ACTIVITY

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ABSTRACT

Purpose: We examined the effects of saw palmetto extract (SPE) on the rat micturition reflex and on autonomic receptors in the lower urinary tract.

Materials and Methods: The effect of SPE was examined on cystometrograms of anesthetized rats induced by intravesical infusion of saline or 0.1% acetic acid. SHR/NDmc-cp (cp/cp) rats received repeat oral administration of SPE and nighttime urodynamic function was determined. The autonomic receptor binding activity of SPE in the rat bladder and prostate was examined by radioligand binding assay.

Results: Intraduodenal administration of SPE (60 mg/kg) in anesthetized rat cystometry caused a significant increase in the micturition interval, micturition volume and bladder capacity during intravesical saline infusion. Also, similar administration of SPE at doses of 12 and 20 mg/kg significantly reversed the shortened micturition interval as well as the decreased micturition volume and bladder capacity due to 0.1% acetic acid infusion in a dose dependent manner. In conscious SHR/NDmc-cp (cp/cp) rats repeat oral administration of SPE (6 mg/kg daily) constantly increased the micturition interval and concomitantly decreased voiding frequency. SPE inhibited specific binding of [^{3}H]NMS ([N-methyl-^{3}H]scopolamine methyl chloride) (bladder) and [^{3}H]prazosin (prostate) with IC_{50} values of 46.1 and 183 μg/ml, respectively.

Conclusions: SPE significantly alleviates urodynamic symptoms in hyperactive rat bladders by increasing bladder capacity and subsequently prolonging the micturition interval. Our data may support the clinical efficacy of SPE for the treatment of lower urinary tract symptoms.

KEY WORDS: bladder; urodynamics; Permixon; rats, Sprague-Dawley

Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) are common disorders in aging men. The 2 main forms of internationally accepted medical treatment are inhibitors of 5α-reductase, such as finasteride or α_1-adrenoceptor antagonists, with the latter being more effective.[1] Nevertheless, plant extracts are widely used for the treatment of BPH and related LUTS. In fact, phytotherapeutic agents, including saw palmetto extract (SPE), are popular in many European countries, where herbal remedies represent up to 80% of all drugs prescribed for these disorders.[2] Debruyne et al reported that Permixon (Pierre Fabre Médicament, Castres, France), a lipid-sterolic extract of SPE, and α_1-blocker were equivalent for the medical treatment of LUTS in men with BPH during 12 months.[3]

Numerous mechanisms of SPE have been proposed, including 5α-reductase inhibition, and antiandrogenic, antiproliferative, anti-inflammatory and anti-edema effects.[4] However, most pharmacological effects were observed at relatively high doses of SPE.[5,6] Thus, none of these mechanisms for SPE has ever convincingly been demonstrated to be therapeutically relevant in vivo.[7,8] We noted the relatively potent smooth muscle relaxant activity by SPE observed in isolated tissues such as the bladder of guinea pigs and rats.[9] However, to our knowledge the smooth muscle relaxation of this extract in the lower urinary tract function has not been previously verified by in vivo pharmacology. Therefore, in this study we clarified the effect of SPE on urodynamic functions in anesthetized rat cystometry, and in conscious and unrestrained SHR/NDmc-cp (cp/cp) rats. Our data demonstrate that SPE is effective for improving the hyperactive bladder response of rats, suggesting the clinical efficacy of this extract for LUTS in patients with overactive bladder. Also, this extract has been shown to bind to autonomic receptors in the rat lower urinary tract.

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MATERIALS AND METHODS

Materials. SPE (Serenoa repens purified extract) was suspended in 0.5% methyl cellulose. [^{3}H]NMS ([N-methyl-^{3}H]scopolamine methyl chloride) (3.03 TBq/mmol) and [^{3}H]prazosin (2.98 TBq/mmol) were purchased from PerkinElmer Life Sciences, Inc., Boston, Massachusetts. All other chemicals were purchased from commercial sources.

Animals. Male Sprague-Dawley rats (SLC Co., Ltd., Hamamatsu, Japan) weighing about 250 to 350 gm were used. Also, the male subline of spontaneously hypertensive/National Institutes of Health corpulent rats [SHR/NDmc-cp (cp/cp)] (SLC Co., Ltd.) was also used. They were housed in the laboratory with free access to food and water, and maintained on a 12-hour dark/light cycle in a room with controlled temperature (about 20C to 26C) and humidity (about 30% to 70%).

Cystometry. The procedure of cystometry in anesthetized rats was performed as previously described.[10] Rats were anesthetized by subcutaneous injection of urethane (1.0 gm/

kg) and the duodenum was cannulated for the administration of SPE. The bladder was exposed through a short midline incision. It was cannulated with polyethylene tubing (PE-50, Clay-Adams, Parsippany, New Jersey) that was heat flared at the end, inserted into the bladder dome and ligated. Using a T connector the bladder catheter was connected to a pressure transducer (DX-360, Nihon Kohden, Tokyo, Japan) and to an infusion pump (STC-521, Terumo Corp., Tokyo, Japan). Saline or 0.1% acetic acid maintained at 37C at a rate of 4.0 ml per hour was instilled into the bladder of rats and urodynamic parameters were monitored by cystometry recording. Voided urine was cumulatively collected into a urine cup containing water that was placed on a microbalance (HF-200, A and D Co., Ltd., Tokyo, Japan). Analog voltage from the microbalance was received by a PowerLab/8sp (AD Instruments Pty Ltd., Castle Hill, New South Wales, Australia) and processed by MacLab8s proprietary client software, version 3.3 (Apple, Sunnyvale, California). After a stable cystometrogram was obtained urodynamic parameters were measured, including maximum micturition pressure, baseline pressure, threshold pressure, micturition interval, mean micturition volume and bladder capacity (infusion rate × micturition interval). The pretreatment period was considered the 20 minutes of saline or 0.1% acetic acid infusion before SPE administration. The effects of SPE (6 to 60 mg/kg) were analyzed 20 to 40 minutes after intraduodenal administration.

Frequency-volume charts. SHR/NDmc-cp (cp/cp) rats were administered SPE (6 mg/kg) orally from 3:00 to 5:00 p.m. for 14 days. The frequency-volume chart was continuously monitored from 6:00 p.m. to 6:00 a.m. on the next day. Voided urine was cumulatively collected into a urine cup containing liquid paraffin that was placed on a microbalance. Analog voltage from the microbalance was received by a PowerLab/8sp and processed by MacLabs8s. Voiding frequency and mean voided volume for 12 hours was calculated by proprietary client software. Water intake was measured before and during the oral administration of SPE.

Binding assay of [^{3}H]NMS and [^{3}H]prazosin in rat tissues. Binding assays of muscarinic and α_1-adrenoceptor receptors were performed using [^{3}H]NMS[11] and [^{3}H]prazosin, respectively. Rats were exsanguinated by taking the blood from the descending aorta under temporary anesthesia with diethyl ether and the tissues were then perfused with cold saline from the aorta. The bladder and prostate were then dissected and the tissues were minced with scissors. For the [^{3}H]NMS binding assay the bladder was homogenized using a Polytron homogenizer (Kinematica AG, Lucerne, Switzerland) in 19 volumes of ice-cold 30 mM Na^+/HEPES buffer (pH 7.5). The homogenate was then centrifuged at 40,000 × gravity for 20 minutes at 4C. The resulting pellet was finally resuspended in ice-cold buffer. For the [^{3}H]prazosin binding assay the prostate was homogenized in 30 volumes of ice-cold 50 mM tris-HCl buffer (pH 7.5). The homogenate was then centrifuged at 40,000 × gravity for 20 minutes at 4C. After suspension in cold buffer the pellet was centrifuged further at 40,000 × gravity for 20 minutes at 4C and the resulting pellet was finally resuspended in cold buffer.

In the presence of various concentrations (10 to 300 μg/ml) of SPE rat bladder and prostate homogenates were incubated with [^{3}H]NMS (456 pM) and [^{3}H]prazosin (516 pM) in 30 mM Na^+/HEPES buffer (pH 7.5) and 50 mM tris-HCl buffer (pH 7.5), respectively. Incubation was done for 60 ([^{3}H]NMS) and 30 ([^{3}H]prazosin) minutes at 25C. The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, Maryland) through GF/B glass fiber filters (Whatman, Brentford, United Kingdom) and the filters were rinsed 3 times with 3 ml ice-cold buffer. Tissue bound radioactivity was extracted from the filters by placing them overnight by immersion in scintillation fluid and radioactivity was then determined. Specific binding of [^{3}H]NMS and [^{3}H]prazosin was determined experimentally from the difference between counts in the absence and presence of 1 μM atropine and 10 μM phentolamine, respectively.

Data analysis. Cystometry and frequency-volume chart data are expressed as the mean ± SEM and analyzed by Student's paired t test. The binding activities of SPE to muscarinic and α_1-adrenocetors were estimated by IC_{50} values, which are the concentrations of SPE necessary to displace 50% of the specific binding of [^{3}H]NMS and [^{3}H]prazosin, as determined by log probit analysis. Statistical significance was considered at $p < 0.05$.

RESULTS

Effects of single administration on cystometric parameters. Figure 1 shows that reproducible micturition patterns were obtained throughout the cystometry period in anesthetized rats. In saline treated rats the intraduodenal administration of vehicle had little effect on urodynamic parameters (see table, fig. 1, *A*). These urodynamic parameters in saline infused rats were unaffected by the intraduodenal administration of SPE at the 6 mg/kg dose but the higher 60 mg/kg dose of SPE exerted a significant increase (40.5% to 42.6%) in micturition interval, mean micturition volume and bladder capacity as well as a significant decrease (8.1%) in maximum micturition pressure (see table, fig. 1, *B*). SPE (6 and 60 mg/kg) had little effect on baseline and threshold bladder pressure.

Following the intravesical infusion of saline containing 0.1% acetic acid in anesthetized rats compared with saline infusion alone the micturition interval (6.22 ± 0.57 vs 8.64 ± 0.73 minutes in 10 and 12, respectively) was significantly shortened (28.0%, $p < 0.05$). Mean micturition volume (0.60 ± 0.05 vs 0.42 ± 0.04 ml) and bladder capacity (0.57 ± 0.05 vs 0.42 ± 0.04 ml) were also significantly decreased (30.0% and 26.3%, respectively, $p < 0.05$).

Following the intraduodenal administration of SPE at doses of 12 and 20 mg/kg compared to results in 0.1% acetic acid treated rats there were significant and dose dependent increases in the micturition interval (15.1% vs 47.7%), mean micturition volume (15.9% vs 65.0%) and bladder capacity (14.0% vs 47.5%) (see table, fig. 1, *C*). In extract administered rats baseline pressure and maximum micturition pressure were slightly but significantly decreased but the effect was not dose related. Threshold pressure in rats was unaffected by SPE administration.

Effects of repeat administration on urodynamic parameters. The effect of repeat oral administration of SPE on urodynamic parameters in conscious and unrestrained SHR/NDmc-cp (cp/cp) rats was examined by recording nighttime micturition frequency-volume charts. Following the oral administration of a relatively low dose of SPE (6 mg/kg) in SHR/NDmc-cp (cp/cp) rats the micturition interval was continuously increased during repeat treatment for 3 to 8 days after the initial transient decrease compared with pretreatment values (46.0 ± 2.3 minutes in 6, fig. 2, *A*). Concomitantly voiding frequency in these rats constantly decreased after the initial transient increase following SPE administration (fig. 2, *C*). On the other hand, the 2 urodynamic parameters during treatment for 9 to 14 days were comparable with pretreatment values. Mean micturition volume tended to constantly increase during oral SPE administration (fig. 2, *B*). Also, total micturition volume was significantly increased for 9 to 14 days after the start of SPE administration (fig. 2, *D*). Water intake was little changed by repeat SPE administration (data not shown).

Effects on bladder muscarinic and prostatic α_1-adrenergic receptors. SPE at concentrations of 10 to 300 μg/ml competed with [^{3}H]NMS and [^{3}H]prazosin for binding sites in the rat bladder and prostate, respectively, in a concentration dependent manner (fig. 3). SPE IC_{50} values of [^{3}H]NMS and [^{3}H]prazosin binding were 46.1 ± 5.6 μg/ml in 7 preparations

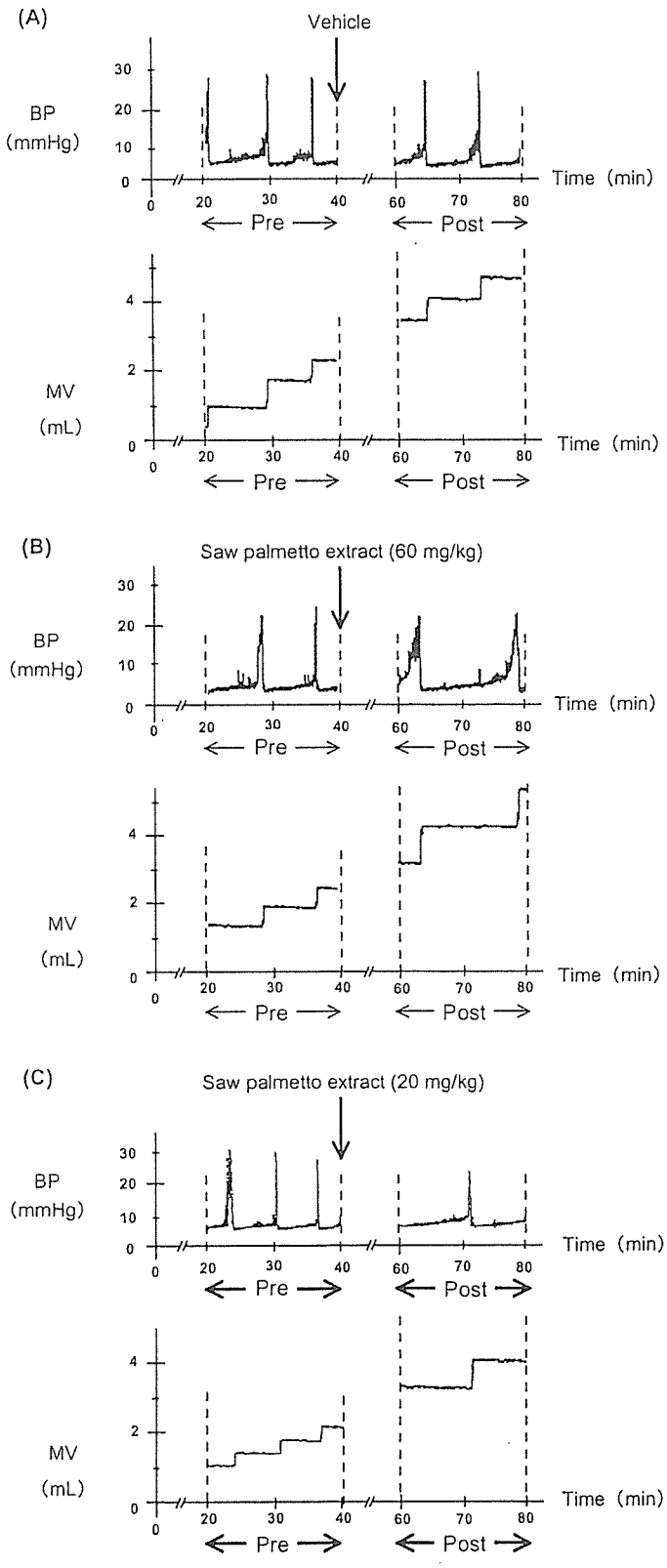

FIG. 1. Effects of intraduodenal administration of 6 to 60 mg/kg SPE on bladder pressure (*BP*) and micturition volume (*MV*) on cystometrograms of saline (*A* and *B*) and or 0.1% acetic acid (*C*) infused anesthetized rats. There were spontaneous contractions during bladder filling. Arrow indicates intraduodenal administration of 0.5% methyl cellulose vehicle (*A*), and 60 mg/kg (*B*) and 20 mg/kg (*C*) SPE. *Pre*, pretreatment. *Post*, posttreatment.

and 183 ± 30 μg/ml in 4, respectively. The SPE IC_{50} value of bladder [^{3}H]NMS binding was significantly lower than that of prostatic [^{3}H]prazosin binding (¼, $p < 0.001$).

DISCUSSION

The intraduodenal administration of SPE at the 60 mg/kg dose significantly increased bladder capacity in anesthetized rats, resulting in a prolonged micturition interval and enhanced mean micturition volume. It is well known that hyperreflexia manifested by enhanced voiding frequency is evoked by stimulating sensory afferents with dilute acetic acid solution in the cystometry infusate.[10,11] Also, in the current study continuous intravesical infusion of 0.1% acetic acid in anesthetized rats produced a hyperactive bladder response, which was characterized by a decrease in bladder capacity and mean micturition volume with a concomitant shortening of the micturition interval. It was evident that such a hyperactive bladder response due to acetic acid infusion was significantly attenuated by relatively low doses (12 and 20 mg/kg) of SPE given intraduodenally in a dose dependent manner. In fact, the values of these urodynamic parameters on rat cystometry after SPE administration at the 20 mg/kg dose were almost identical to control values in saline infused rats without acetic acid.

It should be noted that the intensity of the enhanced micturition interval, mean micturition volume and bladder capacity by SPE at the intraduodenal dose of 20 mg/kg in acetic acid infused rats was almost equivalent to that at the 60 mg/kg dose in saline infused rats (see table). The interpretation of such results is that the hyperactive bladder may be 3-fold more susceptible to SPE than the normal bladder. Thus, it is notable that hyperactive rather than normal bladder appears to be more sensitive to SPE. At any rate the current study may provide the first in vivo functional evidence to suggest that SPE significantly improves bladder instability.

Although the precise mechanism by which SPE attenuates a hyperactive bladder response in acetic acid infused rats remains to be clarified, there are several indications that significantly suppress smooth muscle tone. Previous pharmacological studies have demonstrated the spasmolytic effects of SPE on different isolated smooth muscle organs, such as the ileum, bladder, aorta and uterus in guinea pigs and rats.[9] These investigators speculated that the inhibitory effects of SPE on agonist or KCl induced smooth muscle contractions are mediated at least in part via the antagonistic effects on α-adrenoceptors, muscarinic cholinoceptors and calcium channels with the possible involvement of other activities. In fact, Goepel et al noted that SPE displaced α_1-adrenoceptor radioligands to bind to human prostatic and cloned human α_1-adrenoceptors in a noncompetitive manner and concomitantly suppressed agonist induced formation of [^{3}H]-inositol phosphate.[12] Furthermore, Noronha-Blob et al observed in in vitro and in vivo functional studies that anticholinergic activity rather than spasmolytic or local anesthetic activity of smooth muscle relaxants may have an important role in prevailing therapies for the suppression of bladder hyperactivity.[13] Also, it is worth noting that a relatively low concentration of SPE competed with bladder [^{3}H]NMS binding sites as well as prostatic [^{3}H]prazosin binding sites in a concentration dependent manner and binding activity was significantly (4 times) greater to muscarinic receptors than to α_1-adrenoceptors (fig. 3). Thus, it seems likely that SPE increases bladder capacity, possibly via antimuscarinic and/or spasmolytic effects on bladder smooth muscle, resulting in increased micturition volume and a prolonged micturition interval. Nevertheless, it cannot be ruled out that SPE may have some additional peripheral or central neuronal effects because bladder instability may possibly arise not only from a myogenic, but also from a neurogenic mechanism.[14]

We further examined the urodynamic effect of repeat oral administration of SPE in conscious and unrestrained SHR/NDmc-cp (cp/cp) rats. The SHR/NDmc-cp (cp/cp) strain is a strain of spontaneously hypertensive/National Institutes of

Effects of intraduodenal SPE administration (6 to 60 mg/kg) on urodynamic parameters in cystometrograms of saline and 0.1% acetic acid infused anesthetized rats

	Mean Saline ± SE (mg/kg SPE)		Mean Acetic Acid ± SE (mg/kg SPE)	
	6	60	12	20
Micturition interval (mins):				
Pretreatment	9.93 ± 1.07	7.71 ± 0.90	6.44 ± 1.10	5.99 ± 0.47
Posttreatment	9.07 ± 1.09	10.83 ± 1.77*	7.41 ± 1.25*	8.85 ± 1.07*
Mean micturition vol (ml):				
Pretreatment	0.69 ± 0.07	0.54 ± 0.06	0.44 ± 0.08	0.40 ± 0.04
Posttreatment	0.66 ± 0.09	0.77 ± 0.13*	0.51 ± 0.08*	0.66 ± 0.08*
Bladder capacity (ml):				
Pretreatment	0.65 ± 0.06	0.51 ± 0.06	0.43 ± 0.07	0.40 ± 0.03
Posttreatment	0.60 ± 0.07	0.72 ± 0.12*	0.49 ± 0.08*	0.59 ± 0.07*
Baseline pressure (mm Hg):				
Pretreatment	3.35 ± 0.19	3.17 ± 0.10	3.99 ± 0.26	3.54 ± 0.16
Posttreatment	3.20 ± 0.22	2.93 ± 0.21	3.69 ± 0.23	3.37 ± 0.11*
Threshold pressure (mm Hg):				
Pretreatment	5.02 ± 0.13	4.74 ± 0.16	5.05 ± 0.31	4.44 ± 0.19
Posttreatment	4.78 ± 0.13	4.81 ± 0.15	5.13 ± 0.27	4.62 ± 0.25
Max micturition pressure (mm Hg):				
Pretreatment	26.1 ± 2.2	28.3 ± 0.8	29.3 ± 1.7	25.6 ± 1.4
Posttreatment	23.9 ± 2.0	26.0 ± 0.7*	25.7 ± 2.0†	23.0 ± 0.5

Values in 5 to 7 rats.
* Significantly different vs pretreatment (Student's paired t test $p < 0.05$).
† Significantly different vs pretreatment (Student's paired t test $p < 0.01$).

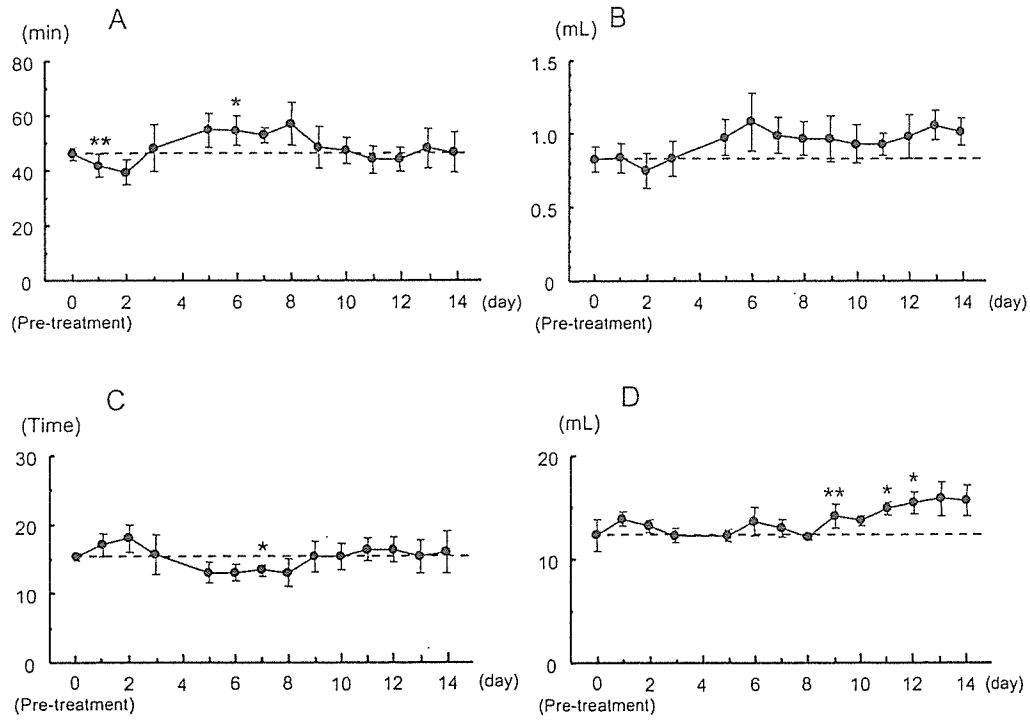

FIG. 2. Effects of repeat SPE oral administration on nighttime urodynamic function from 6:00 p.m. to 6:00 a.m., that is micturition interval (*A*), mean micturition volume (*B*), voiding frequency (*C*) and total micturition volume (*D*), in conscious and unrestrained SHR/NDmc-cp (cp/cp) rats that received 6 mg/kg SPE orally daily for 14 days. Point represent mean ± SE of 3 SHR/NDmc-cp (cp/cp) rats. Dotted lines indicate pretreatment values. Single asterisk indicates significantly different vs pretreatment (Student's paired t test $p < 0.05$). Double asterisks indicate significantly different vs pretreatment (Student's paired t test $p < 0.01$).

Health corpulent rat as a model of type 2 diabetic nephropathy. SHR/NDmc-cp (cp/cp) rats showing significant hypertension retain a common genetic background with nondiabetic SHR rats.[15] Adrenergic innervation is generally increased in the SHR strain but SHR bladders are hyperactive relative to Wistar-Kyoto rat bladders.[16] Clemow et al reported that with an increase in adrenergic efferent innervation there is an increase in sensory afferent innervation in SHR bladders.[17] Their data suggest that the increase in sensory afferent nerve density may underlie an enhanced voiding reflex. It was shown that repeat oral administration of SPE (6 mg/kg daily) in conscious SHR/NDmc-cp (cp/cp) rats for 8 days produced a transient shortening and subsequent sustained prolongation of the micturition interval and concomitantly SPE administration decreased voiding frequency. Thus, it is likely that repeat SPE administration alleviates hyperactivity of the SHR bladder, as observed by a single intraduodenal administration of this extract on anesthetized rat cystometry. However, such urodynamic improvement of SPE in SHR/NDmc-cp (cp/cp) rats apparently disappeared after longer repeat administration more than 9 days in duration. The clear explanation for this result is lacking but it may be in part attributable to the significant increase in total micturition volume associated with extract administration for

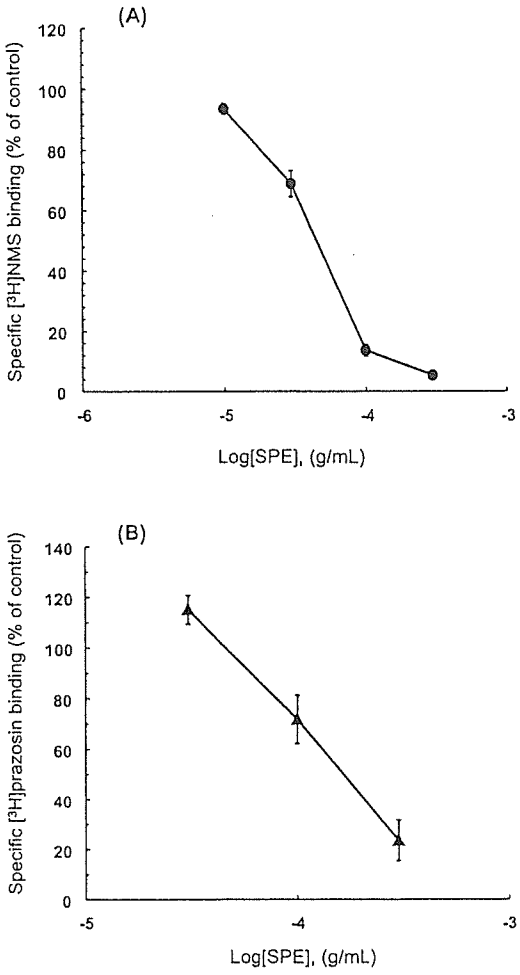

FIG. 3. SPE inhibition of specific binding of [^{3}H]NMS in rat bladder (*A*) and [^{3}H]prazosin in prostate (*B*). Specific binding of [^{3}H]NMS (456 pM) and [^{3}H]prazosin (516 pM) in homogenates of rat bladder and prostate, respectively, was measured in absence and presence of 3 or 4 concentrations (10 to 300 μg/ml) of SPE. Points represents mean ± SE in 4 ([^{3}H]prazosin) or 7 ([^{3}H]NMS) rats.

more than 9 days. To our knowledge basic pharmacological evidence for the diuretic effect of SPE has not been reported previously but a mild diuretic effect in humans has been described.[18] However, until the pharmacological effects of SPE on lower urinary tract function are definitely known we should continue to interpret variable urodynamic effects of this extract in conscious SHR/NDmc-cp (cp/cp) rats with caution.

In the majority of men with BPH and LUTS the major treatment goal is the relief of irritative and obstructive symptoms. These symptoms have been shown to be significantly alleviated by phytotherapeutic agents, including SPE.[3,19] Our data support this clinical usefulness.

CONCLUSIONS

SPE may improve urodynamic symptoms in hyperactive rat bladders by increasing bladder capacity and prolonging the micturition interval. In addition, SPE contains the constituent(s) exerting the binding activities of muscarinic and α_1-adrenergic receptors in the lower urinary tract. Thus, our data may support the clinical efficacy of SPE for the treatment of LUTS accompanying overactive bladder.

SHR/NDmc-cp (cp/cp) rats were established at Disease Model Cooperative Research Association, Kyoto, Japan. Serenoa repens purified extract was provided by Indena Japan Co., Ltd., Tokyo, Japan. K. Sakakura provided technical assistance.

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Effects of Ginkgo Biloba Extract on Pharmacokinetics and Pharmacodynamics of Tolbutamide and Midazolam in Healthy Volunteers

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This study was undertaken to clarify the influence of repeated oral administration of Ginkgo biloba extract (GBE) on CYP2C9 and CYP3A4. CYP2C9 probe (tolbutamide, 125 mg) and CYP3A4 probe (midazolam, 8 mg) were orally administered to 10 male healthy volunteers before and after GBE intake (360 mg/d) for 28 days, and they received 75 g glucose after the dosing of tolbutamide. Plasma drug concentrations and blood glucose levels were measured. The area under concentration versus time curve ($AUC_{0-\infty}$) for tolbutamide after GBE intake was slightly but significantly (16%) lower than that before GBE intake. Concomitantly, GBE tended to attenuate AUC_{0-2} of blood glucose–lowering effect of tolbutamide. $AUC_{0-\infty}$ for midazolam was significantly (25%) increased by GBE intake and oral clearance was significantly (26%) decreased. Thus, it is suggested that the combination of GBE and drugs should be cautious in terms of the potential interactions, especially in elderly patients or patients treated with drugs exerting relatively narrow therapeutic windows.

Keywords: *Ginkgo biloba extract; CYP2C9; CYP3A4; pharmacokinetic interaction*

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Currently, the consumption of dietary supplements containing botanical products and foods is growing at a remarkable speed, in terms of the promotion of health or prevention and therapy of diseases. In particular, elderly people frequently take dietary supplements with prescribed drugs.[1,2] Recent studies have indicated that as many as 16% of prescribed drug users consume herbal dietary supplements.[3] With the widespread use of herbal dietary supplements, the risk of herb-drug interactions is a growing medical concern.[4] The possible interactions would occur in aspects of pharmacokinetics and pharmacodynamics of drugs,[5,6] such as absorption in small intestine, metabolism in the intestine and liver, distribution to target organs, transport across cell membrane, and binding to specific receptors. Among these interactions, induction and inhibition of drug-metabolizing enzymes by herbal medicines or dietary compounds have been investigated. As a well-known example, St John's Wort, an herbal medicine applied for mild depression, has been shown to decrease the blood concentrations of drugs such as cyclosporine, tacrolimus, and midazolam by inducing particularly cytochrome P450 (CYP) 3A4 activity[7-9] and thereby could attenuate the efficacy of drugs. On the other hand, grapefruit juice has been

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reported to increase oral bioavailability of coadministered drugs, such as 1,4-dihydropyridine calcium channel antagonists, benzodiazepines, antihistamines, and cyclosporin, due to a significant inhibition of both metabolism by CYP3A4 enzyme and excretion through P-glycoprotein in the gastrointestinal tract.[10,11]

Ginkgo biloba extract (GBE) has been used in traditional Chinese medicine for thousands of years and contains ginkgo flavonol glycosides and terpenoids.[12,13] Ginkgo biloba extract is easily available both as an over-the-counter herbal supplement in Japan and the United States and as a prescribed drug in some European countries. In clinical practice, it is used mostly for neurological, psychological, and behavioral disorders. Some randomized control trials have shown that oral ingestion of GBE over 24 to 26 weeks compared with placebo improves cognitive function in patients with Alzheimer's disease or vascular dementia.[14] The interaction between GBE and prescribed drugs remains controversial. The previous in vitro study indicated that constituents of GBE inhibited drug-metabolizing enzymes including CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.[15] On the other hand, another study reported that the principal components of GBE preparations in clinical use did not significantly inhibit human CYPs.[16] Furthermore, clinical studies have shown that GBE had little effect on pharmacokinetics and pharmacodynamics of warfarin in healthy volunteers[17] and on warfarin dosage in stable, long-term warfarin-treated patients.[18] Previous clinical studies have been performed by oral administration of GBE for relatively short periods (1-2 weeks) and/or at dose of 120 to 240 mg/d.[17-22] There is little information to indicate the effect of a rather high dose of GBE that is administered for a long period compared to these in the previous trials on pharmacokinetics and pharmacodynamics of drugs. Thus, the present study was designed to assess the influence of repeated treatment with a relatively high dose (360 mg/d) of GBE on the activities of CYP2C9 and CYP3A4 for 28 days. We investigated pharmacokinetics of tolbutamide and midazolam, with respect to in vivo probe substrates of CYP2C9 and CYP3A4, after repeated treatment with GBE in healthy volunteers. In addition, the hypoglycemic effect of tolbutamide in these subjects was determined.

METHODS

Subjects

Ten male healthy volunteers (mean ± SD: age, 24.9 ± 2.6 years; body weight, 68.6 ± 7.1 kg) participated in this study after they had given written informed consent. All subjects were Japanese and in good health as indicated by medical history, routine physical examination, and clinical laboratory tests. There were 9 subjects with extensive metabolizer genotypes of CYP2C9 (*CYP2C9*1/*1*) and 1 subject with *CYP2C9*1/*3* genotype. All subjects were nonsmokers, ate a normal diet, and did not take prescription or nonprescription medications and botanical dietary supplements. The study protocol was approved by the Ethics Committee of Hamamatsu University School of Medicine, Hamamatsu, Japan.

Study Design

In the pretrial phase, following overnight fasting, each subject orally received a standardized 75-g glucose solution (Trelan-G; Takeda Pharmaceutical Co, Osaka, Japan). Blood samples were drawn at 60, 45, 30, and 15 minutes before the intake of glucose and 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, and 180 minutes after the intake, and blood glucose concentrations were quantified by an electrochemical method with FreeStyle Blood Glucose Testing system (Kissei Pharmaceutical Co, Nagano, Japan). After 2 weeks, following overnight fasting, each subject was administered an oral dose of 125 mg tolbutamide (Rastinon; Aventis, Frankfurt, Germany) at 9 AM. At 1 hour after the administration of tolbutamide, they received an oral dose of 8 mg midazolam (Dormicum; Yamanouchi Co, Tokyo, Japan) and 75 g glucose. Blood samples were drawn at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 24 hours after the administration of tolbutamide, urine samples were collected up to 24 hours after dosing, and blood glucose concentrations were quantified as described above. From the next day, each subject took an oral dose of 360 mg/d GBE, which was an EGb 761 formulation manufactured by Dr Willmar Schwabe Company (WSG & Co) distributed by Nature's Way Products (Springville, Utah) under the trade name Ginkgold, as 2 tablets (120 mg) of 60 mg GBE 3 times a day (8 AM, 12 AM, and 8 PM) for 28 days. Ginkgo biloba extract tablets were standardized to the content of 24% Ginkgo flavone glycoside and 6% terpene lactone. This product was chosen for study because of its high degree of standardization and its use in published clinical trials. After the treatment with GBE, subjects received tolbutamide, midazolam, and glucose; blood and urine samples were collected in the same protocol as the previous phase. Plasma samples were isolated from blood samples by centrifugation. Plasma and urine samples were stored at –80°C until the concentrations of drugs were determined.