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Clinica Chimica Acta 367 (2006) 144 – 149

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Effectiveness of activated charcoal and equilibrium dialysis in removing Asian, American, Siberian and Indian ginseng from human serum

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Received 7 November 2005; received in revised form 1 December 2005; accepted 4 December 2005
Available online 31 January 2006

Abstract

Background: Ginsengs are used by the general population worldwide and toxicity of various ginsengs has been reported. We studied the effectiveness of activated charcoal and in vitro equilibrium dialysis for removal of Asian, American, Siberian and Indian ginseng from human serum by measuring digoxin-like immunoreactivity using the fluorescence polarization immunoassay.
Methods: 1× PBS (phosphate buffered saline) or drug free serum pool was supplemented with Asian, American, Siberian or Indian ginseng extract in amount expected in overdose. The aliquots of supplemented buffer or serum pool were treated with activated charcoal (15 or 50 mg of activated charcoal/ml of buffer/serum) for 5, 10, 20 and 30 min and digoxin-like immunoreactivities were compared with the original specimens. Other drug free serum pools were also supplemented with various ginsengs and then passed through a small column packed with activated charcoal or subjected to in vitro equilibrium dialysis against phosphate buffer at pH 7.4.
Results: Complete removal of digoxin-like immunoreactivity from buffer solution or serum pool supplemented with various ginsengs can be achieved by treatment with activated charcoal. Moreover, when serum pools supplemented with various ginsengs were passed through columns packed with activated charcoal, we observed complete removal of digoxin-like immunoreactivity. In addition, significant removal of digoxin-like immunoreactivity was observed when serum pools supplemented with ginsengs were subjected to equilibrium dialysis for 24 h.
Conclusions: Removal of digoxin-like immunoreactivity from buffer solution or serum due to the presence of ginsengs can be achieved by treatment with activated charcoal in vitro but complete removal of digoxin-like activity from serum is not possible even after 24 h of equilibrium dialysis.
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Keywords: Activated charcoal; Equilibrium dialysis; Digoxin-like immunoreactivity; Ginseng

1. Introduction

Various ginseng products are widely used in China, other Asian countries and in the U.S. The Chinese ginseng that grows in Manchuria is *Panax ginseng* which is commonly marketed as "Asian Ginseng". It contains active components ginsenosides which have structural similarity with digoxin. American ginseng is the extract of *Panax quinquefolius*. Siberian ginseng is prepared from a different plant: *Eleutherococcus senticosus*. Hikino et al. isolated 7 glycans from a crude extract of Siberian ginseng [1]. Li et al. isolated a new ligand glycoside named eleutheroside E(2) from Siberian ginseng [2]. Eleutherosides also have structural similarity with digoxin.

More recently Indian ginseng has become available on the US market. Although labeled as "ginseng products", this herbal supplement is prepared from an entirely different plant known as winter cherry or ashwagandha (*Withania somnifera*) and has been used in Ayurvedic medicine for over 3000 years as an aphrodisiac, liver tonic, anti-inflammatory agent and astringent [3]. Recent clinical trials and animal research support the use of Indian ginseng for treating anxiety, cognitive and neurological disorders, inflammation and Parkinson's disease [4]. The major

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0009-8981/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2005.12.005

biochemical constituents of Indian ginseng are steroidal alkaloids and steroidal lactones (withanolides) which have structural similarity with digoxin. More recently other novel withanolides have been isolated and characterized from Indian ginseng [5,6].

Active components of ginsengs can be measured by sophisticated techniques such as HLPC combined with tandem mass spectrometry or UV detection [7,8]. However active components of ginsengs also interfere with the fluorescence polarization immunoassay of digoxin and such assay can be used for rapid but indirect detection of ginseng in human serum [9].

Toxicity and adverse drug reactions from the use of ginseng have been reported. Menorrhagia and sinus tachycardia were reported in a 39-y old female after use of ginseng. Arrhythmia resolved ten days after discontinuation of ginseng [10]. Haller et al. reported that ginseng was ranked number 6 out of 12 herbs associated with toxicity as assessed by the number of calls received by the San Francisco division of the California Poison Control System in 1998. Published adverse effect reports indicated that there were 133 cases of mild toxicity from the use of ginseng, 5 cases of moderate toxicity and 1 case of severe toxicity [11]. In 1979, the term “Ginseng abuse syndrome” was coined as a result of a study on 133 people who took ginseng for 1 month [12].

There is no specific antidote for ginseng toxicity. Activated charcoal is given to prevent further absorption of many toxins and we studied the possibility of removing ginsengs from a buffer solution (pH 7.2, mimicking intestinal fluid) using activated charcoal. We also studied the effectiveness of activated charcoal in removing ginsengs from serum in vitro as well as the effectiveness of equilibrium dialysis in removing various ginsengs from serum as an in vitro model for hemodialysis which is sometimes effective in removing toxins. The effectiveness of activated charcoal and equilibrium dialysis in removing various ginsengs from biological matrix has never been reported before.

2. Materials and methods

Activated charcoal was obtained from Aldrich Chemical Company (Milwaukee, WI). 10× PBS (phosphate buffered saline) concentrate was from EMD Chemicals (Merck, Gibbstown, NJ). Sodium phosphate dibasic was obtained from Fisher Scientific. Equilibrium dialysis was performed using dialysis membrane (Spectra/Por molecular porous membrane with 7.5 mm diameter) with a molecular weight cut-off of 25,000 Da (Spectrum Laboratory Products, Los Angeles, CA). Apparent digoxin concentrations were measured using the fluorescence polarization immunoassay (FPIA) for digoxin and a TDx/FLX analyzer (Abbott Laboratories, Abbott Park, IL). We also used the FPIA assay for procainamide, N-acetyl procainamide (NAPA) and phenytoin for this study. Reagents as well as calibrators and controls were from Abbott Laboratories.

Various types of ginseng were purchased from local herbal stores in Houston, TX. The Indian ginseng was marketed by Herb Pharmacy (Williams, OR) and was sold as a liquid extract of ashwagandha. Siberian ginseng was the Z-T brand liquid extract manufactured in China and distributed by Z-T Universal Inc (Glen Head, NY). Asian ginseng was the Song Shiu Pan brand Panax Ginseng manufactured in China. The North American ginseng was also a liquid extract sold by Herba Natural Products Inc (Brooklyn, NY).

The FPIA digoxin assay requires a sample pretreatment. Two hundred microliters of serum was mixed with 200 μl of precipitating agent (Sulfosalicylic acid in methanol). The precipitated protein was separated from the clear supernatant by centrifugation at high speed and apparent digoxin level was measured using the supernatant. The assay is linear up to a serum digoxin concentration of 5.0 ng/ml and the detection limit of the assay is 0.20 ng/ml. Therefore, any apparent digoxin concentration below 0.20 ng/ml is considered as “none detected” level of apparent digoxin. The FPIA digoxin assay has a good precision with a total CV of $<7\%$.

In order to study whether components of ginsengs cross-react with antidigoxin antibody of the FPIA or interfere with the fluorescence measurement as an artifact, we supplemented drug free serum pool with Asian, American, Siberian or Indian ginseng (100 μl of appropriate ginseng extract per milliliter of serum) and then measured apparent drug levels using FPIA assays for digoxin, procainamide, NAPA and phenytoin. Any value below the detection limit of the respective assay was considered “none detected”.

In the next set of experiments, we diluted 10× PBS buffer with deionized water (1 : 10 dilution) in order to achieve 1× PBS buffer (10 mmol/l phosphate, 137 mmol/l NaCl and 2.7 mmol/l KCl). The pH was adjusted with 2 mol/l HCl in order to obtain a final pH of 7.2 for further experiments. Aliquots of this PBS buffer were supplemented with appropriate ginseng extract (100 μl of extract/ml buffer).

We also supplemented aliquots of drug and DLIS free serum pool with various ginsengs extract (100 μl of extract/ml drug free serum). We used seven different drug free serum pools for this study. Before supplementation, all drug free serum pools were analyzed for any apparent digoxin activity using the fluorescence polarization immunoassay of digoxin to ensure that these serum pools were also free from any endogenous digoxin-like immunoreactive substances (DLIS). None of these serum pools showed any apparent digoxin concentration indicating that such serum pools were virtually free of DLIS. All ginseng products used in this study were liquid extract containing water and various amounts of ethyl alcohol. We added liquid extract directly to drug free serum. We did not attempt to evaporate the alcohol content of the extract under nitrogen because of the potential

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