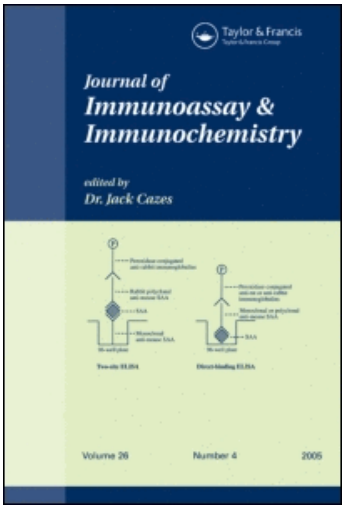

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Direct Determination of Serum Glycyrrhetic Acid by a Monoclonal Antibody-Based Inhibition Elisa Using Ibuprofen for Releasing Serum Albumin-Bound Glycyrrhetic Acid

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DIRECT DETERMINATION OF SERUM GLYCYRRHETIC ACID BY A MONOCLONAL ANTIBODY-BASED INHIBITION ELISA USING IBUPROFEN FOR RELEASING SERUM ALBUMIN-BOUND GLYCYRRHETIC ACID

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ABSTRACT

We found that ibuprofen (IBU) had a potential for releasing serum albumin-bound glycyrrhetic acid (GA). Based on this observation, IBU was used to pretreat samples for the determination of serum GA levels by an inhibition ELISA. This method, termed IBU method was evaluated by the recovery of GA from human serum albumin (HSA) or normal human serum (NHS) that contained the exogeneously added GA (37-1000 ng/ml). The mean recovery of GA from HSA and NHS samples treated with IBU were 104.7 and 105.2%, respectively, whereas those without IBU pretreatment were 2.8 and 10.7%, respectively. Comparison of IBU method and chloroform extraction method revealed that the GA content of serum samples pretreated by each method were almost the same. These results suggest that IBU method is useful as a serum processing procedure for the determination of serum GA levels by an inhibition ELISA.

(KEYWORDS: glycyrrhetic acid, ibuprofen, inhibition ELISA, human serum, drug monitoring)

INTRODUCTION

Glycyrrhizin (GL), a principal constituent of *Glycyrrhizae Radix*, is known to exist in a natural Chinese herb with anti-inflammatory properties (1). The Stronger Neo-Minophagen C (SNMC), a preparation of GL with glycine and cystein, has been widely used in the treatment of chronic hepatitis in Japan (2,3). The patients receiving a large dose of SNMC over a long period often show symptoms such as hypertension, edema, and hypokalemia (4). These side-effects are caused by glycyrrhetic acid (GA), an aglicon of GL, because of its aldosterone-like effects (5). The monitoring of serum GA levels are necessary to avoid such side-effects.

High performance liquid chromatography (HPLC) (6-8), enzyme immunoassay (EIA) (9), and radio immunoassay (RIA) (10) have been reported as quantitative methods to evaluate the serum GA level. Recently we developed a rapid and sensitive ELISA for the determination of serum GL and GA levels (11). Although the extraction and purification of GA from samples are inevitable for accurate determinations, the GA binds tightly to serum albumin making this process complicated and time consuming.

Some drugs are well known to have their binding sites on human serum albumin (HSA). So far the binding sites of warfarin (WAR), ibuprofen (IBU), salicylic acid (SAL), and deoxycholic acid (DCA) have been identified. We have tested the effect of these drugs on the release of serum albumin-bound GA, and it was revealed that IBU had the potential of releasing the serum albumin-bound GA. Here we report that the establishment of the direct immunochemical determination procedure of serum GA levels using IBU as a displacer. Reliability of this procedure, termed the IBU method, was evaluated by the recovery of GA from a serum sample containing a known amount of GA.

MATERIALS AND METHODS

Drugs and serum

GA was supplied by Minophagen Pharmaceuticals (Tokyo, Japan). WAR, IBU, SAR, and DCA as well as HSA were purchased from Sigma (St Louis, MO,

TABLE 1

Effect of drugs on recovery of glycyrrhetic acid from human serum albumin

Drug added (2mg/ml)	Glycyrrhetic acid		Recovery (%)
	Added (ng/ml)	Detected (ng/ml)	
Non		0.0	-
	125	0.5	0.4
	250	2.1	0.8
IBU	0	0.9	-
	125	122.3	97.1
	250	271.2	108.1
WAR	0	0.2	-
	125	1.5	1.0
	250	5.4	2.1
SAL	0	0.0	-
	125	0.6	0.5
	250	2.0	0.8
DCA	0	2.0	-
	125	7.3	4.2
	250	20.2	7.3

*IBU; ibuprofen, WAR; warfarin, SAL; salicylic acid, DCA; deoxycholic acid

U.S.A.). Normal human sera (NHS) were prepared from venous blood collected from healthy adult volunteers.

Sequential treatment of GA and drugs on HSA or NHS

Various concentrations of GA solution (20 μl) supplemented with either HSA or NHS (175 μl) were incubated for 30 min at 37℃. Next, the drug solution (5 μl) was added to the above mixture, and incubated for 30 min at 37℃.

TABLE 2

Effect of ibuprofen on the ELISA system*

GA added to PBS (ng/ml)	Detected (ng/ml)		Recovery (%)	
	-IBU	+IBU	-IBU	+IBU
0	0.0	2.3	-	-
37	35.4	38.3	95.7	97.2
111	103.2	118.7	92.9	104.9
333	322.8	343.1	96.9	102.3
1000	962.5	1083.0	96.3	108.1

*Ibuprofen was used at a concentration of 2 mg/ml.

The GA content in the drug-treated samples were determined by an inhibition ELISA as previously reported (11) without any pretreatment.

RESULTS AND DISCUSSION

First we tested the effects of drugs with known binding sites on serum albumin, on the release of serum albumin-bound GA. GA solutions (125 and 250 ng/ml in PBS) were mixed with HSA (42 mg/ml in PBS), and incubated for 5 min at 37℃. Then the drugs (80 mg/ml in ethanol) to be tested were added to the above reaction mixtures (final concentration 2 mg/ml) and further incubated for 5 min at 37℃. The recovery of GA in the samples were determined by an inhibition ELISA. GA was quantitatively recovered from the samples only when IBU was added to the reaction mixtures (Table 1). Ishida *et al.* (12) reported that the binding sites of GL on HSA were located mainly in both the low-affinity IBU binding site and specific DCA binding site. From these results and observations, the binding sites of GA are not identical to those of GL, but they might partially overlap each other.

The IBU concentration of 2 mg/ml was chosen because it was the lowest concentration with the highest releasing activity (data not shown). We then tested whether 2 mg/ml of IBU had an inhibitory effect on the antigen-antibody reaction in an ELISA system. After adding 2 mg/ml of IBU to a PBS solution containing a known amount of GA, the recovery of GA was determined by an inhibition ELISA. The recovery of GA from samples with the IBU additive was similar with the samples without IBU additives (Table 2).

To confirm the usefulness of the IBU method for the pretreatment of serum samples, the recovery of GA from HSA containing exogeneously added GA (37-1000 ng/ml) was determined. The recovery of GA was found to be between 101.4 and 107.7%, whereas only 2.8% of recovery was obtained from the samples containing 1000 ng/ml of GA without the addition of IBU (Table 3). Using the fresh serum collected from a healthy volunteer, the IBU method was also evaluated. The recovery of GA was found to be between 96.3 and 116.4% (Table 4). Furthermore we did the precision and the method comparative studies. The intra- and inter-assay variations were found to be within 10% (data not shown).

TABLE 3

Effect of ibuprofen on the recovery of glycyrrhetic acid from human serum albumin*

GA added to HSA (ng/ml)	Detected (ng/ml)		Recovery (%)	
	-IBU	+IBU	-IBU	+IBU
0	0.0	12.5	-	-
37	0.0	50.8	0.0	103.5
111	0.4	132.0	0.4	107.7
333	2.8	365.6	0.8	106.0
1000	27.7	1014.0	2.8	101.4

*Ibuprofen was used at a concentration of 2 mg/ml.

TABLE 4

Effect of ibuprofen on the recovery of glycyrrhetic acid from normal human serum*

GA added to NHS (ng/ml)	Detected (ng/ml)		Recovery (%)	
	-IBU	+IBU	-IBU	+IBU
0	43.0	62.2	-	-
37	39.9	99.4	0.0	100.5
111	40.8	191.4	0.0	116.4
333	69.8	419.8	8.0	107.4
1000	149.9	1025.3	10.7	96.3

*Ibuprofen was used at a concentration of 2 mg/ml.

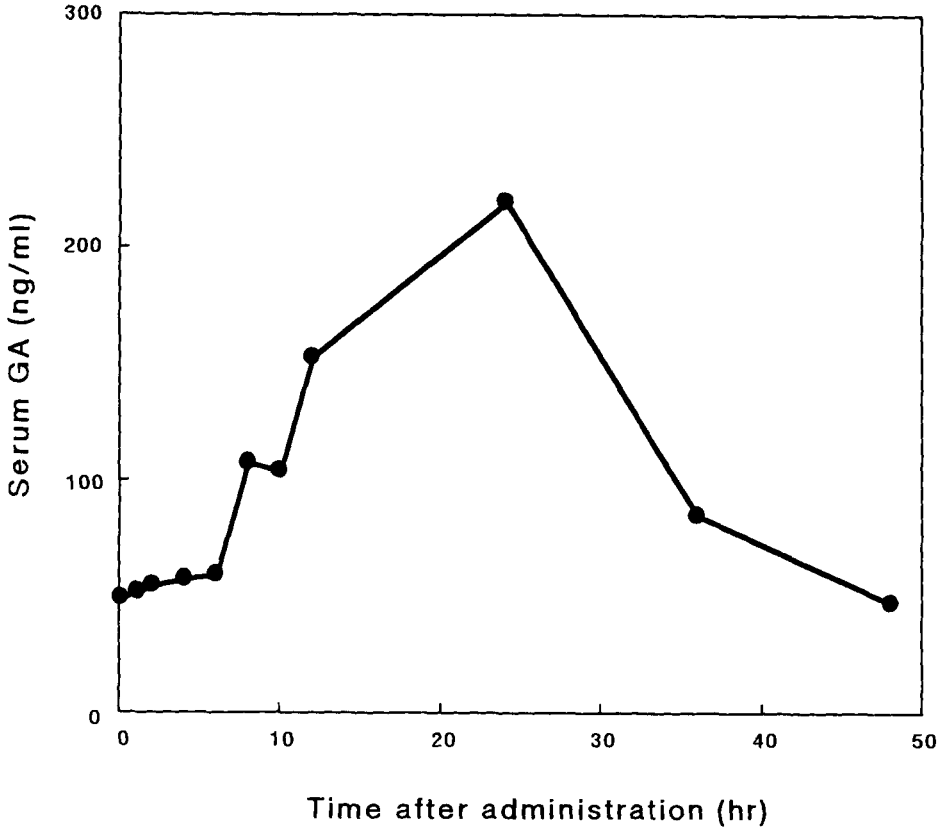

FIGURE 1 Serum GA levels in a healthy individual after intravenous administration of SNMC. Serum samples were pretreated with IBU method, and GA levels were determined by inhibition ELISA.

The mean recovery of GA from NHS containing exogeneously added GA (111-1000 ng/ml) processed by the IBU method and by the chloroform extraction method that is generally used for sample processing was 104.6% and 103.4% , respectively (data not shown). From these results, the IBU method could be applicable as a reliable sample processing procedure to determine the serum GA levels by an inhibition ELISA. In Table 4, certain amounts of GA (43.0-62.2 ng/ml) was detected in NHS samples regardless of the addition of IBU. This is probably caused by the daily food intake because many kinds of foods and seasonings contain GL as an ingredient.

The IBU method was applied to monitor the serum GA levels in a healthy individual after intravenous administration of SNMC. Serum samples were obtained from the venous blood collected at 0, 1, 2, 4, 6, 8, 10, 12, 24, 36, and 48 hrs after administration of 40 ml of SNMC containing 80 mg of GL, and treated with IBU followed by determination of serum GA levels by an inhibition ELISA. The results are shown in Figure 1. The GA levels were successfully determined at all time points, and a single peak appeared at 24 hr after the administration.

In conclusion, serum GA levels can be immunochemically measured by adding IBU to samples without extraction or purification. This procedure is simple to perform and would be applicable for clinical purposes, such as mass-screening or therapeutic drug monitoring. This procedure may thus be applicable for the immunochemical determination of other drugs that bind to serum proteins.

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