Synthetic Polymer Sulphonated Polyisoprene as a Universal Anticoagulant for Laboratory Testing

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To evaluate the feasibility of a synthetic polymer, sulphonated polyisoprene (SPIP), to be used as a new laboratory anticoagulant, hematological items were compared between blood samples anticoagulated with EDTA and SPIP, as were biochemical and electrolyte items between serum samples and SPIP-anticoagulated blood. Among hematological items, the average platelet count of the SPIP group was significantly lower than that of the EDTA group, due to platelet aggregation in the SPIP group. Addition of kanamycin to SPIP, but not of aspirin or prostaglandin E1, restored platelet count to about 80% of that in the EDTA group, and increased coefficients of correlation with the EDTA group for most hematological items

examined. For biochemical and electrolyte items, high (> 0.95) correlation coefficients were obtained for 15 of 18 items between the serum and SPIP-anticoagulated samples. However, for some of these items, the addition of kanamycin decreased coefficients of correlation. Thus, SPIPs are excellent candidates for new anticoagulants that can be used for evaluation of hematological, biochemical, and electrolyte items with a single test tube in routine laboratory work. However, some improvements are required for use of kanamycin as an additive to inhibit platelet aggregation induced by SPIP, and in maintaining reliability of biochemical and electrolyte measurements. *J. Clin. Lab. Anal.* 14:180–187, 2000. © 2000 Wiley-Liss, Inc.

Key words: anticoagulant; sulphonated polyisoprene; synthetic polymer; kanamycin; platelet aggregation; laboratory testing

INTRODUCTION

Recent developments in laboratory technology and transportation systems have helped reduce the time, cost, and manpower required for daily blood examination in hospitals. However, for a long time there has been little change in the methods used to sample blood specimen; ion chelators such as ethylenediaminetetraacetic acid (EDTA) and antithrombin reagents such as heparin have long been employed for hematological evaluation, while serum has been used for blood chemistry and electrolyte examination. A “universal anticoagulant” suitable for the measurement of all these items would be expected not only to decrease the amount of blood needed to be withdrawn from patients but also to significantly improve the time- and cost-effectiveness of laboratory testing.

Recently, a new synthetic polymer, sulphonated polyisoprene (SPIP) was found to have in vitro anticoagulant effects as a result of activities including that of antithrombin (1). However, no other significant effects on plasma protein components have been reported for SPIP, and SPIP does not include free ions in its chemical structure. Thus, SPIP also might be useful for measurement of blood chemistry and plasma

electrolyte components, in addition to measurement of CBC. However, no trial has assessed the use of SPIP as a laboratory anticoagulant. In this study, we therefore examined in detail the feasibility of use of SPIP for assessment of both CBC and blood chemistry parameters. We also studied the effects of known inhibitors of platelet aggregation [which is reported to be induced by SPIP (2)], in order to establish the use of SPIP as a new anticoagulant.

MATERIALS AND METHODS

Anticoagulants

SPIP was kindly donated by Japan Synthetic Rubber Co., Inc. (Tokyo, Japan). SPIP is a negatively-charged water-soluble polymer with sulphonated groups (1,2) (Fig. 1). The anticoagulant activity of SPIP has been reported to be due

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Fig. 1. Chemical structure of sulphonated polyisoprene.

primarily to the concentration of sulphonated groups, but in part to the molecular weight of SPIP (2). In this study, we used SPIP with 2.5–4.6 mmol sulphonate group/g with an average molecular weight of c.57,000 (5,000–80,000) based on the findings of a previous study, in order to obtain maximum anticoagulative activity (2). A test tube containing 1.5 mg of SPIP was prepared to hold 5 ml whole blood in order to obtain a final SPIP concentration of 0.3 mg/ml blood (2).

Vacuum tubes containing EDTA-2K or heparin were purchased from Terumo Co. (Tokyo, Japan).

Inhibitors of Platelet Aggregation

Acetyl salicylate (aspirin), prostaglandin E1 (PGE1), and kanamycin were purchased from SIGMA, Wako Pure Chemical Industries (Osaka, Japan), and Meiji Seika Kaisha, LTD (Tokyo, Japan), respectively. These agents were mixed with SPIP in test tubes as desired.

Specimens

Venous specimens were obtained, after obtaining informed consent, from 50 healthy volunteers (27 males and 23 females)

TABLE 1. Normal ranges of peripheral blood analysis parameters measured in the present study

Items	Normal range
WBC	4.0–11.0 × 10 ⁹ /l
RBC	3.50–5.60 × 10 ¹² /l
Hb	110–180 g/l
Hct	32.0–53.0%
PLT	130–500 × 10 ⁹ /l
Neu	35.0–75.0%
Lym	20.0–55.0%
Mon	2.5–12.0%
Eos	0.0–8.0%
Bas	0.0–2.0%

All the measurements were performed by an NE-8000 blood cell counter with electrical impedance methods. WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; PLT, platelets; Neu, neutrophils; Lym; lymphocytes; Mon, monocytes; Eos, eosinophils; Bas, basophils.

who work in our hospital. The average age of volunteers was 33 years (range: 19–70 years). Blood samples from 30 randomly chosen subjects were used for evaluation of SPIP alone, while the remaining 20 samples were used for that of SPIP plus kanamycin (see Results). For assessment of the feasibility of use of SPIP as a new anticoagulant, EDTA-2K and serum samples were always co-analyzed as controls for hematological and biochemical/electrolyte items, respectively. All the blood samples were obtained in the morning before breakfast and confirmed to exhibit no changes such as hemoly-

TABLE 2. Methods used for determination and normal ranges of biochemical and electrolyte parameters measured

Items	Equipment	Method	Normal range
TP	Hitachi 7170	Biuret method	6.7–8.3 g/dl
ALB		BCG method	4.0–5.0 g/dl
T bil		Alkalineazo bilirubin method	0.2–1.0 mg/dl
TG		Enzyme method (free glycerol method)	50–149 mg/dl
T chol		Enzyme method	150–219mg/dl
BUN		Urease UV method	6–20 mg/dl
CRE		Alkaline pykrate method	0.6–1.3 mg/dl
UA		Urikase POD method	M 3.7–7.6 mg/dl F 2.5–5.4 mg/dl
LDH		Wroblewski–Ladue method	230–460 IU/l/37°C
AST		UV method	10–40 IU/l/37°C
ALT	Hitachi 710	UV method	5–40 IU/l/37°C
ALP		RNP substance method	80–260 IU/l/37°C
LAP		Enzyme method	M 80–170 IU/l/37°C
γ-GTP		(L-Leu-DBHAsubstance)	F 73–125 IU/l/37°C
		L-gammaglutamyl-3-carboxyl-4-nitrosoanhydride method	M < 70 IU/l/37°C F < 30 IU/l/37°C
CHE		Rate assay	M 203–460 IU/l/37°C F 179–354 IU/l/37°C
Na		Electrode method	136–147 nmol/l
Cl			98–109 mmol/l
K			3.6–5.0 mmol/l

TP, total protein; ALB, albumin; T bil, total bilirubin; TG, triglyceride; T chol, total cholesterol; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; LDH, lactic dehydrogenase; AST, asparaginate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LAP, leucine aminopeptidase; γ-GTP, gamma-glutamyltranspeptidase; CHE, cholinesterase; Na, sodium; Cl, chlorine; K, potassium.

TABLE 3. Representative results of hematological analysis of samples anticoagulated with SPIP, SPIP plus PGE1, or SPIP plus aspirin^a

Items	EDTA	SPIP + PGE1 (10 μ M)	SPIP + aspirin (100 μ M)	SPIP + kanamycin (20 mg/ml)
WBC ($\times 10^9$ /l)	5.48	10.56	8.98	5.3
RBC ($\times 10^{12}$ /l)	5.54	5.36	5.41	5.49
Hb (g/l)	172	167	168	169
Hct (%)	48.3	46.7	47.2	47.9
PLT ($\times 10^9$ /l)	198	21	22	134
Neu (%)	53.9	52.1	48.3	59.7
Lym (%)	42.7	43.1	41.6	41.0
Mon (%)	2.4	3.2	7.9	2.1
Eos (%)	0.5	0.6	0.8	1.8
Bas (%)	0.5	1.0	1.4	1.3

^aWBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; PLT, platelets; Neu, neutrophils; Lym, lymphocytes; Mon, monocytes; Eos, eosinophils; Bas, basophils.

sis, coagulation, or fibrin-net formation. Measurements were performed within 6 hours after venipuncture, except for evaluation of the time courses of change in hematological items.

Hematological, Biochemical and Electrolyte Analysis

CBC and the white cell differential were determined using an NE-8000 (Sysmex Corp., Kobe, Japan). The hematological items evaluated in this study and their normal ranges are summarized in Table 1. The biochemical items were mea-

sured with a fully automated Hitachi 7170 multiple analyzer (Hitachi Co., Tokyo, Japan). Serum electrolyte concentrations were measured using a Hitachi 710 (Hitachi Co.). The biochemical and electrolyte items evaluated in this study and their normal ranges are summarized in Table 2.

Statistics

For comparison of mean values between experimental groups, the paired *t*-test was employed. For analysis of correlation, coefficients of correlation were determined following

Fig. 2. Effects of SPIP on cell morphology. Upper pictures show SPIP effects, and lower pictures show effects of SPIP plus kanamycin (from left to right: neutrophils, lymphocytes, and platelets). No morphological changes in white cells were observed with SPIP treatment on Giemsa-stained films, although platelet activation and aggregation were observed by SPIP alone. Such platelet aggregates were observed mostly in the feather-end part of the

film. Addition of kanamycin to SPIP prevented the platelet changes induced by SPIP. Variation of red-cell size among pictures is due to gradient transition of red cells and differences in site of observation on Giemsa-stained films. Red-cell distribution width and mean cell volume were the same in the SPIP and SPIP-plus-kanamycin blood samples.

TABLE 4. Comparison of coefficient of variation^a for EDTA, SPIP, and SPIP plus kanamycin-treated blood samples

Items	EDTA	SPIP	SPIP + kanamycin
WBC ($\times 10^9/l$)	4.9 ± 3.3	13.9 ± 9.9	5.0 ± 3.1
RBC ($\times 10^{12}/l$)	6.5 ± 6.0	10.7 ± 9.4	4.2 ± 2.2
Hb (g/l)	0.5 ± 0.1	10.8 ± 9.9	7.2 ± 1.8
Hct (%)	6.5 ± 6.1	10.5 ± 9.3	6.9 ± 2.0
PLT ($\times 10^9/l$)	8.0 ± 5.6	22.3 ± 9.1	8.3 ± 3.7
Neu (%)	3.2 ± 2.2	4.2 ± 1.3	2.4 ± 0.9
Lym (%)	5.6 ± 3.6	2.9 ± 0.9	3.6 ± 2.1
Mon (%)	30.9 ± 16.6	35.7 ± 19.7	20.5 ± 8.4
Eos (%)	33.7 ± 10.0	20.5 ± 2.3	30.3 ± 17.4
Bas (%)	42.3 ± 16.6	31.4 ± 8.9	39.3 ± 31.3

^aEach value indicates the coefficients of variation (%) $\pm$ SD for 5 repeated measurements ($n = 5$). WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; PLT, platelets; Neu, neutrophils; Lym, lymphocytes; Mon, monocytes; Eos, eosinophils; Bas, basophils.

Z-transformation. In both cases, findings of $P < 0.05$ were considered significant.

RESULTS

Effects of Inhibitors of Platelet Aggregation on Blood Cells From the Samples Anticoagulated With SPIP

Because SPIP was previously reported to induce platelet aggregation (2), we initially screened for the effects of several known inhibitors of platelet aggregation, i.e., aspirin (3–5), PGE1 (6–7), and kanamycin (8) in addition to SPIP. As shown in Table 3, this screening test with a small number of blood samples revealed that the platelet count of samples anticoagulated by SPIP alone was much lower than those of samples anticoagulated by EDTA. However, kanamycin—but neither aspirin nor PGE1 in combination with SPIP—signifi-

Fig. 3. Time courses of change in count of hematological items after sampling of blood. The same samples anticoagulated with EDTA, SPIP, or SPIP with various concentrations of kanamycin were repeatedly measured at various time points after venipuncture. During experiments, samples were left at room temperature. $\blacktriangle$, indicate samples anticoagulated with SPIP alone;

$\blacksquare$, SPIP plus kanamycin (20 mg/ml); $\circ$, SPIP plus kanamycin (30 mg/ml); $\triangle$, SPIP plus kanamycin (40 mg/ml); $\blacksquare$, SPIP plus kanamycin (100 mg/ml). In all cases SPIP was used at 30 mg/dl. On ordinates, values obtained with EDTA just after collection are represented as 1. Abscissas indicate times after venipuncture (hours).

TABLE 5. Comparison of peripheral blood analysis parameters in EDTA-2K with those in SPIP or SPIP plus kanamycin groups^a

Items	EDTA versus SPIP (n = 30)				EDTA versus SPIP + kanamycin (n = 20)			
	EDTA		SPIP		EDTA		SPIP + kanamycin	
	(Mean)	(Range)	(Mean)	(Range)	(Mean)	(Range)	(Mean)	(Range)
WBC ($\times 10^9/l$)	6.70	(4.34–14.05)	5.69 ^b	(3.08–11.3)	5.90	(4.34–7.8)	5.77	(4.29–7.89)
RBC ($\times 10^{12}/l$)	4.49	(3.46–5.86)	4.73	(3.33–7.17)	5.15	(3.98–5.86)	5.19	(4.00–5.86)
Hb (g/l)	149	(108–185)	150	(99.0–179.0)	158	(108–185)	158	(108–187)
Hct (%)	40.0	(29.7–49.3)	40.0	(29.7–43.0)	46.4	(34.9–52.5)	45.5	(33.9–51.0)
PLT ($\times 10^9/l$)	251	(105–763)	17 ^b	(3.0–76.0)	224	(175–302)	185 ^b	(148–245)
Neu (%)	58.4	(28.2–81.2)	44.2 ^b	(12.9–74.3)	57.1	(42.7–81.2)	57.1	(44.1–76.7)
Lym (%)	15.1	(11.0–29.0)	39.6 ^b	(13.7–65.7)	36.0	(16.3–51.6)	36.0	(16.3–51.6)
Mon (%)	4.0	(1.8–9.1)	7.0 ^b	(0.5–21.5)	3.8	(1.8–9.1)	3.6	(1.00–18.5)
Eos (%)	2.5	(0.4–9.2)	4.1 ^b	(1.7–14.9)	2.4	(0.8–8.5)	2.7	(0.7–8.5)
Bas (%)	0.7	(0.2–1.8)	3.3 ^b	(1.3–7.5)	0.7	(0.3–1.2)	0.6	(0.2–1.5)

^aSame sample was measured with different anticoagulants. WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Hc, hematocrit; PLT, platelets; Neu, neutrophils; Ly, lymphocytes; Mono, monocytes; Eos, eosinophils; Bas, basophils.

^b $P < 0.001$.

Fig. 4. Coefficients of correlation for peripheral blood analysis parameters between EDTA and SPIP or EDTA and SPIP-plus-kanamycin groups. Horizontal axis indicates values from EDTA-containing samples. Vertical axis indicates values from blood samples anticoagulated with SPIP (○) or

with SPIP plus kanamycin (●). Thin lines indicate linear correlations between EDTA and SPIP groups, while thick lines indicate those between EDTA and SPIP-plus-kanamycin groups. r, coefficient of correlation. *, $P < 0.0001$.

cantly restored platelet counts. As shown in Figure 2, SPIP alone induced significant platelet aggregation, indicating that low platelet count in SPIP-anticoagulated blood is indeed due to platelet aggregations. However, formation of platelet clumps was significantly prevented by addition of kanamycin to SPIP. On the other hand, neither SPIP nor SPIP plus kanamycin significantly affected the morphology of either red cells or white cells.

Stability of Measurements of Samples Collected With SPIP or SPIP Plus Kanamycin

The stability of hematological parameters determined by five repeated measurements of the same sample anticoagulated with SPIP or SPIP plus kanamycin was determined with the NE-8000. As shown in Table 4, in most of the items, coefficients of variations (CVs) for the SPIP-alone group were larger than those for the EDTA group. However, the CVs for the SPIP plus kanamycin group were nearly the same as those for the EDTA group, indicating that stable measurement could be obtained if kanamycin were added to SPIP.

Time Courses of Change in Hematological Items After Blood Sampling

We also repeatedly measured the same samples anticoagulated with EDTA, SPIP, or SPIP plus various concentrations of kanamycin at different time points after venipuncture. As shown in Figure 3, the values of hematological items obtained with each anticoagulant remained nearly unchanged until at least 6 hours after venipuncture.

Comparison of Results of Hematological Analysis Between Blood Samples Anticoagulated With EDTA and SPIP Alone or EDTA and SPIP Plus Kanamycin

Based on the aforementioned observations, we then increased the number of samples and examined the usefulness of SPIP or SPIP plus kanamycin as a new universal anticoagulant. As shown in Table 5, although red cell indices in the SPIP-alone group were comparable to those in the EDTA group, platelet counts were much lower than those in the EDTA group. In addition, the WBC count was slightly lower in the SPIP group than in the EDTA group. However, WBC counts in the SPIP plus kanamycin group were comparable to those in the EDTA group, and platelet counts were restored to about 80% of those in the EDTA group.

Analysis of Coefficients of Correlation for Hematological Items Between EDTA and SPIP Groups or EDTA and SPIP Plus Kanamycin Groups

As shown in Figure 4, when the EDTA- and SPIP-alone groups were compared, the coefficients of correlation for most

of the hematological items were less than 0.8, indicating that measurements of CBC using SPIP alone are unstable. On the other hand, when the EDTA and SPIP-plus-kanamycin groups were compared, the coefficients of correlation for most of the hematological items improved to above 0.9.

Analysis of Biochemical and Electrolyte Items

Based on the results of hematological analysis, we examined whether SPIP and/or SPIP plus kanamycin is suitable for analysis of biochemical and electrolyte items. As shown in Table 6, although 10 items in the SPIP group differed significantly in value from those in the serum group, all of these differences, except for that in LDH, were less than 10%. When kanamycin was added to SPIP, however, 13 of 18 biochemical/electrolyte items differed significantly from those in the serum group, and 5 of the 13 items (triglyceride, uric acid, alanine aminotransferase, lactic dehydrogenase, and alkaline phosphatase) had differences larger than 10%. As shown in Table 7, 15 of 18 biochemical and electrolyte items had coefficients of correlation between the serum and SPIP-alone groups higher than 0.95; 2 items (creatinine and K) had coefficients between 0.8 and 0.85, while the coefficient for LDH was only 0.62. However, when kanamycin was added to SPIP, the coefficients of correlation for total protein and albumin and particularly those for electrolyte items were clearly lower than those between the serum and SPIP-alone groups, suggesting that addition of kanamycin to SPIP may result in instability of measurement of these items.

DISCUSSION

A series of biopolymers incorporating sulphonated groups and based on the chemical structure of heparin have been tested for anticoagulant activity (9). SPIPs were first synthesized by van der Does et al. and were found to have strong anticoagulant activity (1,10,11). The precise mechanism of this SPIP activity is not well understood. However, it has been speculated that the negative charge of the sulphonic residue of the SPIP polymer inhibits fibrin monomer formation by antithrombin activity (1,2,10). SPIPs may have advantages for laboratory use including: 1) water-solubility; 2) biosafety, as well as ease of handling; 3) no interference with the operation of presently existing laboratory instruments; 4) no chelating effect on divalent cations; 5) no free ions, unlike heparin, EDTA and citric acid compounds; 6) storability for long periods of time; and 7) cost-effectiveness. However, the major problem with SPIP, related to evaluation of hematological items we and others have met (2), is that platelet counts are much lower than with EDTA, due to platelet aggregation. Also, in the present study, although SPIP did not significantly affect individual cell morphologies, the coefficients of correlation for WBC and platelets between the SPIP and EDTA groups were relatively low. In this study, we therefore used a series of known inhibitors of platelet aggregation to deter-

TABLE 6. Comparison of biochemical and electrolyte items for serum versus SPIP and SPIP plus kanamycin groups^a

Items	Serum versus SPIP (n = 30)				Serum versus SPIP + kanamycin (n = 20)			
	Serum		SPIP		Serum		SPIP + kanamycin	
	(Mean)	(Range)	(Mean)	(Range)	(Mean)	(Range)	(Mean)	(Range)
TP (g/dl)	7.7	(6.1–8.3)	7.4 ^c	(6.3–8.5)	7.8	(7.1–8.7)	7.6 ^c	(6.6–8.3)
ALB (g/dl)	4.7	(2.8–5.5)	4.5 ^b	(2.7–5.3)	5.0	(4.7–5.5)	4.6 ^b	(4.0–5.3)
T bil (mg/dl)	0.6	(0.3–1.4)	0.6	(0.3–1.4)	0.7	(0.3–1.1)	0.7	(0.3–1.1)
TG (mg/dl)	118	(40–228)	112 ^c	(36–228)	150	(36–465)	129 ^c	(31–395)
T chol (mg/dl)	184	(107–267)	176 ^b	(103–261)	203	(128–288)	184 ^c	(113–270)
BUN (mg/dl)	14.0	(9.6–22)	13.9	(9.6–21.6)	13.0	(6.6–18.0)	13.0	(6.8–18.6)
CRE (mg/dl)	1.0	(0.6–1.3)	0.9	(0.5–1.2)	1.0	(0.6–1.2)	1.0 ^d	(0.8–1.2)
UA (mg/dl)	4.7	(1.4–8.9)	4.6	(1.3–8.8)	5.4	(2.9–7.1)	4.7 ^b	(2.7–6.2)
LDH (IU/l)	280	(167–429)	346 ^b	(121–546)	362	(249–509)	550 ^c	(190–957)
AST (IU/l)	20	(11–49)	21 ^c	(11–50)	34	(18–84)	36	(18–72)
ALT (IU/l)	20	(3–95)	18	(4–98)	45	(8–146)	52 ^c	(10–165)
ALP (IU/l)	135	(87–245)	126	(85–213)	146	(88–182)	106 ^b	(84–164)
LAP (IU/l)	113	(70–221)	107	(79–215)	116	(52–140)	106 ^b	(46–130)
γ-GTP (IU/l)	43	(9–333)	37	(8–319)	41	(10–78)	37	(9–73)
CHE (IU/l)	316	(173–558)	305 ^c	(167–534)	400	(220–579)	365 ^b	(184–556)
Na (mmol/l)	143	(125–148)	145 ^b	(128–153)	145	(139–152)	146	(129–153)
Cl (mmol/l)	102	(86–107)	106 ^c	(102–128)	105	(100–107)	102 ^d	(96–107)
K (mmol/l)	4.1	(3.5–4.9)	3.8 ^d	(3.3–4.6)	3.9	(3.4–4.3)	4.1 ^c	(3.7–4.6)

^aSame sample was measured with different anticoagulants. TP, total protein; ALB, albumin; T bil, total bilirubin; TG, triglyceride; T chol, total cholesterol; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; LDH, lactic dehydrogenase; AST, asparaginate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LAP, leucine aminopeptidase; γ-GTP, gamma-glutamyltranspeptidase; CHE, cholinesterase; Na, sodium; Cl, chlorine; K, potassium.

^b $P < 0.0001$.

^c $P < 0.001$.

^d $P < 0.01$.

^e $P < 0.05$ versus serum group.

mine whether they are capable of inhibiting the aggregation induced by SPIP. Aspirin is known to inhibit platelet aggregation by inhibiting cyclooxygenase activity (3). PGE1 stimulates adenylyl cyclase and inhibits platelet aggregation and activation (6,7). On the other hand, basic water-soluble antibiotics, including kanamycin sulfate, were recently found to prevent and dissociate EDTA-induced aggregation of platelets in pseudothrombocytopenic subjects, presumably due to positive charges within their chemical structures (8). In our screening experiments, we found that aspirin and PGE1 exhibited almost no prevention of platelet aggregation, suggesting that the thromboxane-prostaglandin system does not play a significant role in the platelet aggregation induced by SPIP. Notably, by contrast, kanamycin significantly prevented platelet aggregation and restored platelet counts to about 80% of values in the EDTA group. Furthermore, kanamycin plus SPIP was sufficiently effective in improving the coefficients of correlation between the EDTA and SPIP groups for most hematological items. Although the reasons why kanamycin prevents aggregation of platelets were not clarified by the present study, the positive electric charge of kanamycin might have helped neutralize interaction among polymer, fibrinogen, and platelets.

The finding of high coefficients of correlation between the serum and SPIP groups for almost all the biochemical and

electrolyte items examined in the present study suggest that SPIP has minimal effects on blood protein components. Although the average values of 10 of these 18 items in the SPIP group were significantly different from those of the serum group, these differences were not due to large differences in values, but to very high coefficients of correlation. However, kanamycin plus SPIP appeared to decrease the coefficients of correlation between the serum and SPIP groups for some biochemical items (total protein and albumin) and for electrolyte items, although it did not decrease the coefficients of correlation for the items of hematological analysis. In particular, the instability of electrolyte measurements in the presence of kanamycin suggests that kanamycin might have affected the ion-selective electrode of the automatic analyzer we used.

In conclusion, we found in the present study that biopolymers such as SPIP are excellent candidates for new anticoagulants usable for simultaneous evaluation of hematological, biochemical, and electrolyte items in routine laboratory work. To overcome the major disadvantage of SPIP—its induction of platelet aggregation—addition of basic water-soluble antibiotics to SPIP might be needed. However, some improvements in the additive, as well as in the choice of automated analyzer, may be required for more reliable evaluation of biochemical and electrolyte items.

TABLE 7. Coefficients of correlation for biochemical and electrolyte items for serum versus SPIP and serum versus SPIP plus kanamycin groups^a

Items	Serum versus SPIP	Serum versus SPIP + kanamycin
TP (g/dl)	0.960 ^b	0.627 ^c
ALB (g/dl)	0.976 ^b	0.646 ^c
T bil (mg/dl)	0.974 ^b	0.931 ^b
TG (mg/dl)	0.994 ^b	0.988 ^b
T chol (mg/dl)	0.994 ^b	0.984 ^b
BUN (mg/dl)	0.994 ^b	0.975 ^b
CRE (mg/dl)	0.827 ^b	0.844 ^b
UA (mg/dl)	0.995 ^b	0.950 ^b
LDH (IU/l)	0.616 ^d	0.607 ^d
AST (IU/l)	0.995 ^b	0.984 ^b
ALT (IU/l)	0.961 ^b	0.996 ^b
ALP (IU/l)	0.996 ^b	0.978 ^b
LAP (IU/l)	0.971 ^b	0.981 ^b
γ-GTP (IU/l)	1.000 ^b	0.995 ^b
CHE (IU/l)	0.999 ^b	0.983 ^b
Na (mmol/l)	0.976 ^b	0.486 ^d
Cl (mmol/l)	0.950 ^b	0.159
K (mmol/l)	0.840 ^b	0.090

^an = 30 for serum versus SPIP and n = 20 for serum versus SPIP plus kanamycin groups. TP, indicates total protein; ALB, albumin; T bil, total bilirubin; TG, triglyceride; T chol, total cholesterol; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid; LDH, lactic dehydrogenase; AST, asparaginate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LAP, leucine aminopeptidase; γ-GTP, gamma-glutamyltranspeptidase; CHE, cholinesterase; Na, sodium; Cl, chlorine; K, potassium.

^bP < 0.0001.

^cP < 0.005.

^dP < 0.05.

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