Kobe J. Med. Sci., Vol. 52, No. 2, pp. 17-26, 2006

Antigenicity and Irritancy Tests of Leukocyte-Reduction Filters Using Animal Models

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Received 28 December 2005 /Accepted 27 January 2006

Key words: non-hemolytic febrile transfusion reactions, leukocyte-reduction filters, Maximization test

We experienced a patient who showed severe allergic symptoms immediately after blood transfusion using a filter for removal of leukocytes. To investigate the cause of this incident, we conducted a maximization test on the antigenicity of substances extracted from filters used for leukocyte removal. The tests were performed in guinea pigs. Acetone extracts were obtained from filters made by three manufacturers (A, B and C) and sensitization and evocation were tested at 10% concentration. It was confirmed that extracts from one filter (B) induced sensitization in guinea pigs. Sensitization of the extracts was also tested at 1%, 0.1% and 0.01%, and was induced at 1% but not at 0.1% and 0.01%. Next, skin irritation and sensitization of the substances were tested using mice. 5%-ethanol extracts were prepared from filters made by 2 manufacturers (A and B) and the extracts (5% or 0.5% concentrations) were injected intradermally into the auricle and the ear swelling was observed 1, 24 and 48 hours and 7 days after injection. Significant ear swelling was induced by the extracts from B-filters. In the skin sensitization test, 5% extracts were injected subcutaneously into the back of mice for 3 continuous days for sensitization, and 0.5% extracts were injected intradermally into the auricle of mice to evoke a response, after which changes in ear swelling were observed. Biphasic ear swelling observed 1 hour (immediate response) and 24 hours (delayed response) after challenge was induced by the extracts from B-filters. In conclusion, our study showed that filters used for leukocyte removal may contain substances that cause skin irritation and sensitization and that antigenicity and irritancy tests of the filters might prevent the adverse reactions after blood transfusion.

Transfusion-related adverse reactions induced by leukocytes in blood products include sensitization to alloantigen, non-hemolytic febrile transfusion reaction (NHFTR), post-transfusion graft-versus-host disease (PT-GVHD), post-transfusion viral infection by CMV, and HTLV-I [1-5]. In particular, non-hemolytic transfusion-related adverse reactions account for 80% of transfusion-induced adverse reactions and medical institutions are currently investigating the cause of these reactions in cooperation with blood centers; however, the reasons for such adverse reactions are often unidentified.

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In patients requiring long-term transfusion of red blood cells and platelets, leukocytes in blood products may act as allogeneic immunogens and induce severe transfusion-related adverse reactions, including production of anti-HLA antibody and platelet transfusion refractoriness (PTR). To prevent these adverse reactions, leukocyte-depleted blood products for subsequent use in patients are prepared using leukocyte-removal filters [6-8] (hereafter referred to as filters). However, transfusion-related adverse reactions also occur following the use of filters, and we experienced a patient who showed severe allergic symptoms in transfusion using a product prepared with a filter supplied by a particular manufacturer (manuscript in preparation). When the patient was transfused with a product prepared using a filter made by another manufacturer, no adverse reactions were found. Using substances extracted from the original filter, a prick test was conducted and the patient showed a positive response, suggesting that interfusion of filter ingredients may have been one of the causes of the transfusion-related adverse reaction and induced allergic response. In this study, we extracted substances from several different filters and investigated whether the filter ingredients showed antigenicity and irritancy.

The commonest skin sensitization test is the so-called guinea pig maximization test, which was developed by Magnusson and Kligman [9]. This test is described in the "Biological evaluation of medical devices (10993-10)" published by the International Organization for Standardization, as a method for examining delayed allergic responses in animals. In this study, the test was conducted in accordance with Annex B of ISO 10993-10 [10, 11]. In addition, the skin irritation and sensitization properties of the filter extracts were tested in mice.

MATERIALS AND METHODS

Preparation of extracts from filters

Acetone extracts: Filters for leukocyte removal made by three manufacturers (A, B and C) were examined. The properties of the filters are shown in Table 1. In accordance with ISO 10993-10, the filters were broken down, cut out, placed in an ultrasonic bath with acetone (Wako Pure Chemicals, Osaka, Japan), and left to stand at room temperature. The fluid obtained was dried using a rotary evaporator and used as the test material.

5%-ethanol extracts: 5% ethanol was passed through each filter and the fluid obtained was freeze-dried and used as the test material.

Animals

Guinea pigs: Hartley guinea pigs aged 5 weeks old were obtained from Japan SLC, Inc. Animals without health problems were used following an 11-day quarantine and acclimation period. Ten animals were assigned to each of the respective test-material groups and 5 animals each were used in the positive and negative (solvent) control groups.

Mice: BALB/c female mice aged 6 weeks old were obtained from Charles River Japan, Inc., and selected and prepared for the study similarly to the guinea pigs. Five animals were assigned to each group.

Chemicals

In accordance with the procedure of the maximization test, 1-chloro-2,4-dinitrobenzene (DNCB, Wako Pure Chemicals, Osaka, Japan) was used as the hapten (positive control material), Freund's Complete Adjuvant (FCA, Gifco) as the response-enhancing agent, and a mixed solution of acetone and olive oil (A/O=1/3,Wako Pure Chemicals, Osaka, Japan) as solvent for the test materials. In pretreatment before secondary sensitization, a petrolatum ointment including 10% sodium lauryl sulfate (Wako Pure Chemicals, Osaka, Japan) was used.

Table 1. Characteristics of filters made by three manufacturers

		Filters for red blood cell products		
		A	B	C
Specifications of main components	Container material	Polycarbonate	Polycarbonate	Acrylic resin
	Filter material	Non-woven surface modified polyester fabric	Non-woven polyester fabric, Micro-porous polyurethane	Non-woven polyester fabric
	Surface charge	Positive	Neutral	Negative
	Priming volume (ml)	35	38	23,(26,35,50)
Sterilization method		Autoclave/ (ETO)	ETO	Gamma rays/ (ETO)

ETO: Ethylene oxide

Skin sensitization test in guinea pigs (guinea pig maximization test)

The day before primary sensitization, the hair on the scapula was shaved over an area of approximately 2×4 cm. For primary sensitization, the following 3 test solutions were intradermally injected into three regions at a dose of 100 μl: (1) an emulsified solution of distilled water and FCA (1:1); (2) extracts from filters prepared at 10% concentration in A/O, or DNCB solution prepared at 0.1% concentration in A/O; and (3) a mixture of extracts from filters prepared at a 20% concentration in A/O and FCA (1:1) or DNCB solution prepared at 0.2% concentration in A/O and FCA (1:1). Solution (1) alone was administered to animals in the negative control group. Six days after primary sensitization, the hair of sensitized regions was shaved; a white petrolatum ointment including 10% sodium lauryl sulfate was then applied, and subsequently wiped off on the following day. Secondary sensitization was performed on the 7^{th} day after primary sensitization. In this procedure, a filter paper containing the extracts or DNCB solution (200 μl) used in (2) above was occlusively patched to the skin for 48 hours. On the 14^{th} day after secondary sensitization, a filter paper containing the extracts or DNCB solution (100 μl) used in (2) above was occlusively patched to the skin for 24 hours, in order to evoke a response. The skin was observed 24 and 48 hours after the removal of this patch and evaluated in accordance with the criteria proposed by Draize. The response induced by the extracts was examined at final concentrations of 1%, 0.1% and 0.01%, as well as at 10%.

Skin irritation and sensitization tests in mice

Extracts in 5% ethanol were prepared as 5% and 0.5% suspensions with sterile PBS. In the skin irritation test, 10 μl of the 5.0% or 0.5% extract suspension was intradermally injected and the thickness of the auricle was measured 1, 24 and 48 hours and 7 days after injection using a dial thickness gauge (Ozaki Co. Ltd, Tokyo, Japan). The difference in the thickness of the auricle at each of these times from that immediately before injection was defined as the ear swelling. In the skin sensitization test, 20 μl of the 5% extract suspension was subcutaneously injected into the back for 3 continuous days. On the 7^{th} day from the beginning of subcutaneous injection, 10 μl of 0.5% extract suspension was intradermally injected into the left auricle and the ear swelling of the auricle was measured 1, 4, 24, 48 and 72 hours later.

Statistical analysis

The statistical significance of differences between the mean values of the experimental and control groups was calculated with a Student *t*-test. Differences were considered to be significant at $p<0.05$.

RESULTS

Skin sensitization test in guinea pigs (guinea pig maximization test)

Table 2 shows the results of skin sensitization tests in guinea pigs (guinea pig maximization test), using extracts from filters made by 3 manufacturers. In the A- and C-filter groups, the positive rate after evocation was 0% and the mean evaluation score was 0. However, in the B-filter group, the positive rates 24 and 48 hours after evocation were 70% and 60%, respectively, and the mean evaluation scores were 0.7 and 0.9, respectively. In the positive control DNCB group, the positive rates 24 and 48 hours after evocation were both 100% and the mean evaluation scores were 4.2 and 6.0, respectively.

The results of the skin sensitization test in individual guinea pigs sensitized with extracts from the B-filter are shown in Table 3. Of the 10 animals tested, 8 showed positive responses 24 or 48 hours after evocation, giving a positive rate of 80%. Table 4 shows the results of skin sensitization tests of extracts from the B-filters at extract concentrations of 10%, 1%, 0.1% and 0.01%. The 10% and 1% extracts induced 50% and 10% positive rates, respectively. However, the positive rate was 0% at extract concentrations of 0.1% and 0.01%.

Skin irritation and sensitization tests in mice

The quantities of substances that were extracted from filters with 5% ethanol are shown in Table 5. The quantity of the extracts from the C-filter was extremely small; therefore, skin irritation and sensitization tests in mice were only conducted using 5%-ethanol extracts from A- and B-filters. In the skin irritation test, changes in ear swelling were observed at two different extract concentrations and differences in swelling were found (Figure 1). The animals in the B-filter group showed significant ear swelling compared with those of the control group ($P<0.001$). In the skin sensitization test, animals in the B-filter group showed a biphasic ear-swelling response at 1 hour (immediate response) and 24 hours (delayed response) after challenge (Figure 2). Animals in the A-filter group showed neither an immediate nor a delayed ear-swelling response; therefore, data for this group are not shown in the figure.

Table 2. Guinea pig skin sensitization tests of extracts from filters made by three manufacturers
(a) 24 hours after completion of response evocation

Group	Sensitization Primary: intradermal injection Secondary: application		Response evocation (Application)		Evaluation 24 hours after completion of response evocation		
	Test substance	Concentration (%)	Test substance	Concentration (%)	Number of positive responses	Positive rate (%)	Mean evaluation score
1	A	10	A	10	0/10	0	0
2	B	10	B	10	7/10	70	0.7
3	C	10	C	10	0/10	0	0
4	P.C.	0.1	DNCB	0.1	5/5	100	4.2
5	N.C.	-	A	10	0/5	0	0
6	N.C.	-	B	10	0/5	0	0
7	N.C.	-	C	10	0/5	0	0
8	N.C.	-	DNCB	0.1	0/5	0	0

(b) 48 hours after the completion of response evocation

Group	Sensitization Primary: intradermal injection Secondary: application		Response evocation (Application)		Evaluation 48 hours after the completion of response evocation		
	Test substance	Concentration (%)	Test substance	Concentration (%)	Number of positive responses	Positive rate (%)	Mean evaluation score
1	A	10	A	10	0/10	0	0
2	B	10	B	10	6/10	60	0.9
3	C	10	C	10	0/10	0	0
4	P.C.	0.1	DNCB	0.1	5/5	100	6.0
5	N.C.	-	A	10	0/5	0	0
6	N.C.	-	B	10	0/5	0	0
7	N.C.	-	C	10	0/5	0	0
8	N.C.	-	DNCB	0.1	0/5	0	0

P.C.: Positive control (DNCB), N.C.: Negative control (distilled water).
An animal with an evaluation score of 1 or more was defined as positive and the positive rate and mean evaluation score were calculated as follows:
Positive rate (%) = (Number of positive animals / number of animals per group) × 100
Mean evaluation score = Total of the evaluation scores per group / number of animals per group
Positive animals were found in the 10% sensitization group using acetone extracts from B-filters and in the positive control (DNCB) group. The mean evaluation score 48 hours after completion of response induction was higher in the sensitization group than in the positive control (DNCB) group.

Table 3. Evaluation scores of individual guinea pigs in skin sensitization tests (erythema and edema) using extracts B-filters

Number *	Evaluation					
	24 hours after completion of response evocation		48 hours after completion of response evocation		Positive rate (%)	Mean evaluation score
	Erythema	Edema	Erythema	Edema		
1	0	0	0	0	(8/10)×100 = 80(%)	0
2	1	0	1	0		1
3	1	0	2	0		2
4	1	0	1	0		1
5	1	0	0	0		1
6	1	0	2	0		2
7	0	0	1	0		1
8	1	0	2	0		2
9	1	0	0	0		1
10	0	0	0	0		0

* Evaluation scores of 10 animals of Group 2 in Table 2

Table 4. Skin sensitization tests of extracts from B-filters at different concentrations

(a) 24 hours after completion of response evocation

Group	Sensitization Primary: intradermal injection Secondary: application		Response evocation (Application)		Evaluation 24 hours after completion of response evocation		
	Test substance	Concentration (%)	Test substance	Concentration (%)	Number of positive responses	Positive rate (%)	Mean evaluation score
1	B	10	B	10	5/10	50	1.0
2	B	1	B	10	1/10	10	0.2
3	B	0.1	B	10	0/10	0	0
4	B	0.01	B	10	0/10	0	0
5	P.C.	0.1	DNCB	0.1	5/5	100	4.2
6	N.C.	-	B	10	0/5	0	0
7	N.C.	-	DNCB	0.1	0/5	0	0

(b) 48 hours after completion of response evocation

Group	Sensitization Primary: intradermal injection Secondary: application		Response evocation (Application)		Evaluation 48 hours after completion of response evocation		
	Test substance	Concentration (%)	Test substance	Concentration (%)	Number of positive responses	Positive rate (%)	Mean evaluation score
1	B	10	B	10	5/10	50	1.1
2	B	1	B	10	1/10	10	0.3
3	B	0.1	B	10	0/10	0	0
4	B	0.01	B	10	0/10	0	0
5	P.C.	0.1	DNCB	0.1	5/5	100	6.2
6	N.C.	-	B	10	0/5	0	0
7	N.C.	-	DNCB	0.1	0/5	0	0

P.C.: Positive control (DNCB), N.C.: negative control (distilled water).
Positive animals were found in the 10% and 1% sensitization groups using acetone extracts from B-filters and in the positive control (DNCB) group.

Table 5. Quantity of substances in 5%-ethanol extracts

Filter	Quantity (mg)/bag
A	20.00
B	5.31
C	0.15

Mean quantities of substances (10 bags) extracted from filters with 5% ethanol and freeze-dried.

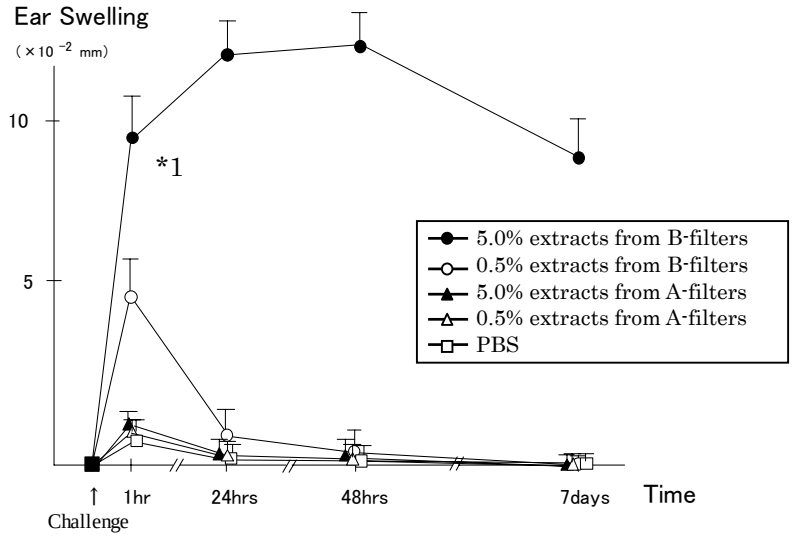

Figure 1. Time-course study of ear swelling in BALB/c mice (skin irritation test)
BALB/c mice were challenged with extracts from filters manufactured by A or B. The ear thickness was measured at various times after the challenge.
(*1, $p<0.001$, 5.0% extract from B filters versus PBS)

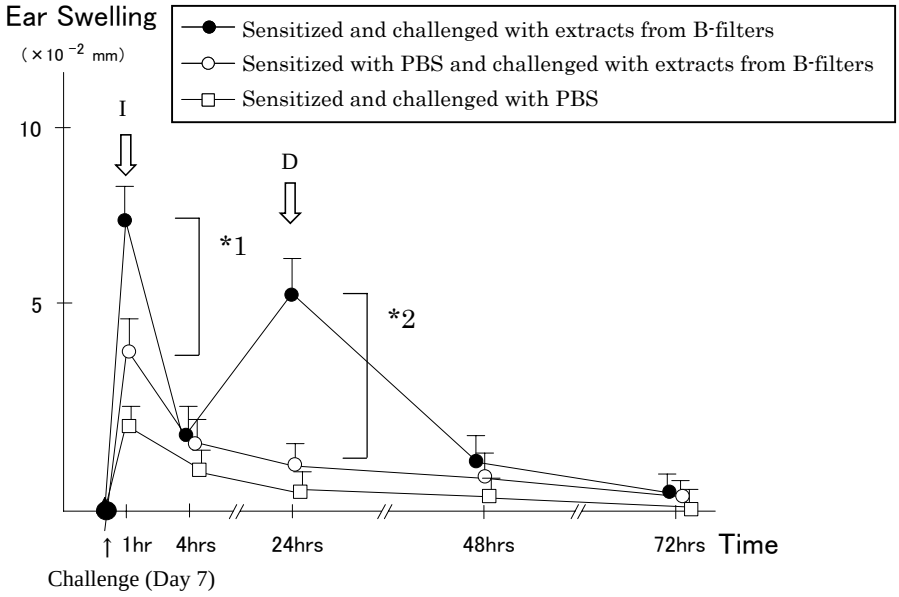

Figure 2. Time-course study of ear swelling in BALB/c mice (skin sensitization test)
BALB/c mice were repeatedly sensitized with extracts from filters manufactured by B (●) or with PBS (○) and challenged with the B-filter extracts on day 7. BALB/c mice were repeatedly sensitized with PBS (□) and challenged with PBS on day 7 as negative controls. Ear thickness was measured at various times after the challenge. I indicates an immediate response, and D indicates a delayed response (*1, $p<0.05$; *2, $p<0.005$).

DISCUSSION

The objective of this study was to investigate adverse reactions that might be induced by filters for leukocyte removal. Identification of the causes of these reactions may allow prevention of transfusion-related adverse reactions induced by leukocytes. We hypothesized that filter components may be inducing sensitization and an allergic response, and therefore we investigated whether substances extracted from filters were allergenic, using animal tests of filter extracts. In this study, we followed the guideline of "Biological evaluation of medical devices" in Annex B of ISO 10993-10. According to the method described in the guideline, not only hydrophilic but also lipophilic substances can be extracted.

The positive rate and mean evaluation score of skin sensitization tests in guinea pigs indicated a delayed response, suggesting that the filter extracts contained antigenic material with sensitizing potency. The results of skin irritation tests in mice showed filter extracts induced skin-irritating inflammatory responses at high concentration. Furthermore, immediate and delayed responses were found in the skin sensitization test, confirming that antigenic material from filter extracts can induce both types of responses.

The results of this study suggest that transfusion-related adverse reactions in humans are induced by components of filters. Thus, these filters contain as yet unidentified antigenic materials that cause an allergic response. Based on the results for filters made by three manufacturers, allergic episodes appear to depend on differences in filter materials, such as polyurethane and non-woven polyester fabric, or treatment chemicals used in the manufacturing process, and not on differences in container materials and sterilization methods. Adverse reactions may also depend on the frequency of filter use, the amount of filter components entering the body, and the patient's predisposition (genetic background) to allergic reaction. A contact hypersensitivity study in mice indicated an immediate response following repeated application of hapten, which initially induced a delayed response [12], and it is possible that frequent exposure to antigenic material causes anaphylactic shock.

It has been reported that materials used in filters might have effects on the intrinsic coagulation system, since they behave as foreign substances in the body [13]. Most filters are made of polyester non-woven fabric, but the fabric surface of some filters is chemically modified, with introduction of positively or negatively-charged functional groups. It has been reported that plasma contact with a negatively-charged material induced production of bradykinin, which may lead to a strong antihypertensive effect mediated by the intrinsic coagulation system [14, 15]. Furthermore, hemocytes such as monocytes and lymphocytes have been shown to produce inflammatory cytokines including IL-1, IL-6 and TNF 3 to 5 days after blood sampling, and this is one of the causes of fever in platelet transfusion [16]. Consistent with this, Heddle et al. reported that acute posttransfusion responses correlated with the storage period of red blood cell products [17]. Shanwell et al. suggested that cytokine production during the storage of red blood cell products could be suppressed by pre-storage leukocyte removal [18], and Nielsen et al. showed that this procedure suppressed an increase in leukocyte- and platelet-derived bioactive substances in fresh frozen plasma (FFP) [19].

Several studies have reported a relationship of cytokine and bradykinin production with transfusion-related adverse reactions, but the current study is the first to examine sensitization and irritation due to filter extracts. The results indicate that following transfusion using a filter, it is important for medical staff to observe the patient's condition and to be prepared to offer appropriate treatment for a severe allergic response. Furthermore, in reporting unexpected transfusion-related adverse reactions, it is important to indicate the

manufacturing process and the type of filter used. It is of note that in a recently introduced process for apheresis platelet products in Japan, leukocytes are removed using a filter before the storage process and blood sampling is conducted using a mixture containing fewer leukocytes to reduce changes in the blood products during storage and prevent adverse reactions. In the near future, it is anticipated that most blood products supplied by the Japan Red Cross Blood Center will be products that are free of leukocytes [20]. The economic effects of pre-storage leukocyte removal are currently under intense discussion in Europe and the United States, and the cost of pre-storage leukocyte removal is a major issue [21]. In this context, the establishment of not only physical but also biological safety of medical devices is of importance to provide safe transfusion for patients.

In conclusion, our study showed that filters used for leukocyte removal may contain substances that cause skin irritation and sensitization and that antigenicity and irritancy tests of the filters might prevent the adverse reactions after blood transfusion.

REFERENCES

1. **Sirchia, G., Wenz, B., Rebulla, P., Parravicini, A., Carnelli, V., and Bertolini, F.** 1990. Removal of white cells from red cells by transfusion through a new filter. Transfusion **30**:30- 33.
2. **Perkins, H.A., Payne, R., Ferguson, J., and Wood, M.** 1966. Nonhemolytic febrile transfusion reactions. Quantitative effects of blood components with emphasis on isoantigenic incompatibility of leukocytes. Vox Sang **11**:578-600.
3. **Dzieczkowski, J.S., Barrett, B.B., Nester, D., Campbell, M., Cook, J., Sugrue, M., Andersen, J.W., and Anderson, K.C.** 1995. Characterization of reactions after exclusive transfusion of white cell-reduced cellular blood components. Transfusion **35**:20-25.
4. **Rinaldo, C.R. Jr., Black, P.H., and Hirsch, M.S.** 1977. Interaction of cytomegalovirus with leukocytes from patients with mononucleosis due to cytomegalovirus. J Infect Dis **136**:667-678.
5. **Verdonck, L.F., de Graan-Hentzen, Y.C., Dekker, A.W., Mudde, G.C., and de Gast, G.C.** 1987. Cytomegalovirus seronegative platelets and leukocyte-poor red blood cells from random donors can prevent primary cytomegalovirus infection after bone marrow transplantation. Bone Marrow Transplant **2**:73-78.
6. **Bordin, J.O., Heddle, N.M., and Blajchman, M.A.** 1994. Biologic effects of leukocytes present in transfused cellular blood products. Blood **84**:1703-1721.
7. **Lane, T.A., Anderson, K.C., Goodnough, L.T., Kurtz, S., Moroff, G., Pisciotto, P.T., Sayers, M., and Silberstein, L.E.** 1992. Leukocyte reduction in blood component therapy. Ann Intern Med **117**:151-162.
8. **Williamson, L.M., and Warwick, R.M.** 1995. Transfusion-associated graft-versus-host disease and its prevention. Blood Rev **9**:251-261.
9. **Magnusson, B., and Kligman, A.M.** 1969. The identification of contact allergens by animal assay. The guinea pig maximization test. J. Invest. Derm. **52**: 268-276.
10. International Standard ISO 1993-10(1995), Biological evaluation medical devices-part10: Tests for irritation and sensitization.
11. **Draize, J. H., Woodward, G., and Calvery, H. O.** 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. **82** : 377–390.

12. **Natsuaki, M., Yano, N., Yamaya, K., and Kitano, Y.** 2000. Immediate contact hypersensitivity induced by repeated hapten challenge in mice. Contact Dermatitis **43**:267-272.
13. **Morris, K., and Bharucha, C.** 1997. Influence of Filtration on Platelet Transfusion Reactions. European Journal of Medical Research **2**:523-526.
14. **Bone, R.C.** 1992. Modulators of coagulation: A critical appraisal of their role in sepsis. Arch Intern Med **152**:1381-1389.
15. **Bonner, G., Preis, S., Schunk, U., Toussaint, C., and Kaufman, W.** 1990. Hemodynamic effects of bradykinin on systemic and pulmonary circulation in healthy and hypertensive humans. J Cardiovasc Pharmacol **15**:S46-56.
16. **Heddle, N.M., Klama, L., Singer, J., Richards, C., Fedak, P., Walker, I., and Kelton, J.G.** 1994. The role of the plasma from platelet concentrates in transfusion reactions. N Engl J Med **8**; 331:625-628.
17. **Heddle, N.M., Klama, L.N., Griffith, L., Roberts, R., Shukla, G., and Kelton, J.G.** 1993. Prospective study to identify the risk factors associated with acute reactions to platelet and red cell transfusions Transfusion **33**:794-797.
18. **Shanwell, A., Kristiansson, M., Remberger, M., and Ringden, O.** 1997. Generation of cytokines in red cell concentrates during storage is prevented by prestorage white cell reduction. Transfusion **37**:678-684.
19. **Nielsen, H.J., Skov, F., Dybkjaer, E., Reimert, C.M., Pedersen, A.N., Brunner, N., and Skov, P.S.** 1997. Leucocyte and platelet-derived bioactive substances in stored blood: effect of prestorage leucocyte filtration. European Journal of Haematology **58**:273-278.
20. **Brown, P., Cervenakova, L., McShane, L.M., Barber, P., Rubenstein, R., and Drohan, W.N.** 1999. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion **39**:1169-1179.
21. **Jensen, L.S., Grunnet, N., Hanberg-Sorensen, F., and Jorgensen, J.** 1995. Cost-effectiveness of blood transfusion and white cell reduction in elective colorectal surgery. Transfusion **35**:719-722.