Clinical Comparison of Two Brands of Heparin for use in Cardiopulmonary Bypass

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Introduction

The importance and complexity of heparin anticoagulation therapy during open-heart surgery with extracorporeal circulation (ECC) has been well documented[1] and widely recognized. Early attempts to regulate the level of anticoagulation led to standardized protocols based on patient weight and heparin activity half-life[2,3,4] along with unit dosage as defined by U.S.P. tests.[5] However, Bull and co-workers[6] have shown these empirical protocols to be frequently inadequate, because of widely variable individual patient response. This variability is further complicated by factors commonly present during cardiopulmonary bypass (CPB) which may affect heparin effectiveness and consumption along with the coagulation process itself, such as hemodilution, hypothermia, blood flow rate, and many others.[1] Previous comparisons of heparin potency have utilized *in-vitro* bioassay methods,[7,8] animal models,[8] or normal, healthy, awake human volunteers[9,11] and have in general concentrated on comparing the effects of heparins from mucosal and beef lung sources.[7,13] Studies involving living subjects have used one or more of the many clotting assays which measure only a portion of the clotting mechanism, such as prothrombin time,[11] activated partial thromboplastin time[9,12,13] and antithrombin III activity.[13] No study could be found comparing heparin potency *in vitro* on the basis of whole blood clotting times, which have been shown to have the greatest applicability to clinical CPB.[14] Further, no study could be found comparing heparins derived from a common source under conditions relating to open heart surgery. This study employs a whole blood clotting time assay[15] to examine the anticoagulant effectiveness of two commercially available brands of beef lung heparin during clinical cardiopulmonary bypass ECC in which the various factors affecting heparin consumption were closely monitored and controlled.

Methods and Materials

Thirty-seven patients undergoing ECC for elective surgical correction of cardiac disease at Shadyside Hospital, Pittsburgh, PA., were entered into the study during a two month period. Study patients were selected prospectively based on surgical procedure and assigned to one of two groups. Eighteen patients received Upjohn beef lung heparin,[a] 1,000 Units/ml U.S.P., for systemic anticoagulation and heparinization of I.V. flush solutions or transfused banked blood, while 19 patients were administered only Arsymed beef lung heparin,[b] 1,000 Units/ml U.S.P. Patient selection and study group assignment provided a balanced, representative sample of the general cardiac surgical population, as listed in Table I. Patients with a history or manifestation of coagulation abnormalities were excluded. Equipment utilized for ECC comprised conventional roller pumps and commercially available disposable circuit components including a bubble oxygenator and microfiltration of both suction and ar-

Presented at the 17th International Conference of the American Society Of Extra-Corporeal Technology, Inc., Houston, Texas, March 1979

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[a] The Upjohn Corporation
[b] Arsymed Inc.

TABLE I
Patient Procedure distribution for the two study groups.

OPERATIVE PROCEDURE	BRAND OF HEPARIN ADMINISTERED UPJOHN	ARSYMED
SVG X 1	4	5
SVG X 2	5	5
SVG X 3	3	3
SVG X 4	1	1
MVR	3	3
AVR	1	1
V.A.+SVG X 1	1	1
	18	19

SVG = Saphenous vein graft; MVR = Mitral valve replacement; AVR = Aortic valve replacement; VA = Left ventricular aneurysmectomy

terial blood. The prime was a balanced electrolyte solution, 20 to 25 ml/Kg of patient weight, with albumin added to constitute a five percent solution. No heparin was added to the priming solution. Surgical procedures were done in a manner routine for this center.

Data were collected preoperatively on patient height and weight, allowing calculation of body surface area.[c] Target ECC blood flow rates were calculated at 2.4 to 2.8 liters/min/m^2 and held in that range throughout perfusion. Venous drainage was through dual caval cannulae. Cardiac decompression was achieved with a left atrial vent cannula. Systemic hypothermia was used in all cases, monitored with rectal and esophageal thermistor probes.[d] Mild hypothermia (mean rectal-esophogeal temperature of 32° to 34°C) was used for single vein graft procedures. Moderate cooling (mean 28° to 32°C) was employed in all other cases. Systemic temperatures were raised to between 35° and 37°C before termination of bypass.

The level of anticoagulation was assessed by manually read whole blood activated clotting times[16] using evacuated tubes containing diatomaceous earth activator[e] which were kept at 37°C by a heating block.[f] Tubes were examined by eye for clot formation beginning 60 seconds after introduction of the blood and every five seconds thereafter. All ACT determinations in the study were performed by the same operator.

Baseline ACT determinations were made on entry of the patient into the operating room. All patients received 300 units/Kg initial heparin dose just prior to cannulation. ACT assays were made three to ten minutes after the initial heparin dose and every 20 minutes thereafter throughout the anticoagulation period. Additional heparin was administered as needed to maintain ACT values in the range of 480 to 600 seconds. Protamine (10 mg/ml) from a single source[g] was administered for reversal of anticoagulation by slow I.V. push. Initial dosages were calculated by the method of Bull and associates.[16] Supplemental doses were given if required to reach a target ACT of 94 to 120 seconds. Both heparin and protamine were refrigerated until just prior to use.

Results

Patient perioperative course, conduct of perfusion and surgical procedure and results were unremarkable. No patients were returned to the operating room for any reason. There were no deaths. Thirteen patients underwent uncomplicated aorto-coronary saphenous vein grafting (SVG) in the Upjohn group, receiving an average of 2.1 grafts per patient. In the Arysmed group fourteen patients had SVG only, averaging 2.0 grafts per patient. Valve replacements were evenly divided between the groups with one aortic and three mitral replacements in each. Both groups also contained one combined left ventricular aneurysmectomy and single SVG. The age of the Upjohn group was 56 ± 7 years, while the Arysmed group was 58 ± 7 years ($p > .9$, all p values for Student's two-tailed t test at 95% confidence level).[17] Both groups were predominantly male (Upjohn 13/5, Arsymed 11/8).

Other study data are summarized in Table II. Body weight and surface area were the same in both groups. Preoperative ACT values were equivalent between groups. There was no statistical difference between the two groups in duration of anticoagulation, defined as the time from the beginning of initial heparin bolus infusion to the beginning protamine administration. Magnitude of hypothermia, derived by plotting the difference between 37°C and mean rectal-esophogeal temperature as a function of time during anticoagulation and integrating the area under the curve, showed the two groups to be equivalent.

Mean ACT during anticoagulation, shown in Figure 1, was lower in the Arsymed group than for the Upjohn patients, but the difference was not statistically significant ($p > 0.1$). However, when displayed as a histogram in Figure 2, a striking difference in the distri-

[c] Collins Pulmonary Function Computer, the Warren Collins Company
[d] Yellow Springs Instruments, Inc.
[e] Vacutainer 3206XF534, Becton-Dickenson Inc.
[f] Drybath Type 5900, Thermoline Inc.
[g] Ely Lilly Co.

TABLE II

Summary of data from the clinical comparison of the effectiveness of two brands of beef lung heparin during cardiopulmonary bypass.

PARAMETER MEASURED	BRAND OF HEPARIN UPJOHN	ARSYMED	P VALUE*
NUMBER OF PATIENTS	18	19	—
BODY WEIGHT, Kg	70.5 ± 12	68.4 ± 12	> 0.6
BODY SURFACE AREA, m^2	1.79 ± .22	1.78 ± .25	> .09
PRE-OP ACT, sec	117 ± 16	117 ± 17	> 0.9
POST-OP ACT, sec	113 ± 29	116 ± 10	> 0.35
ANTICOAGULATION TIME, min	128 ± 29	116 ± 31	> 0.25
MAGNITUDE OF HYPOTHERMIA, °min	519 ± 244	537 ± 314	> 0.9
HEPARINIZED ACT, sec	546 ± 116	496 ± 100	> 0.1
PROTAMINE REQUIREMENT, mg	347 ± 78	353 ± 74	> 0.9
HEPARIN REQUIREMENT RATE, Units/Kg/min	3.3 ± 77	4.2 ± 1.1	< 0.01

* Student's "t" test, 95% confidence level

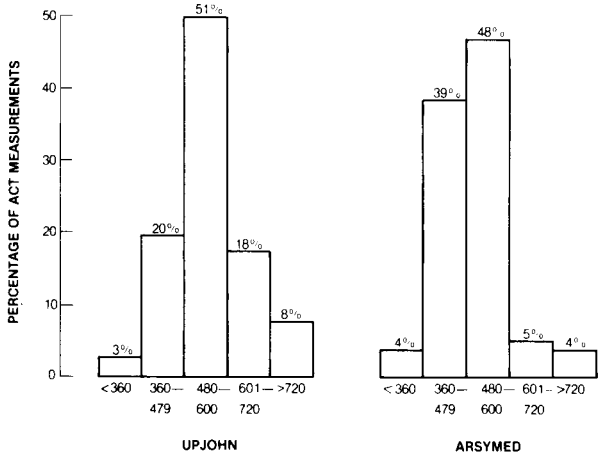

FIGURE 2. Distribution of activated whole blood clotting time measurements during systemic anticoagulation with two heparin brands.

bution pattern of the two groups is apparent. The Upjohn group manifests a nearly normal random distribution about the target range. Half the ACT measurements in this group fell within the target times, while one-fifth were in the range immediately below and one-fifth were in the range immediately above target values. By comparison, the Arsymed ACT values were markedly skewed toward the lower range. Again, half the measurements were within the target times.

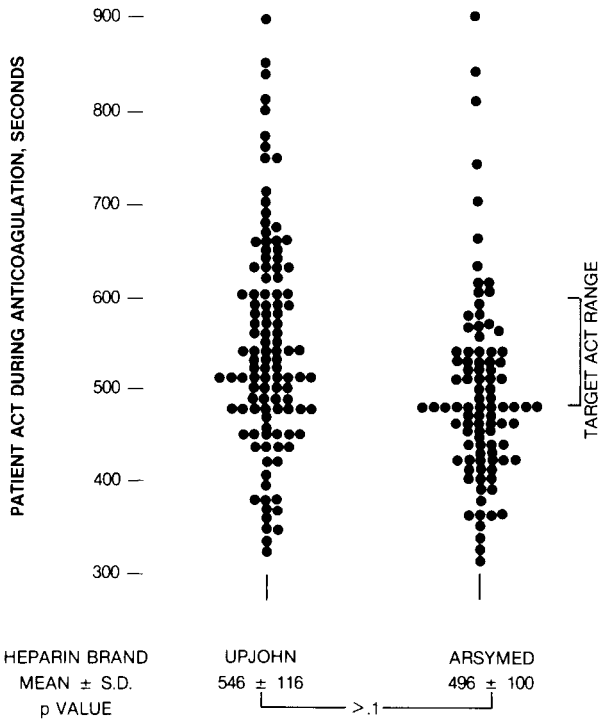

FIGURE 1. Activated whole blood clotting times determined during systemic anticoagulation with two brands of heparin.

But in contrast to the Upjohn distribution, two-fifths of the Arsymed determinations fell in the range immediately below the target times while very few Arsymed ACT's were above the target range. The higher average ACT of the Upjohn patients was not associated with significantly increased operative blood loss (Upjohn = 1211 ± 696 ml, Arsymed = 996 ± 566 ml, $p > 0.25$.)

Diligent pursuit of the target ACT range resulted in ACT measurements taken just prior to protaminization which were similar in the two groups, Upjohn = 487 ± 55 and Arsymed = 476 ± 102 seconds ($p > .9$). This was reflected in nearly identical protamine requirements, which resulted in a return of ACT values to the normal range in both groups. Heparin administered from all sources during the systemic anticoagulation period was totaled for each patient. This total was converted to a heparin requirement rate, Units/Kg/min., by dividing the patient total heparin dose by body weight and duration of the anticoagulation period. The individual values thus derived are plotted in Figure 3.

Statistical analysis showed a significant difference between the means of the two groups ($p < 0.01$). The Arsymed patients required an average of 27% more heparin than did the Upjohn group, in terms of units per kilogram of body weight per minute of systemic anticoagulation. Both groups displayed a wide range of heparin requirement rates. Those in the Upjohn group varied from 2.2 to 4.7 U/Kg/min, a multiple of 214%. The Arsymed patients ranged from 2.6 to 6.5 U/Kg/min, a multiple of 250%.

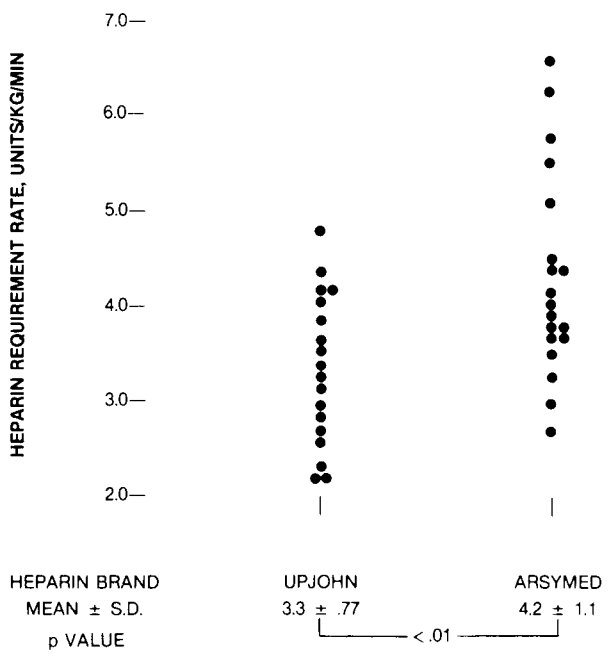

FIGURE 3. Patient heparin requirement rates during heparinization for cardiopulmonary bypass.

Discussion

Heparin is the family name for a heterogenous admixture of components which vary considerably in physical and biological properties. The various components which make up the heparin complex can range in molecular weight from 3,000 to 38,000 AMU,[18,19] and the many fractions have been shown to differ widely in biological activity.[18] Even though the same animal by-product may be used for the heparin source there are known differences in manufacturing processes,[22] levels of residual contaminants[21] and preservatives,[26] molecular weight distributions,[13] net electrical charge, structural differences in molecular chain length and the formation of acid groups on the heparin and accompanying polysaccharides, all of which may cause differences in biologic effect.[20] In addition, the mechanics of heparin action in the circulatory system are poorly understood.[21] Patients undergoing CPB present particular complications in anticipating individual response to heparinization, for many reasons. Plasma proteins and clotting factors, with which heparin interacts directly, are altered in concentration and activity by hemodilution. Hypothermia can have a wide effect, reducing metabolic rate and the ability of the reticuloendothelial system to remove heparin,[21] disturbing the reaction kinetics of the various molecular combinations involved, and affecting the character of rheology in small vessels.[1] Blood flow rate itself can alter the clotting mechanism, with areas of stasis being more prone to fibrin formation.[23] The presence of widespread cardiovascular disease is often associated with elevated levels of plasma lipids and triglycerides, both of which may interact with heparin.[21]

An attempt to standardize the potency of heparin solutions has led to the institutionalization in this country of the U.S.P. method for determining heparin unit strength. But there is no reason to presume that the U.S.P. methods bears any direct correlation to the action of heparin in either a normal or diseased human blood stream.[24] The U.S.P. utilizes sheep plasma which is citrated, fractionated, frozen and stored, then thawed and recalcified for use as a substrate. Varying volumes of heparin solution to be tested are added to the reconstituted sheep plasma and the delay in clotting time is used to derive unit potency.

This is all well and good until one considers the dependence of heparin's biological activity on heparin-plasma protein interactions.[21] Eighty-five percent of the biological activity of a heparin mixture is derived from only one-third of the material present, which is bound to plasma proteins. The other two-thirds, which is unbound, accounts for the remaining 15% of activity.[25] Small differences in heparin-protein binding constants[13] could therefore result in considerable alteration of heparin functionality. It does not seem rash to suggest that such subtle differences in character may exist between proteins in citrated, fractionated, frozen, stored, thawed, recalcified sheep plasma and those in the hypothermic blood stream of a human being with advanced cardiovascular disease undergoing extracorporeal circulation. Indeed, considerable variation may exist in the U.S.P. method itself. A collaborative study[7] involving the international standard for heparin (1958), which uses human plasma substrate processed similarly to the U.S.P. sheep plasma, found that estimates of heparin strength could vary as much as 40 to 50% with different substrate batches. There is no reason to doubt that deviations of equal proportion could occur with U.S.P. techniques.

Brief mention should be made of the relevance of protamine requirements. The amounts of protamine needed for neutralization is, of course, by itself unrelated to the assay of heparin potency. Tests which rely on the protamine reaction alone may be misleading in the derived concentration of heparin because protamine can be bound by any strong acid, including many non-anticoagulating components of commercial heparin such as chondroiten.[22] The combination, however, of statistically equivalent end perfusion ACT values,

protamine requirements, and post-neutralization ACT argues strongly that equal levels of whole blood anticoagulant activity were reached in both groups, albeit with the use of a much higher unit dosage rate for one of the brands.

Summary and Conclusions

Two brands of heparin, both derived from a beef lung source and both having the same potency as defined by an *in vitro* test utilizing processed sheep plasma, were found to have sharply different levels of anticoagulant activity when used for clinical systemic anticoagulation in the presence of hemodilution, hypothermia, extracorporeal circulation, and cardiovascular pathology. An average of one-fourth more Arsymed heparin was required to reach target ACT levels than was needed with Upjohn heparin. Additionally, Arsymed values during anticoagulation averaged lower than Upjohn values, although the difference was not statistically significant.

The span of heparin requirement was large in both groups, with the highest consumption rates two to two and one-half times the lowest rates.

U.S.P. unit activity equivalence between the two brands of beef lung heparin did not directly translate into clinical activity equivalence during cardiopulmonary bypass, as determined by whole blood activated clotting times.

Because of the wide divergence and unpredictability of patient response to heparin administration along with the variable effect extracorporeal circulation and associated maneuvers on heparin metabolism and patient coagulation functions, no empirical protocol for heparin administration will ensure safe levels of anticoagulation for all patients undergoing CPB.

The brand to brand inequality of clinical effectiveness demonstrated in this study, in spite of equivalent U.S.P. unit strength rating, is a further argument for the necessity of continuous assessment of patient anticoagulation level during ECC. We have found the whole blood ACT to be a convenient, reliable method to achieve this aim.

Acknowledgments

The authors wish to thank Pamela Finley, B.S., for her diligence in performing the many ACT determinations, and Andrew G. Hood, B.A., for his assistance in preparation of the manuscript. We are also grateful to the cardiac surgery staff and the perfusion staff of Shadyside Hospital, and to the staff and students of the Shadyside School of Perfusion, without whose help and cooperation this work would not have been possible. Appreciation is extended to Arsymed Inc. for their kind donation of heparin used in the study.

References

1. Jaberi, M., et al: Control of Heparin Therapy in Open-Heart Surgery. *J. Thorac. Cardiovasc. Surg.* 67:133, 1974.
2. Galetti, P. M. *Heart-Lung Bypass*, Grune & Stratton, Inc., N.Y., N.Y., 1962.
3. Rygg, I. H.: *Studies in Extracorporeal Circulation*, Fadls Forlag, Pub., Kobenhavn, 1973.
4. Stoufer, R. C. *A Technic For Extracorporeal Circulation*, Thomas, Pub., Springfield, Ill., 1968.
5. *United States Pharmacopea*, XVII, p. 611.
6. Bull, B. S., Korpman, R. A., Huse, W. M., et al: Heparin Therapy During Extracorporeal Circulation, I. Problems Inherent in Existing Heparin Protocols. *J. Thorac. Cardiovasc. Surg.* 69: 674, 1975.
7. Bangham, D. R., Woodward, P. M.: Collaborative Study of Heparin from Different Sources, *Bull WHO* 42:129, 1970.
8. Lowary, L. R., et al: Comparative Neutralization of Lung Derived and Mucosal Derived Heparin By Protamine Sulfate Using *In Vitro* and *In Vivo* Methods. *J. Pharm. Sci.* 60:638, 1971.
9. McMahon, F. G., et al: Anticoagulant Potency of Mucosal and Lung Heparin *Clin. Pharmacol. Ther.* 17:79, 1975.
10. Battes, B. J., et al: Comparison of Anticoagulant Activity of Two Preparations of Purified Heparin, *Clin. Pharmacol. Ther.* 15:287, 1973.
11. Gomez-Perez, F.; Anticoagulant Activity of Two Commercially Available Heparin Preparations. A Controlled Study. *J. Clin. Pharmacol.* 12:413, 1972.
12. Koepka, J. A., et al: The Effect of Heparin on the Activated Partial Thromboplastin Time. *Am. J. Clin. Pathol.* 70 (suppl. 3): 556, 1978.
13. Barrowcliffe, T. W., et al: Anticoagulation Activities of Lung and Mucous Heparins. *Thromb. Res.* 12:27, 1978.
14. Bull, M. H., et al: Evaluation of Tests Used to Monitor Heparin Therapy During Extracorporeal Circulation. *Anesth.* 43:346, 1975.
15. Hattersley, P. G.: Activated Coagulation Time of Whole Blood. *JAMA* 196:436, 1966.
16. Bull, B. S., et al: Heparin Therapy During Extracorporeal Circulation, 2: The Use Of A Dose Response Curve to Individualize Heparin and Protamine Dosage, *J. Thorac. Cardiovasc. Surg.* 69:685, 1975.
17. Armitage, P.: *Statistical Methods in Medical Research*, Wiley and Sons, Pub, N.Y., N.Y., 1971.
18. Lasker, S. E.: The Heterogeneity of Heparins. *Fed. Proc.* 36:70, 1977.
19. Johnson, E. A., Mulloy, B.: The Molecular Weight Range of Mucosal Heparin Preparations. *Carbohydr. Res.* 51:119, 1976.
20. Jaques, R. A.: Heparin From Different Sources. *Lancet* 2:1262, 1972.
21. Estes, J. W.: The Fate of Heparin in the Body. *Curr. Ther. Res.* 18:45, 1975.
22. Silverglade, A.: Biological Equivalence of Beef Lung and Hog Mucosal Heparins. *Curr. Ther. Res. Clin. Exp.* 18:91, 1975.
23. Ward, B. D., Hood, A. G., Hershgold, E. J.: Pathophysiology and Mechanics of Membrane Lung Performance Deterioration. *Trans ASAIO* 21:206, 1975.
24. Engelberg, H.: Probable Physiologic Functions of Heparin. *Fed. Proc.* 36:70, 1977.
25. Lam, L. H., et al: Biochem. Biophys. *Res. Commun.* 69:570, 1976.
26. Reiff, R., et al: Reducing Substances in Certain Heparin Preparations. *Am Heart J.* 92:542, 1976.