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Etude comparative de l'activité anticoagulante de trois héparines sodiques

Comparison of the activity of three heparin brands in hospital use

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SUMMARY

The variability existing in the biological characteristics of the heparins used in different commercial preparations, which may affect their anticoagulant action, and the hospital department's preference for a specific commercial brand, suggest to assay three sodium heparins (A, B and C) used in the clinic, evaluating their activity by two different methods : chromogenic substrate for determining anti-factor Xa activity, and evaluation of ability to inhibit coagulation of sheep plasma. The anticoagulant activity of all three heparins was within the margins accepted by the United State Pharmacopoeia (USP) XXI. However, there were statistically significant differences between them, with heparin C being the least active. The values obtained by the anti-factor Xa test were 11.8 and 13.5 % higher than those obtained by sheep coagulation plasma inhibition in the cases of heparins A and B. Heparin C did not show such pronounced differences between the two methods, but a second batch presented even less activity by chromogenic substrate than for the sheep plasma coagulation inhibition method.

Key-words : *Heparin, anti-factor Xa activity, coagulation inhibition.*

RÉSUMÉ

La variabilité qui existe dans les différentes formes commerciales d'héparine, pouvant affecter leur action anticoagulante, et la préférence de certains services pour une marque donnée, nous ont amenés à faire l'essai de 3 héparines sodiques (A, B et C) utilisées en clinique, par l'évaluation de leur activité par 2 méthodes différentes : Substrat chromogénique pour déterminer l'activité antifacteur Xa et inhibition de la coagulation du plasma de mouton.

L'activité anticoagulante de ces 3 héparines s'est révélée conforme aux limites acceptées par la Pharmacopée Américaine (USP) XXI. Cependant nous avons trouvé des différences significatives entre les trois : l'héparine C étant la moins active, les valeurs obtenues par le test antifacteur Xa sont supérieures de 11,8 et 13,5 % à celles obtenues par la méthode d'inhibition de la coagulation du plasma de mouton dans le cas des héparines A et B. Pour l'héparine C, de telles différences n'ont pas été retrouvées avec les deux méthodes, mais un second "lot" a même montré une activité encore moindre avec le substrat chromogénique.

Mots clés : *Héparines sodiques, activité antifacteur Xa, inhibition de la coagulation, essai inhibition.*

INTRODUCTION

Heparin is an anticoagulant widely used in prophylaxis and treatment of thromboembolic pathology. This substance is not made up of one single chemical, but is rather a biological mixture formed by a group of polymers of an heterogeneous family of variable mucopolysaccharides.

For a number of years there has been an important controversy in literature about possible structural and biological differences of heparins in relation to their origin and molecular weight.

It has been suggested that the origin of the commercial heparins, principally from ox lung or pig intestinal mucose, i.e. if they come from American or European firms respectively, may influence their anticoagulant action [1, 2].

The greatest difference between the biological properties of heparins of higher or lower molecular weights seems to lie in their different abilities to inhibit thrombin and Factor Xa [4, 6, 7].

The fact that there may be variability in the biological characteristic of different commercial brands of heparins, which may influence their anticoagulant action, led the Pharmacy Department of the Bilbao Hospital in collaboration with the Unidad de Trombosis y Arteriosclerosis del Consejo Superior de Investigaciones Cientificas (CSIC), to consider

carrying out a comparative study of 3 heparins from different commercial firms, in order to evaluate their actions objectively and answer the enquiries which are occasionally made by a Clinical Department about variying different effectiveness of some of prestations used.

MATERIAL AND METHODS

Material

In this study, the anticoagulant actions of three specialities of sodium heparin (A, B and C), were compared. Three different batches of each heparin were studied, and three vials per batch were evaluated. In all cases anticoagulant action was evaluated both for inhibition of sheep plasma coagulation and for inhibition of factor Xa.

The action of the different samples was evaluated against a standard heparin (1780 UI/ampoule. Ref. porcine heparin 82/502) from the National Institute for Biological Standards and Control in London (Holly Hill, Hampstead, London).

The samples of 1 % sodium heparin, in 5 ml vials, with a theorical activity of 100 UI/ml, are from the following commercial firms, and in each case the batches studies :

Heparin A : Heparin Leo, batches V-45, X-9, X-14

Heparin B : Heparin Roger, batches V-28, X-18, X-22

Heparin C : Heparin Rovi, batches V-4, V-3, X-9

Sheep plasma (Bioibérica)

Chromogenic substrate kit for determining anti-factor Xa

Substrate S-2222 (Bz-Ile-Glu-(Gamma-Or)-Gly-Arg-pNa HCl)

kindly supplied by Kabi Vitrum, Mölndal, Sweden

Methods

A - Inhibition of sheep plasma coagulation (USP XXI, 1985, p. 485-482): the technique is based on determining what is the minimum concentration of heparin that is capable of maintaining fluid 1 ml of sheep plasma for 1 hour after the addition of 0.2 ml of 1 % $CaCl_2$ solution.

B - Evaluation of the anti-factor Xa activity by chromogenic substrate according to the technique described by Teien and Lie (1977) [10].

The principle of this technique is based on the determination of heparin as a complex with an excess of factor Xa. The remaining f-Xa catalyses the split of the p-nitroaniline from the chromogenic sustrate S-2222, producing a colored emission which is read at 405 nm.

C - Determination of the volume contained in each vial by measuring density and weighing.

Table 1

Estimated activities for coagulation inhibition and anti-Factor Xa for each of the different batches studies ; n = 3 vials batch

BATCH	HEPARIN A U/ml		HEPARIN B U/ml		HEPARIN C U/ml	
	Coagulation Inhibition	Anti-Factor Xa	Coagulation Inhibition	Anti-Factor Xa	Coagulation Inhibition	Anti-Factor Xa
1	1032 ± 8,5	1225 ± 87	1024 ± 5,5	1175 ± 50	933 ± 7,3	1050 ± 43
2	1058 ± 9	1083 ± 38	1014 ± 7,5	1196 ± 76	982 ± 3,5	958 ± 52
3	1023 ± 6	1125 ± 87	996 ± 8	1070 ± 128	1032 ± 3	1041 ± 123

Statiscal study carried out by the variance analysis test.
Difference between vials (1, 2, 3) not significant. Difference between heparins (A, B, C) significant for α 0.01
Difference between methodologies used to evaluated heparin activity (plasma coagulation inhibition and anti-factor Xa) statistically significant (α = 0.05)

RESULTS

In table 1 the results obtained from the heparin activity for each batch are shown comparatively, and table 2 shows the averages and SD of the activities obtained for the heparins studied from the different commercial firms.

Table 2

Average activities and relationship of anti-factor Xa activity : coagultion inhibition in the different heparin preparations

	HEPARIN A	HEPARIN B	HEPARIN C
Coagulation inhibition U/ml	1023 ± 40	1011 ± 14*	982 ± 49
	NS	NS	
	NS		
Anti Factor Xa activity U/ml	1144 ± 72**	1147 ± 67**	1016 ± 50
	NS	NS	
	NS		
% Difference anti-factor Xa coagulation inhibition	+ 11,8	+ 13,5	+ 3,5

The average avtivity values obtained for each heparin (A, B, C) were statistically compared (student's T test) with the expected theoretical value (U/ml).
NS = not significant
* significant for $0.05<2p<0.01$
** significant for $2p<0.005$

The differences in the activity obtained by inhibition of coagulation and anti-factor Xa methods for each one of the nine batches studied are within the limits established by the USP XXI (activity obtained from anti-factor Xa should not differ more 20 % in relation to that obtained from inhibition of coagulation).

The three batches showed no differences in activity in any of the heparins studied (A, B or C). On comparing the potency values of the three heparins obtained by the anti-factor Xa method, and that of sheep plasma coagulation inhibition, the differences between the two methodologies were statistically significant (table 1). Heparins A and B showed differences in activity of 11.8 % to 13.5 % between anti-factor Xa evaluation and sheep plasma coagulation inhibition. For heparin C, the

differences between the two methods were not so pronounced, and the second batch even presented a potency 2.4 % lower by chromogenic substrate than by the coagulation inhibition method.

In all three cases, the activity found by chromogenic substrate was higher than the indicated theorical value (1000 UI/ml), though only heparin A (Δ = 14 %) and B (Δ = 15 %) showed statiscally significant differences with regard to the theorical value ($2p > 0.005$). When activity was evaluated by coagulation inhibition, heparin C presented 2 % less activity than the theorical value indicated.

Comparing the three heparins studied among themselves, the differences obtained were statiscally significant ($\alpha = 0.01$). Heparin C presented the least activity in both methodologies, 3 % and 11 % less than heparin A by coagulation inhibition and anti-factor Xa methods respectively, and 4 % and 12 % less than heparin B.

Table 3 shows that the content of the vials of heparin was higher than 5 ml in all cases. Although the differences were minimal, and always within the margins accepted by USP XXI, heparin B presented a content which was statistically higher than heparins A and C.

Table 3

Average heparin volume (ml/vial)

HEPARIN A	HEPARIN B	HEPARIN C
5,33	5,40	5,30
± 0,026	± 0,020	± 0,030
	$2p < 0,05$	$2p < 0,01$
	NS	

Statiscally method Student's T test

DISCUSSION

The sheep plasma coagulation inhibition test, a classic method employed in different official compendiums to evaluate antithrombin activity of heparin, only permits evaluation if the activity shown by the different preparations studied is within the accepted theorical anticoagulant limits (90-100 %) in relation to the theorical described activity (criterion of the British Pharmacopoeia). In our case, all batches were within these limits, so that heparins A and B with a theorical activity of 1000 UI/ml, had an average activity of 1023 and 1011 UI/ml theorically described. However, despite the fact that all the preparations were within accepted limits, heparins A and B demonstrated higher anticoagulant (antithrombin) action than heparin C.

The USP XXI (1985) incorporated the possibility of measuring the anticoagulant action of heparins not only by the classical method of inhibition of sheep plasma coagulation, but also through evaluation of anti-factor Xa activity by chromogenic substrate.

In 1979, Anderson [2] described how anticoagulant activity, evaluated by the APTT, decreased as the molecular weight diminishes, anti-factor Xa activity increases. This fact, confirmed by various authors [8, 9] could explain why in the case of heparins A and B the value obtained by chromogenic substrates (anti-factor Xa activity) is 11.8 % and 11.5 % respectively higher than that obtained through the coagulation inhibition method, whereas for heparin C there is only 3.5 % difference between the two method.

The use of two methods allows us to evaluate more objectively the activity of commercial heparins. In the case of heparins A, B and C, the differences obtained by the two methods suggest that preparations A and B may contain a heparin of smaller molecular size than preparation C. Nevertheless, all heparins meet requirements of USP XXI, which does not explain the preference of a Clinical Department for a particular brand.

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