DAC-Coated Resins for Hemoperfusion

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SYNOPSIS

This paper reports the use of diacetylchitin (DAC)-coated macroreticular resin HS-12 as an adsorbent in hemoperfusion. Sixteen dogs acutely intoxicated with pentobarbital were hemoperfused, either with the coated resin (n = 8) or raw resin (n = 8). The results showed the coating greatly improved resin hemocompatibility. The clearance rates for the coated and raw resins were 195 mL/min for the first 5 min at a flow rate of 200 mL/min. In clinical practice, 12 patients who were acutely intoxicated by drugs, such as pentobarbital and phenobarbital, were hemoperfused and saved. © 1992 John Wiley & Sons, Inc.

INTRODUCTION

The research on the use of adsorption resins in hemoperfusion began with the work of Rosenbaum in 1970. In 1976, he succeeded in treating patients suffering from acute drug intoxication with Amberlite XAD-4, an adsorption resin produced in America.[1] At present, this resin has become the pattern of adsorption resins for hemoperfusion. The equipment for adsorption has been commercialized. Starting from the 1980s, domestic units such as Nan Kai University and Zhong Shan Hospital[2] (Shanghai) worked on this problem, but products of coated resins are still not known at present.

The improvement of the blood compatability of resins by coating has been a topic of concern for people working in the field of hemoperfusion. Uncoated resins cause medium destruction to leukocytes and thrombocytes and affect their use in cases such as liver failure and even in certain cases of acute drug intoxication that causes serious shock.[3] The Amberlite XAD-4 already commercialized is a kind of uncoated resin: Its pore radius is small (50 Å) and can be used only for the therapy of intoxication due to overdosage of molecular weight less than 1000. The use of albumin, agarose, and diacetylchitin[4] as coatings for improving the blood compatibility of resins have been reported, but all are still in the experimental stage. The method of using human plasma for the treatment and coating resins has been patented in Japan,[5] but due to the large quantity of plasma used and the inconvenience in storing the resin after coating, difficulties occur in actual applications. Simultaneously with the preparation of highly efficient adsorption resins, we also studied materials for blood compatibility and methods for coating of resins. On the basis of studies on the synthesis and antithrombogenic properties of diacetylchitin (DAC), we used it as coating material for resins. The coated resins obtained have been used for hemoperfusion.

EXPERIMENTAL

1. The preparation of adsorption resins: Adsorption resins were obtained from suspension polymerization of technical divinylbenzene in the presence of porogenic agents. The basic formula of the polymerization process was

Oil phase	
Divinylbenzene (industrial grade)	100 parts
Porogenic agent	120 parts
Benzoyl peroxide	1 part
Aqueous phase	
Deionized water	500 parts
Hydroxyethyl cellulose	1.2 parts
Water solution of methylene blue	0.01 part (1%)

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Table I The Physical Structure of HS-12 Resins

Title of Determination	Skeletal Density (g/mL)	Apparent Density (g/mL)	Pore Volume (mL/g)	Specific Surface Area (m^2/g)	Pore Radius (Å)
Values determined	1.07	0.487	1.09	568	77

In this case, the ratio porogenic agent : monomer = 1.2 : 1 and oil phase : water phase = 1 : 2.3. The procedure of polymerization was as follows: The oil and aqueous phases were mixed and stirred at 50°C, and the reaction proceeded at 70°C for 2 h. The temperature was maintained at 80–85°C for 10 h; the product was washed with water, steam-distilled, and sifted to give macroporous pearly resins (50–20 mesh); and HS-12 type resin was selected from the products of nearly 100 prescriptions and was strictly purified before coating.

2. Coating of the resin: HS-12 resin was coated by means of dispersive precipitation. DAC was dissolved in mixed solvents, giving a 1–2% solution. The resin was soaked in it for 1–2 h, filtered, dried, and put into a large excess of a 40% aqueous solution of ethyl alcohol. The resin dispersed in the ethyl alcohol, and a layer of DAC membrane separated out on its surface, resulting in DAC-coated resin.
3. Radiation tests on DAC-coated resin: DAC-coated resin was radiated with r-ray (0.8–1.0 mrad).
4. Determination of Clotting Time.

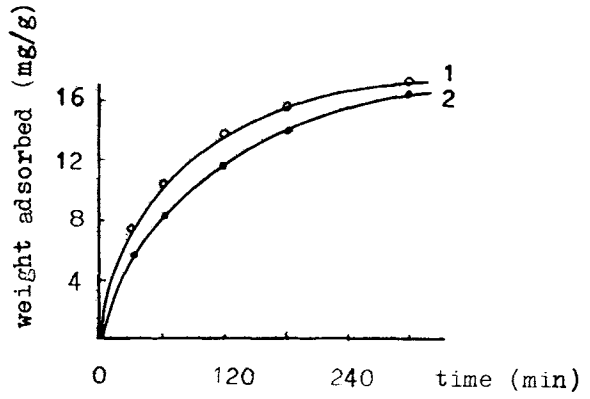

Figure 1 The adsorption of amyl barbital by HS-12 (1) and XAD-4 (2).

RESULTS AND DISCUSSION

Physical Structure and Adsorption Properties of HS-12 Resin

The physical structure of HS-12 resins selected from different prescriptions is shown in Table I.

Comparison was made between HS-12 and XAD-4 on the adsorption of substances of low molecular weight (pentobarbital, MW = 226; phenylbarbital, MW = 232), of medium molecular weight (vitamin B_{12}, MW = 1355), and of large molecular weight (complex of bilirubin and albumin, MW = 69,000). The results are shown in Figures 1–3 and Table II.

From Figures 1–3 and Table II it is evident that the adsorption rate and amount adsorbed for materials of low, medium, and high molecular weights are higher in the case of HS-12 than in XAD-4. Furthermore, with the increase in the molecular weight of the adsorbate, HS-12 is better.

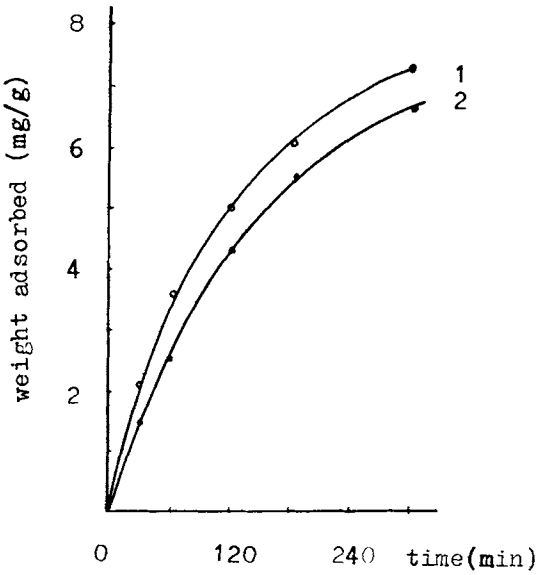

Figure 2 The adsorption of phenylbarbital by HS-12 (1) and XAD-4 (2).

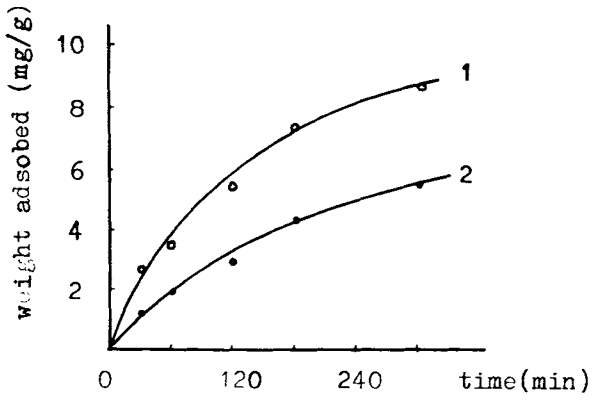

Figure 3 The adsorption of vitamin B_{12} and XAD-4; 1: HS-12, 2: XAD-4.

The Blood Compatibility and Detoxifying Efficiency of DAC-coated Resin

DAC possesses good antithrombogenic properties. Some acylated chitins,[6] such as formyl acetyl, butyryl, and caproyl chitin, were considered to have good antithrombogenic properties, and the differences between the acylated chitins were dependent on the type of the acyl group attached to chitin. In our research[7] it was found that the antithrombogenic properties of acetylchitins were related to their degree of acylation and reduced viscosity (relating to molecular weight of DAC), and DAC (degree of acylation = 2) with high reduced viscosity showed the best antithrombogenic properties. Furthermore, DAC was found to be able to form a porous structure[4] and a spongelike film that permits the passage of adsorbates, such as pentobarbital and vitamin B_{12},[7] whereas the cell fraction in blood cannot pass when blood passes through it. So, we tried using DAC as coating materials for HS-12 resin, and the DAC-coated resins were used in the hemoperfusion tests.

DAC-coated HS-12 resin was examined by SEM. This showed that complete coating was obtained.

After coating, there occurred changes in the pore structural parameters of the resins, the magnitude of the change being dependent on the reduced viscosity of DAC. Table III shows the change in specific surface area after being coated with DAC of high, medium, and low reduced viscosity (designated as DAC-H, DAC-M, and DAC-L). It showed that by selecting DAC of as high a reduced viscosity as possible for the coating material the least drop in specific surface area will occur.

Table II Adsorptivity of HS-12 and XAD-4 toward Bilirubin (%)

	Resin			
	HS-12		XAD-4	
Adsorption time (h)	2	6	2	6
Adsorptivity	13.0	21.5	7.0	12.0

Table III Change in Specific Surface Area of Resins when Coated with DAC of Different Reduced Viscosity

	Uncoated Resin	Material for Coating		
		DAC-H	DAC-M	DAC-L
Specific surface area (m^2/g)	516	476	442	405

The results of animal hemoperfusion tests are shown in Figures 4 and 5. Figure 4 represents results of hemoperfusion on rats. Fourteen white rats, each weighing about 250 g, were divided into two sets. After anesthesia, they were hemoperfused with coated and uncoated resins. Flow rate was 2 mL/min. After 2 h of perfusion, the fall in thrombocyte and leukocyte using coated resins were 13 and 12%, while the fall using uncoated resins were 42% and 37%, respectively. Figure 5 shows the results of hemoperfusion for dogs. Sixteen dogs, each weighing 10–15 kg, were divided into two series, poisoned (pentobarbital 2 × 30 mg/kg) and, after lethargy, hemoperfused with coated and uncoated resins, the flow rate being 200 mL/min. After perfusion, the

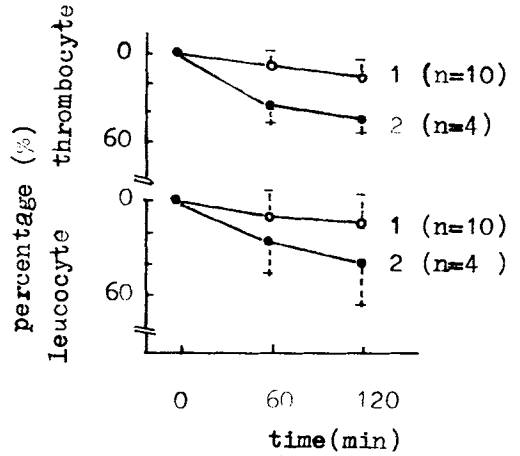

Figure 4 Rate of decrease of thrombocytes and leukocytes of white rats after hemoperfusion with resins. 1: coated resins, 2: uncoated resins; n is number of rats in each series.

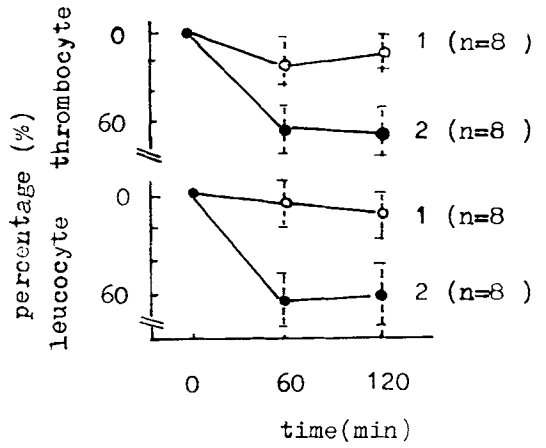

Figure 5 Rate of decrease of thrombocytes and leukocytes of dogs after hemoperfusion with resins. 1: coated resins, 2: uncoated resins; n is number of dogs in each series.

drop in thrombocyte and leukocyte for the series with coated resins are 5 and 12%, while those for the series with uncoated resins amounted to 67% and 60%, respectively. Evidently, the blood compatibility of coated resins are better than the uncoated ones.

During the hemoperfusion for dogs, the change in drug concentration in the blood and that of the clearance rate of the resins within a definite period of time were further determined, as shown in Tables IV and V. Both series of resins show very high rates of cleansing. At 5 min after the beginning of perfusion, all clearance rates were high, up to 195 mL/min, approaching the flow rate of blood during hemoperfusion. At the end of perfusion, the clearance rate against pentobarbital of coated and uncoated resins still remained at 159 and 156 ml/min, respectively, showing that after DAC coating, the resins still keeps high detoxifying efficiency.

The Stability of DAC-coated HS-12 Resins

To use DAC-coated resins as the adsorbent for hemoperfusion, they must allow sterilization and treatment for the removal of pyrogens without changes in properties. They should be able to be stored for a comparatively long time without loss of its original efficiency. This requires the possession of corresponding chemical and physical stability by the coated resin.

There are usually three methods of sterilization, one using high-pressure steam, the other using ethylene oxide vapor, and still another by irradiation. Since resins can be used only in the moistened state, sterilization by ethylene oxide is not suitable. After sterilization with steam under high pressure, both coated and uncoated resins show no change in adsorption effects. Furthermore, the blood compatibility of coated resins is definitely better than that of the uncoated ones (see Figures 4 and 5), which shows that the coated resins are stable toward heat.

Sterilization by irradiation is advantageous for production by lots. Therefore, we have tested the effect of sterilization by r-radiation on the properties of coating material DAC, on the dissolved substances from the coated resins, and on the adsorption properties of coated resins in order to offer basis for the management of sterilization in enlarged tests. The reduced viscosity of DAC dropped down (from 1030 to 519 mL/g), but the change in strength is not large (tensile strength from 318 to 299 kg/cm^2). The infrared spectrum of DAC before and after irradiation proved that there is no considerable change in its chemical structure. There is no change in pH value of aqueous soaking liquid for the coated resins before and after irradiation. There seems to be some decrease in adsorption values of the coated resins after irradiation, but the value of decrease is rather

Table IV Concentration of Pentobarbital in Dogs during Hemoperfusion with Resins (mg/dL, X ± SD)

	Time (min)				
	0	5	35	65	125
Coated resin (n = 8)					
C_0	3.65 ± 0.30	2.98 ± 0.44	1.50 ± 0.20	1.08 ± 0.25	0.50 ± 0.23
C_1		0.08 ± 0.08	0.06 ± 0.05	0.13 ± 0.08	0.13 ± 0.10
Uncoated resin (n = 8)					
C_0	3.81 ± 0.42	3.17 ± 0.45	1.56 ± 0.28	1.29 ± 0.31	0.69 ± 0.17
C_1		0.09 ± 0.11	0.10 ± 0.09	0.19 ± 0.06	0.15 ± 0.05

C_0 = Concn at the entrance; C_1 = concn at the end.

Table V The Clearance Rate of Resins for Pentobarbital during Hemoperfusion (mL/min, X ± SD)

	Time (min)			
	5	35	65	125
Coated resins (n = 8)	195 ± 5.3	192 ± 6.1	190 ± 13.8	159 ± 30.2
Uncoated resins (n = 8)	195 ± 6.0	185 ± 13.8	170 ± 6.4	156 ± 15.7

Blood flow rate: 200 mL/min.

small (Table VI). All these results show that sterilization by r-radiation is suitable for DAC-coated resins.

The change in degree of acylation and reduced viscosity of DAC membranes after being soaked in acids and bases are listed in Tables VII and VIII. Since the degree of acylation is related to antithrombogenic properties in general and reduced viscosity to molecular weight, these two parameters are related to the antithrombogenic properties and strength of DAC coatings.

The data of Tables VII and VIII shows that in 1*N* NaOH the chemical structure of DAC rapidly changes, making it lose its antithrombogenic properties. But in 1 or 2*N* HCl, the rates of deacetylation and degradation of the molecular chain are all rather small.

Table VI Adsorption for Pentobarbital (mg/g) by DAC-coated Resins before and after Irradiation

	Adsorption Time (min)				
	30	60	120	180	200
Before irradiation	9.5	10.5	12.8	14.7	16.4
After irradiation	9.4	9.9	12.5	14.5	16.0

Table VII The Degree of Acylation[a] of DAC after being Soaked in Acids and Bases (Temperature of Soaking 25°C)

	Time of Soaking (h)					
	0	1	2	4	6	20
1*N* HCl	1.95	1.86	1.82	1.74	1.73	1.61
2*N* HCl	1.95	1.85	1.80	1.75	1.71	1.39
1*N* NaOH	1.95	0.79	0.63	—	—	—

[a] The number of acetyl groups per *N*-acetylglucosamine residue.

To estimate the antithrombogenic properties of DAC after treatment with hydrochloric acid, we determined kinetically the clotting times[6] on the surfaces of 2*N* HCl-soaked DAC and on the surfaces of glass and siliconized glass as comparing materials. In Table IX, the clotting times on the surfaces of 2*N* HCl-soaked DAC were normalized to those of the comparing surfaces. We can see that DAC being soaked in 2*N* HCl for 2 h actually kept its good antithrombogenic properties as its clotting time was still nearly twice as long as that of siliconized glass, although the antithrombogenic properties of DAC evidently decreased after DAC was soaked for 20 h.

Clinical Applications of DAC-coated HS-12 Resin

Zhong Shan Hospital is the principal unit trying out the usage of coated resins. Besides, the First Affiliated Hospital of Zhong Qing Medical University, the 94th Hospital of the Liberation Army, and the

Table VIII The Reduced Viscosity of DAC after being Soaked in HCl (mL/g)

	Soaking Time (h)					
	0	1	2	4	6	20
1*N* HCl	616	632	639	637	638	590
2*N* HCl	616	638	634	632	629	420

Table IX Relative Clotting Times of DAC being Soaked in 2*N* HCl for Different Times

Soaking Time (h)	Clotting Time Ratio	
	DAC/Glass	DAC/Siliconized
0	3.0	1.9
2	2.8	1.8
20	1.6	1.0

Second Affiliated Hospital of Jiang Si Medical College have altogether rescued 13 patients, among whom 12 were of drug intoxication and one of liver failure. Intoxicating drugs were pentobarbital, Valium, Miltown, chloropromagin, etc., altogether 10 kinds. The amounts taken were much higher than the lethal dosage and the period of intoxication was 3.5–72 h. Generally, the patients had been rescued by the internal medicine departments, but there was no improvement in bodily characteristics of life and patients were under deep lethargy and in a dangerous state. After perfusion, all 12 cases were saved and left the hospital after several days. In one case, perfusion was done twice; for all other cases, it was done only once. The case suffering from liver failure was saved at first after hemoperfusion, but 5 days later bled seriously due to break of the vein at the bottom of the stomach causing death.

CONCLUSIONS

1. DAC-coated resin is of high detoxifying efficiency, good blood compatibility, and stable properties. It can be used in hemoperfusion therapy for urgent drug intoxication. Compared with the same type of products, it reaches or surpasses the typical hemoperfusion adsorption efficiency, but evidently surpasses XAD-4 in blood compatibility (the loss in thrombocyte is about 40% when XAD-4 is used once for perfusion).
2. DAC-coated resin has strong adsorption power, and thus the amount of resin used for each perfusion is reduced. In clinical therapy, the amount of DAC-coated resin used to fill in a perfusion apparatus is only 500 mL, thus reducing the blood capacity of the perfusion apparatus.

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