Effects of Chondroitin Sulfates on Serum Lipids and Hexosamines in Atherosclerotic Patients : with Special Reference to Thrombus Formation Time

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SUMMARY

Long term effects of chondroitin sulfates A and C on the lipid and hexosamine levels were studied for 22 weeks in the patients with atherosclerosis. Chondroitin sulfates A and C both kept serum cholesterol levels low in the patients in comparison with the controls during the course of treatment.

Short term effects of chondroitin sulfate C injected intravenously on the thrombus formation time were also studied in the patients by Chandler's method in connection with thrombogenic causes of atherosclerosis. The prolongation of thrombus formation time was observed at 30 and 60 min. after the injection of the substance.

Additional Indexing Words :

Acid mucopolysaccharides Chondroitin sulfates A, B and C Glucosamine Galactosamine Cholesterol Thrombosis

THE inhibitory effects of chondroitin sulfate, an acid mucopolysaccharide (AMPS), on the occurrence of experimental atherosclerosis have been studied in the past decade by many investigators.¹⁾⁻⁶⁾ Oshima and his co-workers⁶⁾ have reported in their incipient clinical studies that serum cholesterol levels were decreased with daily intravenous or peroral administrations of chondroitin sulfate in patients with hypercholesteremia for short periods. Although this finding has been supported by succeeding studies, not much work has been done in studying the long-term effects of chondroitin sulfate (CS) in atherosclerotic patients. In this experiment, comparative studies of the effects of the long-term administrations of chondroitin sulfates A and C (CS-A and CS-C) on the serum lipid fractions such as cholesterol, phospholipid and triglycerides have been conducted in the patients with cerebral atherosclerosis. The changes of components of CS, hexosamine and galactosamine, were also

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investigated under the peroral administration of CS in the patients.

On the other hand, many investigations have called attention to thrombosis as a cause of arteriosclerosis, since Duguid had set forth the thrombogenic theory of arteriosclerosis in 1946.^{7), 8)} For example, in 1958 Chandler devised a simple method for the production of a thrombus *in vitro*.⁹⁾ In this connection, we have also examined the short-term effects of CS-C injected intravenously on the thrombus formation time¹⁰⁾ by means of a modified Chandler's method.

This preliminary study, therefore, deals with 2 experiments regarding the studies of CS in arteriosclerotic patients: 1. the long-term peroral administration of CS-A and CS-C, 2. the short-term effects of CS-C injected intravenously on thrombus formation time.

MATERIALS AND METHODS

Experiment 1

The 30 atherosclerotic patients including hemiplegic subject, in both sexes, ranging in age 40 to 72 years, were selected by the determination of the serum cholesterol levels 4 times prior to the experiments. The serum cholesterol levels were more than 180 mg./100 ml. in the 4 determinations. They were divided into 3 groups. Six patients, in group 1, were treated with 4.5 Gm./day of CS-A* for the initial 4 weeks, then 6 Gm./day for further 18 weeks. Six patients, in group 2, were given CS-C* in the same manner. Eighteen subjects, well matched with the CS-treated patients in age, sex, and serum lipid levels were used as the control.

The serum cholesterol, triglycerides, phospholipid and β -lipoprotein were determined by the methods reported by Zak,¹¹⁾ Handel,¹²⁾ Allen¹³⁾ and Heiskell et al.,¹⁴⁾ respectively. Serum hexosamine and galactosamine were measured by a modification of the methods of Boas¹⁵⁾ and Cessi.¹⁶⁾ During the course of the investigation, serum cholesterol levels were determined at monthly intervals in the CS-treated patients and in the control subjects. Serum hexosamine, galactosamine, phospholipid and triglyceride levels were measured before the experiment and at the 4th, 14th and 18th weeks of the treatment and after the withdrawal of the CS.

The patients were put on diets containing 1,900 Calories with 8 Gm. of sodium chloride. Three patients in the CS-treated groups received a daily dosage of 0.4 to 0.8 mg. of reserpine to control their systolic and diastolic pressure below 160/90 mm. Hg.

Experiment 2

Investigations were performed on the effects of an intravenous injection of 5 mg. per Kg. of body weight of CS-C** dissolved in approximately 10 ml. of saline solution

* CS-A and CS-C, which were prepared from the cartilages of whale and shark, respectively, are gifts from Seikagaku Kogyo Co. The analytical data of the CS-A and CS-C are as follows: sulfur, 5.80% and 6.66%; nitrogen, 3.07% and 2.65%; and the optical rotation, -35° and -17° , respectively.

** CS-C used for intravenous injection, which was prepared from shark cartilage, is a gift from Kakenyaku Kako Co. The available data indicate; sulfur contents, 5.8-6.8%; nitrogen contents, less than 2.9%.

on the thrombus formation time by the method described by Chandler⁹⁾ during the fasting time. The 45 patients were selected and injected CS-C for the determination of thrombus formation time; a control group consisted of patients who were injected intravenously with 10 ml. of saline solution. The bloods were drawn before and at 30, 60, 90 and 180 min. after the injection of CS. The thrombus formation time was measured by Chandler's technique in which we used platelet-rich silicone plasma rather than whole blood. The blood was drawn from the antecubital veins of subjects using the syringe containing 3.8% citrate solution in the ratio of 1 to 9 of blood and centrifuged for 5 min. at 2,000 rpm. in centrifuge tubes. A 0.1 ml. of 0.25 M CaCl_2 was added to 1 ml. of plasma and poured into a vinyl tube (27.5 cm. long and 0.3 cm. in diameter). The tube was then attached end to end to an adaptor and was revolved on a slanted turntable; 45 degrees in angle, rotating speed 16 rpm. (the linear velocity 440 cm./min.). The thrombus formation time was measured from addition of a CaCl_2 solution to plasma to the thrombus formation, which was manifested by the contraction or convolution of the fibrin to form a white mass at the advancing edge of the serum within the looped tube. The experiment was conducted at a room temperature of 25 to 27°C.

RESULTS

Experiment 1

The data obtained, as illustrated in Fig. 1, show that the peroral administration of CS-A and CS-C lowered serum cholesterol levels for 4 months. The cholesterol levels of the two CS-treated groups were significantly reduced at a p level of 0.01 or 0.05 after 4 weeks from the start of the treatment. The levels of serum cholesterol in the CS-A treated group returned to the initial levels in

Fig. 1. Alterations of serum cholesterol levels during the treatment of chondroitin sulfates A and C. Significant differences were obtained between the value of the treatment groups and the controls as follows: * <0.01 , CS-A in Feb.; $p<0.05$, CS-A in Mar. and CS-C in Feb. and April.

Table I. Effects of Long Term Administration of Chondroitin Sulfate A and C on Hexosamine and Lipid Levels in Serum

Treatment	Duration of Treatment				
	0 w	4 w	14 w	18 w	22 w 2w after withdraw
Hexosamine (mg./100 ml.)					
CS-A	116±6 ⁺	122±2	118±5	133*±4	107±6
CS-C	113±5	107±6	123±7	126±2	106±7
Galactosamine (mg./100 ml.)					
CS-A	23.6±1.1	26.8±2.2	—	23.0±0.7	20.2±0.7
CS-C	18.5±1.5	18.5±0.8	—	22.4±1.3	21.1±1.4
Phospholipid (mg./100 ml.)					
CS-A	318±12	312±17	221**±11	296±15	299±18
CS-C	330±13	331±18	251**±25	270±21	292±19
Triglyceride (mg./100 ml.)					
CS-A	109±17	96±21	141±26	129±21	102±18
CS-C	135±25	132±17	178±16	171±21	135±19

⁺ Means and standard errors.

Statistical comparisons are made between the pretreatment values and the levels of indicated periods. Significant differences are indicated as follows:

* $p < 0.05$, ** $p < 0.01$.

April, while those of the CS-C treated group reached the levels of the controls in June. No differences could be found after that time. The serum phospholipids in CS-treated groups, as shown in Table I, gave a significant reduction ($p < 0.01$) at the 14 week of the treatment over the initial value and then returned to the pre-treatment levels. The serum triglycerides showed no much changes in both CS-treated groups.

The changes of serum hexosamine and galactosamine levels were shown in Table I. The hexosamine values in CS-C treated group rose progressively after the treatment and returned to the starting levels. The hexosamine of CS-A treated group showed a significant rise ($p < 0.05$) at the 18 week over the pretreatment values. The serum galactosamine in both CS-treated groups maintained the initial values during the course of the study. No alteration was found by the administration of reserpine.

Experiment 2

The thrombus formation time was prolonged, as illustrated in Fig. 2, at 60 min. after the injection of CS-C when a comparison was made with the controls: Twenty three % increase in an averaged time formed thrombus in vitro

Fig. 2. Effects of chondroitin sulfate C on thrombus formation time, hexosamine and galactosamine in plasma.

—— Control, injected saline solution in an equivalent amount.

- - - - Intravenous injection of 5 mg./Kg. of body weight of chondroitin sulfate C.

was found over the values of the pre-injection and of the controls at 60 min.

The serum hexosamine levels showed a trend of a reduction after the injection of CS, but increased again at 60 min. and then returned gradually to the initial levels. No significant changes were observed in the levels of serum cholesterol, galactosamine and triglycerides in the condition used here. β -Lipoproteins showed a reduction trend at 60 and 90 min. after the CS injection.

DISCUSSION

The present data show that the long-term oral administration of CS-A or CS-C gave a fall in the serum cholesterol levels in the patients with atherosclerosis. On the other hand, the single intravenous injection of CS-C resulted in a prolongation of the thrombus formation time shortly after the injection.

In 1955, Kurita²⁾ has observed that the daily intravenous administration of CS-C of 5 mg. per Kg. of body weight lowered serum lipid levels and prevented atherosclerosis in cholesterol-fed rabbits. Ohdoi³⁾ has reported that daily peroral administrations of CS-C of 20 mg./Kg. of body weight reduced serum cholesterol levels, total lipids, produced restoration of β/α lipoprotein ratio and

inhibited atheromatous aortic lesions in cholesterol-fed cockerels. Murata⁴⁾ has observed that chondroitin polysulfate which was prepared by sulfation of CS-C from shark cartilage prevented the elevation of serum cholesterol and total lipid levels and suppressed the atherosclerosis in cholesterol-fed rabbits when injected intravenously at a level of 5 mg./Kg. of body weight per day. More recently, Morrison and Murata⁵⁾ have reported that daily subcutaneous injections of CS-A prepared from bovine cartilage at a level of 20 mg./Kg. of body weight reduced the serum lipids and inhibited the atherosclerotic lesions in squirrel monkeys fed a diet containing cholesterol and butter.

The present findings, that the peroral administration of CS-A and CS-C to the atherosclerotic patients showed the reduction of serum cholesterol, will be in agreement with the above experimental observations. There was not shown any difference in serum cholesterol between CS-treated groups and the controls in June. This observation may be referred, in part, to the seasonal fluctuations of the serum cholesterol. The remarkable fall of phospholipid as well as cholesterol in both CS-treated groups was noted in May (14 week), without any changes of triglyceride levels. This resulted in a temporary rise of the cholesterol phospholipid ratios in the treated groups. Further prolonged studies should be required before we can discuss this in details.

There are not much data available dealing with the alterations of the serum hexosamine and galactosamine during the course of CS-administration. Our data show that the serum hexosamine in the CS-treated groups progressively increased after the start of the treatment and returned to the initial levels. This may reflect an increase of the absorption of CS. However, no much changes were noticed in the galactosamine levels, indicating an increase of glucosamine following the continuous administration of CS. This finding should be pertinent to the report by Kaplan and Meyer¹⁷⁾ that AMPS with glucosamine were excreted in urine in addition to galactosamine at 5 to 7 weeks following the continuous intravenous injection of CS-B. A presumption was made that urinary excretion of a glucosamine-containing polymer was induced by CS-B injection.

Much of the evidence in respect to the role of arterial thrombosis in atherosclerosis has been reported. In 1948, Duguid⁸⁾ has established a theory upon examination of lesions in the coronary and aorta: the microthrombi can be incorporated into the vessel walls, give rise to initial thickening and form lesions identical with atherosclerosis. Chandler⁹⁾ has described in 1958 a method of a thrombus formation *in vitro*. Poole¹⁸⁾ has successively confirmed the reliability of the method using a modified technique. Present findings will be in accordance with the observations of Poole¹⁸⁾ and Cunningham,¹⁹⁾ who found the prolongation of the thrombus formation time when heparin was added to blood *in*

vitro. Cunningham¹⁹⁾ further observed the delay of thrombus formation time in patients receiving anticoagulant drugs such as warfarin and phenindione.

Recently Morrison and his colleagues²⁰⁾ reported that a single intravenous injection of CS-A at a level of 80 mg./Kg. of body weight prolonged thrombus formation time in rabbits. The prolongation by CS-A injection was related with the concentration and was maximum at 12 hours after the injection. Present results that the delay of thrombus formation time occurs at shorter periods after the intravenous injection of CS-C will be pertinent to the previous findings by Murata²¹⁾ that the physiological activities of chondroitin polysulfate are exhibited shortly after the intravenous injection, when determined by anticoagulant activity, turbidity reducing activity and electrophoretic mobility of the substance. As pointed by Morrison et al.,²²⁾ however, the effects of CS on hyperlipemia or atherosclerosis would be different in species or by amounts used in experiments. Further studies should be necessary to understand the detailed mechanisms of the efficacy of CS as anti-atherogenic agents on the basis of sulfur content or molecular weight.

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