## Effects of Chondroitin Polysulfate on Thrombus-Formation and Serum Lipid Levels

In recent years attention has been focussed on possible roles of glycosaminoglycans in arterial walls in relation to aging and atherogenesis[1-4]. In 1962, MURATA[5] had reported that chondroitin polysulfate (CPS), a galactosaminoglycan which was prepared from chondroitin 6-sulfate, possesses preventive effects on lipemia and atherosclerosis experimentally induced in animals. The CPS also has lipid clearing and anticoagulant properties which have been shown to be at their maximum shortly after intravenous administration[6]. These physiological activities are partly due to the sulfate contents: IR spectroscopic evidence indicated that CPS with 15.7% sulfur shows sulfate groups at least at positions 4 and 6 in the galactosamine moiety[7]. However, the nature of the inhibitory mechanisms of CPS on atherogenesis still remains to be clarified.

The present paper reports the pronounced preventive effects of CPS on the formation of thrombus in vitro judged by both thrombus-formation time[8] and thrombus-weight[9]. The study includes the changes in serum lipids after CPS administration.

*Materials and methods.* Sodium CPS with 15.6% of sulfur was synthetically prepared by sulfation of chondroitin 6-sulfate from shark cartilage[7]. The determination of thrombus-formation time was conducted by a slight modification[10] of the method devised by CHANDLER[11], as follows. The CPS was injected i.v. into albino rabbits at 5–30 mg/kg body weight. The blood was withdrawn from the ear veins before and at the desired times following the injection using siliconized needles and syringes containing 3.8% citrate solution (1:9 v/v) and centrifuged 10 min at a speed of 500 rpm in siliconized tubes. The determination was started by addition of 0.1 ml of 0.25 *M* $CaCl_2$ to 1 ml of plasma and poured into a vinyl tube (27.5 cm in length and 0.3 cm in diameter). The circled tube was immediately revolved at 16 rpm on a slanted turntable at an angle of 45 degrees. Thrombus-formation time was measured when the contraction of fibrin formed a white mass at the advancing edge of the tube. In addition, the thrombus-weight was measured after being fixed with 10% formalin. For serum lipid determination, a number of rabbits were daily fed cholesterol-containing diet at the rate of 200 mg/kg body weight for 4–8 weeks.

*Results and discussion.* The data obtained here are illustrated in the Table which shows that thrombus-formation was progressively delayed when the concentration of CPS rose and, moreover, thrombus-weight was appreciably reduced. A dose of 5 mg CPS/kg body weight caused slight, if any, changes, but doses greater than 10 mg CPS/kg body weight caused remarkable prolongation of thrombus-formation time and significant reduction of thrombus-weight over pre-injection levels during $^1/_2$ to 6 h after the injection, being slightly effective for a further 6 h. The activity was found to be 1/10–1/30 of


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Effects of chondroitin polysulfate on thrombus-formation time, thrombus-weight and serum triglyceride levels

| Amount of chondroitin polysulfate (per kg body weight) | No. of cases | Before | Time after single i.v. injection 30 min | 60 min | 3 h | 6 h |
|---|---|---|---|---|---|---|
| | Thrombus-formation time (min) | | | | | |
| 30 mg | 18 | 14.7 ± 1.4 | 113.5[b] ± 20.8 | 56.5[b] ± 9.4 | 27.7[a] ± 4.6 | 21.2[a] ± 2.4 |
| 10 mg | 13 | 14.6 ± 1.9 | 68.1[b] ± 11.8 | 50.7[b] ± 12.9 | 22.7 ± 5.3 | 22.4 ± 4.3 |
| 5 mg | 6 | 14.2 ± 2.4 | 23.0 ± 3.9 | 18.5 ± 1.6 | 14.5 ± 1.3 | – |
| Heparin 1 mg | 6 | 17.0 ± 1.5 | 99.7[b] ± 12.0 | 45.6[a] ± 16.7 | 17.8 ± 4.7 | – |
| | Thrombus-weight (mg) | | | | | |
| 30 mg | 8 | 19.5 ± 2.4 | 1.3[b] ± 0.9 | 4.1[b] ± 3.6 | 13.1 ± 3.6 | 17.6 ± 5.1 |
| 10 mg | 7 | 19.9 ± 4.5 | 4.7[a] ± 3.8 | 5.3[a] ± 1.3 | 14.8 ± 5.3 | 17.6 ± 5.7 |
| 5 mg | 4 | 22.0 ± 8.1 | 18.0 ± 2.9 | 17.0 ± 6.2 | 18.6 ± 4.1 | – |
| Heparin 1 mg | 6 | 21.0 ± 3.8 | 2.3[b] ± 1.2 | 7.2[a] ± 2.5 | 18.7 ± 2.4 | – |
| | Serum triglyceride (mg/100 ml) | | | | | |
| 30 mg | 7 | 87.4 ± 13.3 | 27.1[b] ± 9.3 | 20.0[b] ± 4.9 | 28.5[a] ± 11.7 | 45.0 ± 17.7 |

Figures represent means and the standard errors. Comparisons were made between the mean values and the corresponding initial mean values. Significant difference was expressed as follows: [a] $p < 0.05$. [b] $p < 0.01$.

that of sodium heparin (150 U/mg) when it was injected i.v. 0.5–1 mg/kg body weight. The duration of the activity of heparin was somewhat shorter than that of CPS. No appreciable side effects of CPS were observed at the concentrations used here.

The serum lipid levels after the CPS injection at a dose of 30 mg/kg body weight were determined in 7 cases for triglyceride, total cholesterol and phospholipid by the same method as reported in a previous paper[10]. It is surprising to note that a striking suppression of triglyceride levels was demonstrated in the serum taken $^1/_2$, 1 and 3 h after CPS administration (Table). The suppression appears to be coincidental with the inhibitory effect of CPS on thrombus-formation. Only a slight reduction was observed in total cholesterol levels after injection when initial levels were lower than 310 mg/100 ml. However, when an initial value of total cholesterol was as high as 670 mg/100 ml, a considerable suppression occurred: ranging in value from 304 mg/100 ml to 573 mg/100 ml during 1–9 h after injection. Approximately 20% reduction was found in phospholipid during 1–9 h after injection when initial levels were 95 mg/100 ml to 125 mg/100 ml. In a case which showed 286 mg/100 ml of an initial phospholipid level, it was reduced in range of 138 mg/100 ml to 223 mg/100 ml after the treatment. No appreciable changes were shown in thrombus-formation and lipid levels after i.v. injections of an equivalent amount of physiological saline.

The duration of the effects of CPS was similar to those of the physiological properties reported previously[6]. The serum hexosamine and galactosamine levels, determined by the methods of BOAS[12] and CESSI[13] respectively, reached their maximal levels immediately after injection and decreased to initial levels within 3 h.

The present results clearly demonstrate that CPS interferes with thrombus-formation in relation to reduction of serum lipid levels, triglyceride level in particular. Further studies indicated that the inhibitory effects of CPS are associated with the sulfate contents: less inhibition is noticed with lower sulfate amounts.

In connection with antilipemic and anti-atherogenic activities of CPS[5], the present data would be correlated with our recent study that CPS inhibits lipid synthesis, particularly neutral fat synthesis, in cultured chick aortic cells[14]. These physiological activities of CPS should be of interest in our more recent findings that over-sulfated chondroitin sulfate (molar ratio of uronic acid to sulfur 1.00:2.04) exists in human aortic tissue[4,15]. Significance of the presence of the over-sulfated chondroitin sulfate in vascular tissue is not clear. However, these observations suggest that the over-sulfated chondroitin sulfate, which would possess an anti-thrombogenic property, may play important roles in atherogenesis[16].

*Zusammenfassung.* Chondroitinpolysulfat führt nach i.v. Injektion bei Kaninchen kurzfristig zu Verlängerung der Thrombusbildungszeit und zu einer Verminderung des Thrombusgewichtes. Möglicherweise besteht eine Beziehung zwischen der Hemmung der Thrombusbildung und der reduzierenden Wirkung von Chondroitinpolysulfat auf Serum Triglyzerid.

K. MURATA, K. IZUKA and T. FURUHASHI

*Department of Physical Therapy and Medicine, Faculty of Medicine, University of Tokyo, and Seikagaku Kogyo Co., Kurihama, Yokosuka (Japan),*
*30 January 1969.*

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[16] Acknowledgments. We are grateful to Prof. Y. OSHIMA for his interest and advice in this study. We are also indebted to Dr. T. OKUYAMA for supplying chondroitin polysulfate and to Miss K. OKUBO for skilful assistance.

## Über die Wirkung von Sr-Ionen auf den $Ca^{2+}$-Austausch am Meerschweinchenvorhof

REUTER und SEITZ[1] haben Veränderungen im $^{45}Ca$-Efflux von Meerschweinchenvorhöfen unter der Annahme einer Carrier-vermittelten Austauschdiffusion quantitativ beschrieben. Danach sollen Ca- und Na-Ionen um Bindungsstellen des hypothetischen Carriers an der Aussenseite der Myokardzellmembran konkurrieren. Um den Austauschmechanismus weiter zu kennzeichnen, wird in der vorliegenden Arbeit die Wirkung extrazellulärer Sr-Ionen auf den $Ca^{2+}$-Austausch am Vorhof des Meerschweinchenherzens untersucht.

*Methodik.* Die Versuche wurden an linken Meerschweinchenvorhöfen durchgeführt. Die Zusammensetzung der Lösungen ist in der Tabelle aufgeführt. Je nach Versuchsbedingungen enthielten die Lösungen zusätzlich 1,8 bzw. 7,2 m$M$/l $CaCl_2$ und/oder äquimolare Mengen $SrCl_2$. Die Versuchstemperatur betrug 35 °C. Die Methodik der $^{45}Ca$-Abgabe-Experimente und der Aktivitätsmessung wurde bereits beschrieben[1].

In den $^{45}Ca$-Aufnahme-Experimenten wurden die Vorhöfe in Tyrodelösung mit 1,8 m$M$/l $CaCl_2$ 30 min bei 35 °C äquilibriert. Eine Gruppe von Präparaten wurde in $^{45}Ca$-haltige, $Na^+$-freie Lösung eingebracht, die 1,8 oder 7,2 m$M$/l $CaCl_2$ enthielt. Eine zweite Gruppe kam in $^{45}Ca$-haltige, $Na^+$-freie Lösung mit zusätzlich 1,8 oder 7,2 m$M$/l $SrCl_2$. Die spezifische Aktivität beider Lösungen war gleich. Zu festgelegten Zeitpunkten wurden die Vorhöfe den aktiven Lösungen entnommen. Die weitere Aufarbeitung erfolgte nach Angaben von REUTER und SEITZ[1]. Von der gesamten $^{45}Ca$-Aktivität der Präparate wurde der auf den Extrazellulärraum (30% des Feuchtgewichtes[2]) entfallende Anteil abgezogen.

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