β_2 -Glycoprotein I in Rheumatoid Arthritis

SHIRO KOSAKA

*The First Department of Internal Medicine, Aomori Prefectural
Central Hospital, Aomori 030*

KOSAKA, S. *β_2 -Glycoprotein I in Rheumatoid Arthritis*. Tohoku J. exp. Med., 1977, 122 (3), 223-228 — Serum β_2 -glycoprotein I of the patients with rheumatoid arthritis was studied by means of single radial immunodiffusion method. There was a significant lowering of β_2 -glycoprotein I concentration in patients with rheumatoid arthritis. An inverse proportional correlation was seen between the concentrations of β_2 -glycoprotein I and of α_1 -antitrypsin, and between the former and C-reactive protein (CRP) rates in individual specimens. Slightly positive relationship was observed between the concentrations of β_2 -glycoprotein I and of α_2 -HS glycoprotein. The β_2 -glycoprotein I concentrations in healthy adults were significantly higher than those of previous reports — serum β_2 -glycoprotein I; rheumatoid arthritis

In the past decade, several new proteins were isolated from the β -globulin fraction of human serum, e.g., β_2 -glycoprotein I (Schultze et al. 1961; Haupt and Heide 1966), β_2 -glycoprotein II (Haupt and Heide 1965) and β_2 -glycoprotein III (Schwick et al. 1968). They can be distinguished by certain physico-chemical and immunological properties.

In 1961 Schultze and associates demonstrated the presence of so far unknown β_2 -globulin which they called β_2 -glycoprotein I. This is a low molecular weight protein (molecular weight, 40,000) with a sedimentation constant (S_{20}°, W) of 2.9 (Schultze et al. 1961). This molecule consists of a protein moiety (82%) and carbohydrate chains composed of hexoses (6.7%), acetyl hexosamine (5.8%), sialic acid (4.4%) and fucose (0.2%) (Schultze et al. 1961). In contrast to its heterogeneity in starch gel electrophoresis, there is only one precipitation line in immunoelectrophoresis which proves the immunological uniformity of the individual components. Purified β_2 -glycoprotein I (Haupt and Heide 1966) has been used for the production of specific antisera and for the construction of calibration curves, thus making possible its quantitative immunological determination.

Although there have been several reports on the genetic analysis and the population frequency of serum β_2 -glycoprotein I concentration, little is known about the biological function as well as the pathological condition of this protein. In the present study, the concentrations of β_2 -glycoprotein I were determined and were compared with other glycoproteins belonging to α -globulin fraction and C-reactive protein (CRP) in the sera of patients with rheumatoid arthritis.

MATERIAL AND METHODS

Determination of β_2 -glycoprotein I in serum was carried out by the single radial immunodiffusion method as follows (Mancini et al. 1965): 10 ml of warm 1.25% agar gel were mixed with 0.25 ml of anti- β_2 -glycoprotein I rabbit serum (Behringwerke/Marburg) and poured on a Partigen plate to give a 2 mm thick layer. After congelation, circular wells with a diameter of 2 mm were punched out. Exactly 2 μ l of test serum diluted 1:3 were introduced into each of the wells by means of a micropipette. After the plate was incubated for 48 hr at room temperature, the formation of a visible ring of precipitate around the wells was examined. The β_2 -glycoprotein I concentration was estimated from the calibration curve which was designed for a series of dilution of standard human serum (Behringwerke) in reference to variations of the ring diameter.

Serum concentrations of α_1 -antitrypsin, α_2 -HS-glycoprotein and α_1 -acid glycoprotein were simultaneously measured with Partigen plate kit (Behringwerke) according to the procedure of single radial immunodiffusion method. In order to quantify the CRP, commercially available anti-CRP prepared from sheep and goat (Hyland Lab./Los Angeles) was used.

RESULTS

 β_2 -Glycoprotein I concentrations in sera from healthy persons

Serum samples of controls were taken from 43 males and 46 females at the age of 20 to 25.

The β_2 -glycoprotein I levels of these cases were in the range between 21 and 52 mg/100 ml, and the values seemed to represent the normal range. The average values for males and females were 35 mg/100 ml and 30 mg/100 ml, respectively. Thus the average value for the males seems to be somewhat higher than that for the females. No case with a β_2 -glycoprotein I concentration above 38 mg/100 ml was found in females (Fig. 1).

Fig. 1. β_2 -Glycoprotein I concentrations in healthy controls. Open column for male; shaded column for female.

 β_2 -Glycoprotein I concentrations in sera from patients with rheumatoid arthritis

In 65 cases of classical or definite rheumatoid arthritis which were composed of 18 males and 47 females, the β_2 -glycoprotein I concentrations were between 18 and

Fig. 2. β_2 -Glycoprotein I concentrations in patients with rheumatoid arthritis. Open column for male; shaded column for female.

42 mg/100 ml with an average of 28 mg/100 ml and were below 30 mg/100 ml in most of the patients (Fig. 2). This indicates that the values were significantly decreased in patients with rheumatoid arthritis as compared with the normal healthy persons ($p < 0.01$).

Relationship between β_2 -glycoprotein I and other glycoprotein in rheumatoid arthritis

Kosaka and Kitabatake (1970, 1971) reported significant increases in α_1 -antitrypsin and α_1 -acid glycoprotein concentrations, and a decrease in α_2 -HS glycoprotein concentration in the sera of patients with rheumatoid arthritis.

There was an inverse high correlation between β_2 -glycoprotein I and α_1 -

Fig. 3. Correlation between β_2 -glycoprotein I and α_1 -antitrypsin.

Fig. 4. Correlation between β_2 -glycoprotein I and α_2 -HS glycoprotein.

Fig. 5. Correlation between β_2 -glycoprotein I and α_1 -acid glycoprotein.

antitrypsin but was a low correlation between β_2 -glycoprotein I and α_2 -HS glycoprotein, showing -0.29 of coefficient of correlation for α_1 -antitrypsin and 0.20 for α_2 -HS glycoprotein (Figs. 3 and 4). On the other hand, there was no definite relationship between β_2 -glycoprotein I and α_1 -acid glycoprotein (Fig. 5).

Relationship between β_2 -glycoprotein I and CRP in rheumatoid arthritis

CRP tests were performed in 54 cases. An inverse correlation was found between the β_2 -glycoprotein I levels and degrees of the CRP rates, since the β_2 -glycoprotein I concentrations for the positive group of CRP were significantly lower than for the negative group of CRP ($p < 0.001$, Fig. 6).

Fig. 6. Correlation between β_2 -glycoprotein I and C-reactive protein.

DISCUSSION

Cleve (1968) showed that, although the concentration of β_2 -glycoprotein I in serum is influenced by factors like age, diseases (e.g., chronic liver diseases such

as liver cirrhosis) and pregnancy, the individual levels of this protein are also controlled by genetic factors. The results of family studies suggested that these concentrations are controlled by a pair of autosomal co-dominant alleles: Bg^N and Bg^D . Bg^N and Bg^D with individual homozygous for the common allele Bg^N were found to have β_2 -glycoprotein I levels between 16 and 30 mg/100 ml, and heterozygous ones between 6-4 mg/100 ml. Individuals homozygous for the allele Bg^D showed deficiency of β_2 -glycoprotein I (less than 5 mg/100 ml). Haupt et al. (1968) have also demonstrated such a case.

Apart from the original data of Cleve (1968) and Cleve and Rittner (1969), there were observed population frequencies for several Caucasoid, Mongoloid and Negroid populations by Koppe et al. (1970), who showed all samples of Caucasoid origin to have approximately similar mean values, whereas the Negroid and Mongoloid races had a much lower frequency, of the more common Bg^N allele. Subsequently, Atkin and Rundle (1974) reported that the levels of serum β_2 -glycoprotein I were estimated on 381 healthy adult subjects of English origin and the frequency of Bg^N gene was found to be 0.941 (s.e. 0.09), which is in close agreement with pooled data of Caucasian subjects of 0.937 introduced by Koppe et al. (1970).

Cleve (1968) demonstrated that serum concentrations of β_2 -glycoprotein I in 94% of healthy persons varied from 16-30 mg/100 ml with a mean value of 21.3 ± 3.6 mg/100 ml, whereas the lower concentrations were found in 6% of the sera: $\bar{x} = 10.0 \pm 1.3$ mg/100 ml. Similar observations were published by Cohnen (1970), who found 22.6 mg/100 ml for a mean value of the serum concentrations in healthy adults. However, the normal values of the healthy Japanese adults in the present study are remarkably higher than those in the previous reports. On the contrary, it is of interest that Koreans belonging to the same oriental as Japanese have considerably low β_2 -glycoprotein I concentrations in their sera (Koppe et al. 1970).

Besides the genetic mechanism, the concentration of β_2 -glycoprotein I is influenced by other factors such as age, sex and various pathological conditions. In newborn serum, the concentration is about a half of that of adults (Cleve 1968). Men are reported to have slightly higher β_2 -glycoprotein I levels than women (Cleve 1968). This tendency corresponds to the present finding.

Serum concentrations of β_2 -glycoprotein I are significantly decreased in the patients with rheumatoid arthritis as compared with the healthy control persons in the present study. This result did not agree with the earlier observations by Cleve (1968), who showed values corresponding to the normal range in 25 patients with active rheumatoid arthritis. It is the known fact that the α_1 -antitrypsin and α_1 -antichymotrypsin belong to the group of the acute phase proteins which respond to inflammatory stimuli with a substantial rise in concentration (Kueppers 1968; Aronsen et al. 1972; Kosaka and Tazawa 1976). In rheumatoid arthritis, CRP is present in practically all patients with clinical evidence of disease activity. It tends to parallel the sedimentation rate closely (Ziff and Baum 1966). Because β_2 -glycoprotein I levels are inversely proportional to α_1 -antitrypsin concentrations

and CRP rates in rheumatoid arthritis, the present findings suggest that this new glycoprotein has also some relation with the acute phase protein and the activity of the disease in the patients.

Acknowledgment

I wish to thank Prof. T. Sugiyama, Narugo Branch of Tohoku University School of Medicine, for his kind advice.

References

- 1) Aronsen, K.F., Ekelund, G., Kindmark, C.O. & Laurell, C.B. (1972) Sequential changes of plasma proteins after surgical trauma. *Scand. J. clin. Lab. Invest.*, **29**, Suppl. 124, 127-136.
- 2) Atkin, J. & Rundle, A.T. (1974) Serum β_2 -glycoprotein I phenotype frequencies in an English population. *Humangenetik*, **21**, 81-84.
- 3) Cleve, H. (1968) Genetic studies on the deficiency of β_2 -glycoprotein I of human serum. *Humangenetik*, **5**, 294-304.
- 4) Cleve, H. & Rittner, C. (1969) Further family studies on the genetic control of β_2 -glycoprotein I concentration in human serum. *Humangenetik*, **7**, 93-97.
- 5) Cohnen, J. (1970) Immunochemical quantitation of β_2 -glycoprotein I in various diseases. *J. Lab. clin. Med.*, **75**, 212-216.
- 6) Haupt, H. & Heide, K. (1965) Isolierung und Eigenschaften eines β_2 -Glykoproteins aus Humanserum. *Clin. chim. Acta*, **12**, 419-424.
- 7) Haupt, H. & Heide, K. (1966) Isolierung und Kristallisation von β_2 -Glykoprotein I aus Humanserum. *Clin. chim. Acta*, **14**, 418-421.
- 8) Haupt, H., Schwick, H.G. & Störko, K. (1968) Über einen erblichen β_2 -Glykoprotein I-Mangel. *Humangenetik*, **5**, 291-293.
- 9) Koppe, A.L., Walter, H., Chopra, V.P. & Bajatzadeh, M. (1970) Investigations on the genetics and population genetics of the β_2 -glycoprotein I polymorphism. *Humangenetik*, **9**, 164-171.
- 10) Kosaka, S. & Kitabatake, T. (1970) α_1 -Antitrypsin and α_1 -acid glycoprotein in rheumatoid arthritis. *Ryumachi (Jap.)*, **10**, 143.
- 11) Kosaka, S. & Kitabatake, T. (1971) α_2 -HS Glycoprotein in rheumatoid arthritis. *Ryumachi (Jap.)*, **11**, 134.
- 12) Kosaka, S. & Tazawa, M. (1976) α_1 -Antichymotrypsin in rheumatoid arthritis. *Tohoku J. exp. Med.*, **119**, 369-375.
- 13) Kueppers, F. (1968) Genetically determined differences in the response of alpha I-antitrypsin levels in human serum to typhoid vaccine. *Humangenetik*, **6**, 207-214.
- 14) Mancini, G., Carbonara, A.O. & Heremans, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235-254.
- 15) Schultze, H.E., Heide, K. & Haupt, H. (1961) Über ein bisher unbekanntes niedermolekulares β_2 -Globulin des Humanserums. *Naturwissenschaften*, **48**, 719.
- 16) Schwick, H.G., Haupt, H. & Heide, K. (1968) Isolierung und charakterisierung von β_2 -Glykoprotein III aus Humanserum. *Klin. Wschr.*, **46**, 981.
- 17) Ziff, M. & Baum, J. (1966) Laboratory findings in rheumatoid arthritis. In: *Arthritis and Allied Conditions*, edited by J.L. Hollander, Lea & Febiger, Philadelphia, p. 236.