The "So-Called" Somatomedin B and Growth Hormone Measured by Radioimmunoassay in Normal and Cirrhotic Individuals

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Summary

Serum somatomedin B (SmB) levels in cirrhotic individuals, 3.3 ± 1.5 mg/l, were strikingly lower (P < 0.001) than in normal subjects, 9.0 ± 1.7 mg/l. SmB levels were clearly related to the levels of α1-globulins in the cirrhotics (r = + 0.8, P < 0.002). Serum SmB and growth hormone correlated negatively in a group of normal and cirrhotic individuals (r = -0.67, P < 0.001). Direct measurements of serum SmB failed to reveal differences between hepatic, renal and peripheral veins. These findings suggest that: 1) SmB is produced by liver and/or normal liver function plays an important role in maintaining normal serum SmB levels; 2) SmB carrier proteins are reduced in the cirrhotics and 3) SmB is part of a negative feed-back system involving growth hormone.

Key-Words: Somatomedin – Growth Hormone – Carrier Proteins – Feed-Back – Liver Cirrhosis

Introduction

The "so-called" somatomedin B (SmB) a protein of MW 5000, was identified during studies dealing with somatomedin fractionation from outdated human plasma (Uthne 1973). It was later purified (Fryklund, Uthne, Sievertsson and Westermark 1974) and its primary structure determined by Fryklund and Sievertsson (1978). Initially it was characterized by its property of stimulating the growth (Uthne 1973) of human glial cells in culture, which was, however, later demonstrated to be due to contaminating epidermal growth factor (Heldin, Wasteson, Fryklund and Westermark 1981). In addition, SmB does not stimulate cartilage (Uthne 1973) and has no insulin-like activity (Sievertsson, Fryklund, Uthne, Hall and Westermark 1975). Therefore, although SmB levels are dependent upon growth hormone (GH) status (Yalow, Hall and Luft 1975), there is not sufficient evidence to justify the term somatomedin for this substance (Heldin et al. 1981).

SmB is structurally related to small trypsin inhibitors and, indeed, it is able to inhibit trypsin action (Fryklund and Sievertsson 1978). Nonetheless, SmB function and role is presently unknown.

Clinically, SmB levels are high in acromegaly and superposable to the normal population in growth hormone deficient patients (Rudd, Rayner, Schalbe and Boddem 1979; Wajchenberg, Liberman, Gomes and Pieroni 1980). The levels are high in pregnancy but low in cord blood and neonates, indicating that little, if any, transplacental passage of immunoreactive SmB occurs (Svan, Hall, Riten, Takano and Skottner 1977). It was found to be high in Turner's Syndrome, for reasons that remain unknown (Benker, Spira, Zah, Tharandt, Hackenberg and Reinwein 1979). SmB is specific to primate sera and the highest concentrations are found in humans, where it circulates bound to carrier proteins (Yalow, Hall and Luft 1975).

Radioceptor assays for SmB failed to demonstrate any binding with various rat and monkey tissues (Takano, Hall, Fryklund and Sievertsson 1976).

Thus, despite a series of biochemical and clinical studies concerning SmB, almost nothing can be inferred at present with regards to basic questions such as the regulation of physiological levels and the organ(s) in which it is synthesized.

The present investigation into the role of the liver in the production of SmB was motivated by the fact that liver has GH receptors (Carr and Friesen 1976) and SmB shows GH dependency and circulates bound to carrier proteins.

The results reported here include analyses of the sera of 14 cirrhotic patients for SmB and GH content, and parameters of liver function. Sera from hepatic, peripheral and renal veins of 20 normal individuals were also analyzed for SmB.

Material and Methods

The first group included fifty-nine individuals, of which forty-five were normal controls and fourteen patients, the latter with liver cirrhosis diagnosed by clinical, laboratory and biopsy findings. Surface antigen or antibody of hepatitis B virus were positive in 4 of 8 patients tested. Alcoholism occurred in 6 of the 14 cases. Blood samples were obtained after an overnight fast. In a second group, 20 individuals, which were subjected to haemodynamic exploration for diagnostic purposes, had hepatic, renal and peripheral venous blood collections to measure SmB.

Serum SmB was determined by radioimmunoassay using a Kabi Diagnostica (Stockholm, Sweden) kit. Additional reagents for the SmB radioimmunoassay were kindly provided by Dr. L. Fryklund of Kabi. Rabbit second antibody was prepared by Dr. V.C. Borghi at IPEN. For the cirrhotics and normal controls the assays were developed using SmB labelled according to the lactoperoxidase
procedure (Thorell and Johansson 1971) as recommended in the kit. For the hepatic, renal and peripheral venous serum determinations, the specific activity of SmB was increased from 50 to 260 μCi/μg by increasing the quantity of 111In (2 mCi instead of 0.5 mCi) and decreasing that of 5mB (1 μg instead of 2 μg) in the labelling reaction. With these modifications, the intra-assay coefficient of variation (C.V.) decreased from 9% to 5%. Each sample was quantified in triplicate.

Serum growth hormone was determined by radioimmunoassay using human growth hormone prepared in this laboratory (Bartolini, Assis, Schwarz and Pieroni 1977). The separation of bound and free hormone was accomplished with PEG (Desbuquois and Aurbach 1971). For the correlation studies, the levels of GH and SmB were determined intra-assay (C.V. < 10%) for both the cirrhotic patients and a matched group of fourteen normal individuals. The SmB values of the remaining normal controls were derived from inter-assay determinations (C.V. < 20%).

Standard laboratory methods were used to determine total protein (T. Prot), serum albumin (Alb), α1, α2, β and γ-globulins, total bilirubin (T. Bil), direct bilirubin (D. Bil), indirect bilirubin, (I. Bil.), alkaline phosphatase (Alp), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

GH and SmB levels of normals and cirrhotics were statistically analysed respectively by the Mann-Whitney non-parametric U-test and the Student's t-test. The significance of the differences between the means of SmB of hepatic, peripheral and renal veins, were assessed by variance analysis.

**Results**

The levels of SmB and GH in normal subjects and cirrhotic patients are presented in Figure 1. The mean ± SD serum SmB concentration was 9.0 ± 1.7 mg/l in the normal controls, with no significant difference between males and females. The SmB concentration in the cirrhotic patients, 3.3 ± 1.5 mg/l, was significantly lower (P < 0.001) than in normal subjects. On the other hand, serum GH in the cirrhotic patients was significantly higher than in 14 age- and sex-matched normal individuals (α < 0.01).

The clinical and laboratory findings for the cirrhotic patients are shown in Table 1.
The SmB and GH results were correlated with the biochemical findings for the cirrhotic patients (see Table 2). Regarding serum proteins, SmB correlated positively with \( \alpha_2 \) - and \( \beta \)-globulins, whereas GH correlated negatively with these indices. GH also correlated negatively with total protein. With the bilirubins, there was a positive correlation only between GH and total bilirubin. In relation to the enzyme activities, GH correlated positively with aspartate aminotransferase and alanine aminotransferase, whereas SmB correlated negatively with the latter. Thus, in most cases SmB and GH correlated inversely in relation to the various indices of hepatic function (see Table 2).

The correlation between SmB and GH was negative for both cirrhotic patients (r = -0.74, P < 0.003) and normal controls (r = -0.59, P < 0.05). The combined results for both groups (r = -0.67, P < 0.001) are shown in Figure 2.

Serum SmB levels in the hepatic vein, 9 ± 2.3 mg/l, renal vein, 9.5 ± 1.8 mg/l and peripheral vein, 9.9 ± 2.9 mg/l, of 20 individuals were not significantly different. There was also no evidence of an overall distribution pattern for relative SmB levels in the 3 veins of any given individual.

The diagnoses of the fourteen cirrhotic patients were confirmed by liver biopsy. All presented very low levels of albumin, pointing to extensive loss of liver function (MacDonald, Taylor, Johnstone, Walsh and Bogoch 1973). In addition, almost all patients had signs indicative of ongoing hepatocyte destruction, as evidenced by high levels of AST and ALT enzyme activities (MacDonald et al. 1973). Bilirubins were also elevated, indicating inadequacy of liver cell function (Sherlock 1975).

The mean levels of SmB in the cirrhotics was reduced about 3-fold relative to the controls. On an individual basis, the level in the cirrhotics appeared to be related to the intensity of the ongoing hepato-cellular lysis, the cases with the higher levels of ALT, AST and total and direct bilirubin systematically falling into the group with the lower values of SmB. This relationship between higher indices of hepatocyte destruction and lower SmB levels is confirmed by the negative correlation between ALT and SmB. Thus, the present findings suggest that SmB may be produced or regulated in the liver.

The strong positive correlation between immunoreactive SmB and \( \alpha_2 \)-globulins values (r = +0.8, P < 0.002) is of particular interest. From paper electrophoretic analysis of normal, hypopituitary and acromegalic sera, Yalow, Hall...
The higher GH levels in the cirrhotics confirms previous
results in the literature (Conn and Daughaday 1970; Wu, Grant, Hambley and Levi 1974; Schimpf, Lebrec and Donnadieu 1977; Borzio, Caldara, Ferrari, Barbieri, Borzio and Romussi 1981). The correlations in Table 2 further indicate that these levels are highest in the cases with the most severe liver dysfunction and hepatocyte injury. In addition, a negative correlation was found between GH and SmB suggesting the existence of a negative feed-back system between GH and SmB.

An attempt to obtain more direct evidence of liver SmB production was made by measuring SmB levels in hepatic, peripheral and renal veins. However, with the method used there was no evidence of significant difference between the results. A cell culture system which permits measurement of SmB in liver explants could eventually resolve the question as to whether SmB is synthesized by liver.

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References


Takano, K., K. Hall, L. Fryklund, H. Sievertsson: Binding of somatomedins and insulin to plasma membranes prepared from rat and monkey tissue. Horm. Metab. Res. 8: 16–24 (1976)


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