The human urinary gastric inhibitor displays blood-group activity

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Summary. The human urinary glycoprotein with gastric antisecretory activity displays a marked blood-group activity corresponding to the blood-group of the subject examined. Several studies exclude the possibility that the activity is due to contaminants.

During the last few years, a human urinary glycoprotein with gastric antisecretory activity (human urinary gastric inhibitor or HUGI) has been isolated and partially characterized[1,2].

The antisecretory activity, quite relevant in the rat, is extremely high when assayed in the Heidenhain pouch dog, where about 0.25 µg glycoprotein/kg i.v. causes a 50% reduction of the acidic gastric secretion induced by proteic meal[3].

The urinary origin, and the fact that the carbohydrate content is high (37%) and similar to that of blood-group glycoproteins[4,5], prompted us to test if the HUGI displays blood-group activity.

Material and methods. a) Purification of the human urinary gastric inhibitor (HUGI). The purification procedure[1] was as follows: chromatography of urine through a column of IONAC A-540 (Hellige, Inc.) and benzoic acid extraction (step 1), fractionated precipitations with organic solvents (step 2), gel filtration on Sephadex G-200 or Bio Gel A-1.5 m (step 3). HUGI was isolated from pooled urines of several individuals as well as from urine of single individuals.

b) Analytical methods. Aminoacid and carbohydrate analyses were performed as previously reported[1].

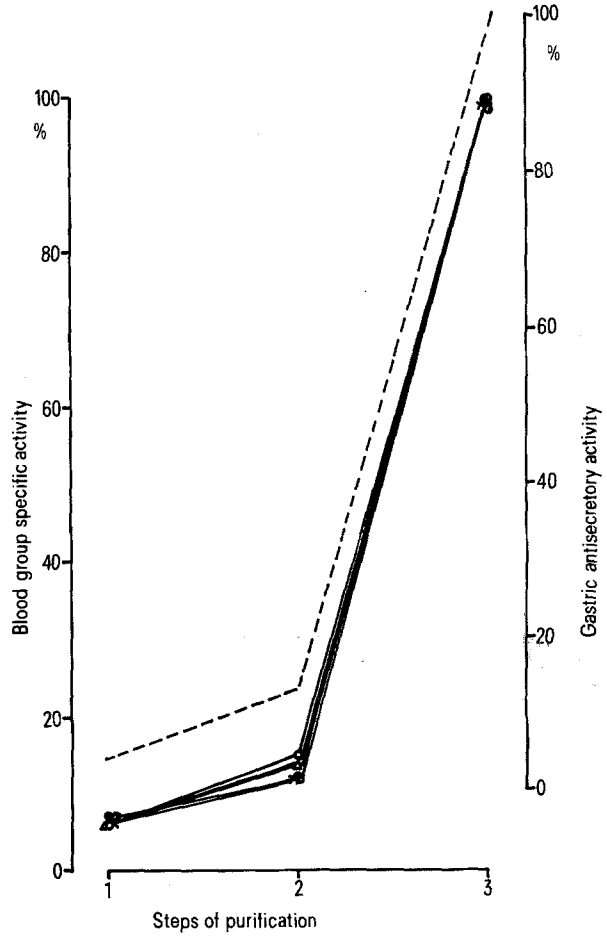
c) Gastric antisecretory activity determination. The assay was performed in pylorus-ligated rats, as previously described[6]. The antisecretory activity is defined as the amount of HUGI (in µg) i.v. which causes 50% reduction (ED_{50}) of the gastric secretion volume per kg body weight.

d) Blood-group activity determination. The presence of group specific activity (A, B, Lewis, M, N, S, s, P_1 and 1) was checked by the haemagglutination inhibition test[7] using human agglutinating anti-sera and red blood cells carrying the corresponding antigen. For the determination of the H-antigen-like activity, anti-H lectin *(Ulex europaeus)* and 0 target red blood cells were used. Positive and negative controls were run in parallel using human A and B substance and saline respectively.

Serial 2-fold dilutions in saline of HUGI were prepared. 30 µl of these preparations were mixed with an equal volume of antiserum at twice the concentration necessary to agglutinate completely the target cells.

The glycoprotein-antibody mixtures were incubated for 30 min at room temperature (for A, B, Lewis, M, N, S, s, P_1 and H-like activity) or at 4 °C (for I-like activity), then the appropriate detector cells were added. After an additional 1-h incubation at the same temperature, the tubes were read for macro- and microscopic agglutination. Blood-group activity is defined as the amount of HUGI (µg/30 µl) which causes 100% inhibition (ED_{100}) of haemoagglutination.

Results and discussion. Purified HUGI was examined for A, B, H, Lewis, I, M, N, S, s and P_1 blood-group activities by the haemagglutination inhibition technique. A marked



Specific blood-group activity (●, A; ○, B; □, H; △, Le^a; ◐, Le^b; ×, I) and gastric antisecretory activity (-------) of the human urinary gastric inhibitor at different steps of purification. Both the specific blood group activity and the antisecretory activity are expressed as 100% of step 3.

Table 1. Gastric antisecretory activity and A, B, H, Le^a, Le^b and I blood group activity of human urinary gastric inhibitor (HUGI) at various purification steps, starting from pooled 100 l urine

Step	Dry weight (g)	Carbohydrate content (g)	Antisecretory activity (ED_{50}, in µg/kg)	Blood group activity (ED_{100} in µg)					
				A	B	H	Le^a	Le^b	I
1	4.53	-	2530	43	37	150	90	75	100
2	1.89	0.270	917	25	19	75	45	37	43
3	0.22	0.081	127	3	4	9	5	4	6

cross reactivity with blood-group A, B, H, Lewis and I substances was detected, while no M, N, S, s and P_1-like activity was found.

Table 1 summarizes the results of haemagglutination inhibition tests performed on the urinary glycoprotein at different stages of purification. It appears that the blood-group activity of the HUGI increases as the gastric antisecretory activity increases. This direct relationship between the two activities is better illustrated in the figure.

The following evidence indicates that blood-group activities are an intrinsic property of HUGI: a) the HUGI appears to be homogeneous by several criteria (C and N-terminal amino acid, ultracentrifugation, sodium dodecyl sulphate polyacrylamide electrophoresis and gel filtration, etc.)[2]; b) the blood-group activity of the purified HUGI is marked (table 1); c) the increase of all the group specific activities found strictly parallels the increase of the gastric antisecretory activity during the various steps of purification. It seems very improbable that the blood-group activities are due to spurious materials. These contaminations should behave in the same way as HUGI, throughout the purification procedure.

Further, when other urinary glycoproteins, as for instance the Tamm-Horsfall glycoprotein which is present in 20–40 mg/l amount, were examined, no blood-group-like activity was detected after the mere precipitation with 0.58 M sodium chloride. The presence of A, B and H blood-group specificity in the same glycoprotein preparation is explained by the fact that the HUGI was obtained from pooled urines of individuals of different blood-group.

When HUGI was purified from urine of a single secretor individual, the activity corresponding to the ABO blood-group of the subject examined was detected. In this case too, a strict parallelism between the increase of antisecretory activity and blood-group activity during the purification was found. These results are reported in table 2.

When HUGI was purified from urines of non-secretor subjects, a strong gastric antisecretory activity was detected in the absence of blood-group specific activity. This observation suggests that the Secretor gene (Se gene) is involved in synthesis of a part of the HUGI molecule. However, neither the presence or the absence of various blood group determinants influences the biological activity of the molecule. The fact that HUGI, which is a compound with a possible pharmacological interest, possesses blood-group specific activities is an occurrence to be considered to avoid anaphylactogenic implications. Together with intestinal disaccaridase[8] and human chorionic gonadotrophin which however displays A activity alone[9], HUGI is the only example of blood-group antigenicity associated with a functioning glycoprotein molecule.

Table 2. A- and B-like blood group activity of HUGI from 2 secretor and 2 non-secretor subjects, respectively

Subjects		Blood group activity (ED_{100} in µg) A	B
Secretors	blood group A	12	absent
	blood group B	absent	17
Non-secretors	blood group A	absent	absent
	blood group B	absent	absent

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Relation between fibrinolytic activity and prostacyclin generation of atherosclerotic artery and dacron prosthetic graft

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Summary. The newly formed tissue of dacron vascular prosthetic grafts implanted in humans demonstrates prostacyclin generation and fibrinolytic activity comparable to that of the atherosclerotic artery in the vicinity. This provides some evidence that both activities important for haemostasis run parallel.

Quantitative studies of fibrinolytic activity of the normal vascular wall[1] and atherosclerotic tissue[2–5] discovered an important self-regulation mechanism of endothelium in haemostasis, which is changed considerably under different metabolic conditions[6]. Since Moncada's group[7,8] first described a new metabolite of arachidonic acid metabolism, prostacyclin (PG I_2), the most potent known endogenous inhibitor of platelet aggregation, which prevents platelet thrombus formation in vivo, the question arose, whether there is a relation between fibrinolytic activity and prostacyclin formation in vascular prosthetic grafts and the arteries in the vicinity. Recently D'Angelo and coworkers[9] demonstrated a comparable diminution of prostacyclin generation and fibrinolytic activity over an atherosclerotic plaque.

Material and methods. The vascular tissue was obtained from the iliac arteries of 8 male human (age: 45–77 years) in the vicinity of dacron vascular grafts, which were removed after being implanted for 6 up to 56 weeks. The fibrinolytic activity was estimated using Todd's fibrinolysis autography technique[10,11] (incubation time 60 min) and the quantitative evaluation described by Fischer[12] (endothelial