Specific activity of adenosine deaminase in patient with 20q- polycythaemia rubra vera

Patient	Sex	Haematocrit %	Red cell mass ml/kg	Percentage of marrow cells 20q	Adenosine deaminase (nmoles/h mg protein) Erythrocyte	Leukocyte	Lymphocyte
1	M	77	59	100	60	2,060	4,450
2	F	50	ND	100	53	913	1,670
3	M	49.5	44	60	97	1,390	2,180
4	F	71	37	0	105	1,950	3,650
Normal	F 37-47 M 42-52		F 22-29 M 25-32		63 ± 24	750 ± 280	2,205 ± 1,170

NP = not performed.

the intercovertibility between the molecular forms of ADA[6,8], suggest that the heterogeneity of ADA in human tissue is the result of modification of a single gene locus product[6,9]. Cell hybridization studies assign this locus to chromosome 20[10]. Because a deletion of the long arm of this chromosome, karyotypically designated as 20q-, occurs in marrow haemopoetic precursors of subgroups of patients with polycythaemia rubra vera and refractory sideroblastic anaemia[11], we thought it would be of interest to determine the activity of ADA and its molecular forms in cells of the peripheral blood of these patients.

Methods. Routine haematological procedures were performed by standard techniques. Bone marrow chromosome prepartions were made by a modification[12] of the direct method of Kiossoglou et al.[13]. The Giemsa banding technique used was according to Summer et al.[14].

Erythrocytes, leukocytes and lymphocytes from heparinized peripheral blood were separated and processed as described previously[5,15]. Adenosine deaminase activity was assayed by the conversion of radiolabelled adenosine to inosine and hypoxanthine[6], and this activity in leukocyte and lymphocyte extracts were chromatographed on Biogel A-0.5 M[5].

Results and discussion. Results are shown in the table. In marrow precursors of 3 of 4 patients with polycythaemia rubra vera studied deletions varying in size from $\frac{1}{3}$ to $\frac{2}{3}$ of the long arm of chromosome 20 were found. 2 patients (No. 1 and 2) had the partially deleted chromosome (20q-) in 100% of cells analysed whilst patient No. 3 showed a mosaic cell pattern, i.e. 60% with the 20q- chromosome and 40% with no abnormalitiy. Patient No. 4 had a completely normal karyotype in all cells examined.

The specific activities of ADA in cells of peripheral blood of all 4 patients were within the normal range and gel filtration of leukocyte and lymphocyte extracts demonstrated the presence of both the 296,000 and 34,000 molecular forms of ADA[5].

In the light of assignment of the locus for the catalytic unit of ADA to chromosome 20[10] the present data are of interest. If the ADA gene was located on the deleted portion of chromosome 20, in the absence of translocation, the ADA activity in peripheral blood erythrocytes would either be absent or markedly reduced. That this is not the case suggest that the locus for human ADA is either on the short arm or close to the centromere on the long arm of chromosome 20.

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Destruction of the platelet aggregating activity of ristocetin A[1]

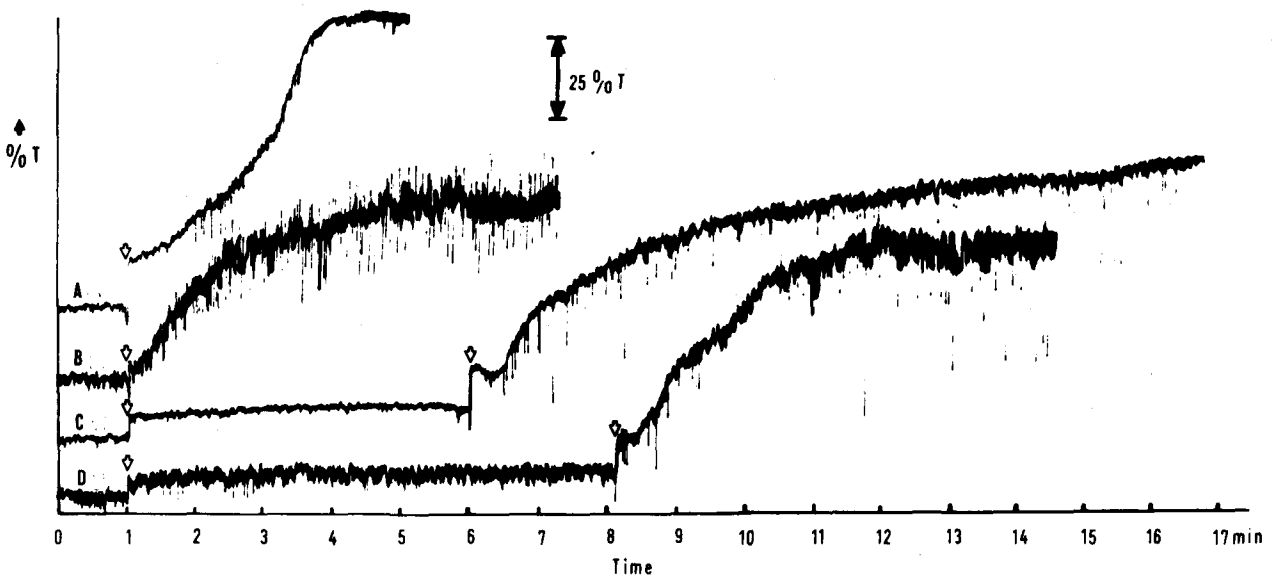
St. S. Kuwahara and W.T. Chambers

Bureau of Disease Control and Laboratory Services, Michigan Department of Public Health, Box 30035, Lansing (Michigan 48909, USA), 29 August 1977

Summary. Hydrolysis of ristocetin A in 0.1 N HCl at 37 °C for 2 h resulted in the loss of its ability to induce platelet aggregation in platelet-rich plasma derived from guinea-pigs and humans. However its antibiotic activity against *Staph. aureus* was not lost.

The glycopeptide antibiotic[2], ristocetin A, underwent extensive testing which demonstrated its efficacy against gram positive organisms[3-7]. Its use as an antibiotic was later discontinued partially as a result of its ability to induce thrombocytopenia and thrombophlebitis[5,8,9]. Howard and Firkin[10] found that platelets in plasmas derived from patients with von Willebrand's disease would not aggregate in its presence. It was later demonstrated that the defect lay in the factor VIII molecules present in plasmas from von Willebrand's patients[11].

While studying the mechanisms of ristocetin induced platelet aggregation, we found that mild acid hydrolysis of



Aggregometer tracings showing: *A* 1.0 mg/ml native ristocetin in diluted human PRP; *B* 1.0 mg/ml 35 min acid-hydrolyzed ristocetin in diluted PRP; *C* 1.0 mg/ml 2 h acid-hydrolyzed ristocetin in diluted human PRP followed by 0.91 mg/ml of native ristocetin; *D* 1.8 mg/ml of 2 h acid-hydrolyzed ristocetin in diluted human PRP followed by 0.91 mg/ml of native ristocetin. Diluted human PRP was prepared by mixing 5 vol. of PRP with 1 vol. of 0.5 M Tris-Cl, pH 7.2, in 0.9% (w/v) NaCl, and 4 vol. of 0.9% (w/v) NaCl. For aggregometry, 0.05 ml of hydrolyzed or native ristocetin was added to 0.45 ml of diluted PRP, except in the case of *D* where 0.1 ml of aggregating agent was added. Open arrows show points at which material was added.

ristocetin destroys its ability to aggregate platelets while allowing the survival of its antibiotic activity.

Material and methods. Ristocetin, containing in excess of 90% ristocetin A, was obtained from Abbott Laboratories (North Chicago, IL; Lots 071 and 57-509-BS) and hydrolyzed by dissolving 100 mg in 5 ml of 1 N HCl. The solution was held at 37 °C for 2 h, and 1 N NaOH was added to neutralize the mixture. This solution contained the equivalent of 10 mg/ml of native ristocetin and was used without fractionation. For intermediate aliquots, 0.1 ml of the HCl solution was mixed with 0.1 ml of 1 N NaOH.

The antibiotic activity of the hydrolysates was determined by broth dilution assays in trypticase-soy medium (BBL; Cockeysville, MD) using 10 fold dilutions. The cultures were inoculated with a 1:100 dilution of a 24-h culture of *Staphylococcus aureus* (ATCC No. 6538P), or a local strain of *Bacillus subtilis* (Strain 5230; Continental Can. Co., Midland, MI). *B. subtilis* cell walls were obtained by sonic disruption followed with washing in 40 mM Tris-Cl, 0.15 M NaCl, pH 7.4 (TBS). Platelet-rich (PRP) and platelet-poor plasmas, containing EDTA, were prepared from 2 male volunteers and from albino guinea-pigs. Their aggregation was studied by a previously described method[12].

Results and discussion. The results (figure) showed that hydrolysis destroyed the ability of ristocetin to induce aggregation in PRP's from humans and guinea-pigs. The activity was usually lost after 1 h, but incubation for 2 h was used to ensure complete hydrolysis. The hydrolysate was unable to induce aggregation when added at final concentrations of 1.0 and 1.8 mg/ml, but the subsequent addition of 0.91 mg/ml of native ristocetin to these mixtures resulted in prompt aggregation. This showed that aggregation was not being inhibited by a component of the neutralized hydrolysis mixtures.

Phillip et al.[13] showed that hydrolyzed ristocetin was a more potent antibiotic than the parent compound. We were able to confirm their results. The growth of *Staph. aureus* and *B. subtilis* was inhibited in the presence of 10 µg/ml of the hydrolysate, but not at 1 µg/ml.

We also confirmed the work of Best and Durham[14] who showed that divalent cations reversed the binding of native and hydrolyzed ristocetin to *B. subtilis* cell walls.

If the side effects associated with the therapeutic use of ristocetin are solely due to its ability to induce platelet aggregation[8,9], our results suggest that a hydrolyzed preparation would be free of these defects while still acting as an effective antibiotic.

With respect to the aggregation of platelets, the work of others combined with our observations show that the ability to induce aggregation is not an intrinsic property of the aglycone. This suggests that mechanisms of ristocetin-induced platelet aggregation, based on analogies with the interaction of ristocetin with bacterial cell walls, cannot be totally correct. Others have used similar procedures for hydrolyzing ristocetin and isolating the aglycone[13–16]. They showed that the aglycone can interact with bacterial cell walls and bind peptides[17,18]. Best and Durham[14] proposed an ionic mechanism for these interactions.

Coller et al.[19] proposed a similar mechanism for the binding of ristocetin to platelet membranes. Coller[20] further proposed that ristocetin is bound by an interaction involving the phenolic residues of the aglycone. This conflicts with the results of Baugh et al.[21] who were unable to find ^{125}I ristocetin in platelet aggregates. Our results also conflict with the proposed mechanisms[19,20]. The procedure used here will cleave glycosidic bonds but should not affect the aglycone. However, the hydrolysate does not induce platelet aggregation, nor does it inhibit the ability of native ristocetin to induce aggregation (figure).

Thus it appears that the electrostatic effects noted by others[19,22] are only a part of the mechanism whereby ristocetin induces aggregation. The present results suggest that the carbohydrate portion of this glycopeptide is important and a recognition of carbohydrate structures is a part of the mechanism.

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Stimulation of methemoglobin reduction by selenium: A comparative study with erythrocytes of various animals

H. Iwata, T. Masukawa, S. Kasamatsu and S. Komemushi

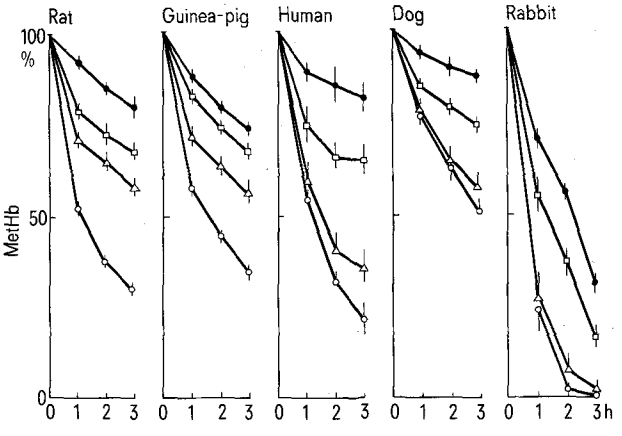
Faculty of Pharmaceutical Sciences, Osaka University, 133-1, Yamada-kami, Suita-shi, Osaka 565 (Japan), 29 September 1977

Summary. The extent of stimulation of methemoglobin (metHb) reduction by selenite depends upon the level of reduced glutathione (GSH) in the erythrocytes. The reason for the species difference in the effect of selenite was discussed with respect to species differences in the GSH levels in erythrocytes.

Recently, we reported that selenite stimulates metHb reduction in rat erythrocytes and that its action may be to catalyze the reduction of metHb by GSH[1,2]. Selenite was shown to accumulate in erythrocytes, where it was metabolized by GSH-dependent systems[3–6]. Thus, the GSH level in erythrocytes is thought to be closely related to the selenite effect. This paper is on the relationship between the effect of selenite on metHb reduction and the GSH level in the erythrocytes of various animals.

Materials and methods. Heparinized blood samples from male adult Sprague-Dawley strain rats, guinea-pigs, rabbits, dogs and humans were used. The methods used for isolation of erythrocytes and conversion of hemoglobin to metHb were as described previously[1]. Erythrocytes containing metHb were depleted of GSH by treatment with N-ethylmaleimide (NEM) using a modification of the method of Morell et al.[7]: namely, equal volumes of packed, metHb-containing cells and 4 or 8 mM NEM in isotonic phosphate buffered saline (pH 7.4) (PBS) containing 10 mM glucose were mixed and incubated for 20 min at 37 °C, and then the cells were washed 4 times with PBS. The basal mixture used to study the reduction of metHb consisted of 25% erythrocytes plus 10 mM glucose in PBS. MetHb was determined by the method of Evelyn and Malloy[8] and GSH by the method of Beutler et al.[9].

Results. The table shows the relationship between the endogenous GSH level in erythrocytes and the rate of enhancement of metHb reduction observed on adding selenite. The GSH level in the cells decreased on preincubation of the cells with NEM, the decrease depending on the concentration of NEM, and the GSH did not reappear during the 60-min incubation period. In cells with normal GSH levels, about 45% of the metHb was reduced on addition of selenite, whereas in cells with half the normal GSH level preincubated with 2.0 mM NEM, the effect of selenite was considerably less, and in cells completely depleted of GSH (preincubated with 4.0 mM NEM), selenite did not enhance metHb reduction at all. Jenkins and Hidiroglou[10] reported the NEM inhibited the uptake of



Species differences in enhancement by selenite of metHb reduction in erythrocytes from various animals. No addition, ●——●; Na_2SeO_3 10^{-5} M, O——O; 3×10^{-6} M, △——△; 10^{-6} M, □——□.
Means±SE of values in 5 experiments are shown.

Effect of GSH depletion on enhancement of metHb reduction by selenite in rat erythrocytes

Pretreatment	Addition	GSH (mM) Initial	After incubation	Reduced metHb (%)
None	None	2.22±0.16	2.14±0.04	5.2±1.9
None	Na_2SeO_3	2.22±0.16	1.45±0.08	44.5±3.6
NEM (2 mM)*	Na_2SeO_3	1.16±0.10	0.69±0.01	29.2±4.4
NEM (4 mM)*	Na_2SeO_3	0.28±0.06	0.16±0.04	3.4±1.9

Means±SE of values in 3 experiments are shown. Cells were incubated for 60 min at 37 °C with or without 10^{-5} M selenite.
* Final concentration.