Fluorescence microscopical study of 5-hydroxytryptamine storage organelles in mepacrine-incubated blood platelets of beige mice (Chediak-Higashi syndrome)

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Summary. The number and fluorescence intensity of fluorescent granules (5-HT storage organelles) of mepacrine-incubated blood platelets of beige mice (Chediak-Higashi syndrome) are decreased compared to those of control mice platelets. This indicates both quantitative and qualitative changes of the 5-HT organelles, namely a reduced number and a reduced storage capacity, respectively.

Recently, fluorescent probes, e.g. mepacrine, have been utilized to study 5-hydroxytryptamine (5-HT) storage organelles of blood platelets. Mepacrine accumulates selectively in these organelles[1], allowing their visualization in the fluorescence microscope[2,3]. The exploitation of this fluorescence microscopical method to congenitally abnormal platelets in storage pool disease(s) and Hermansky-Pudlak syndrome[4,5] confirmed previous results of ultrastructural studies[6], namely a reduced number of 5-HT storage organelles. In the present investigation, mepacrine was used to study Chediak-Higashi syndrome (CHS) mice platelets. In man CHS is characterized by large anomalous granulations in leukocytes and many tissue cells, partial oculocutaneous pseudoalbinism, frequent pyogenic infections and early death due to infection, hemorrhage or infiltrative processes of the accelerated (lymphoma-like) phase of the disease[7]. Prolonged bleeding time, abnormal platelet aggregation, reduced platelet nucleotides (mainly ADP) and 5-HT, and decreased platelet uptake of 5-HT were also observed in CHS patients[8-10]; ultrastructural studies revealed a reduced number of 5-HT storage organelles in the platelets[11]. In mice, mutations to beige (bg) have occurred, e.g. in strain C57BL/6J[12], which are accepted as an animal model of the CHS of man on the basis of a similar phenotype[13-15]. Similarly, beige mice showed prolonged bleeding and impaired storage and uptake of 5-HT by platelets, along with an adenine nucleotide storage deficiency[16], and no typical 5-HT storage organelles were observed in electron photomicrographs of platelet sections[16,17]. However, electron-lucent cores surrounded by a clear halo were noted[16], possibly representing modified storage organelles deficient in 5-HT. This issue prompted the present fluorescence microscopic study.

Materials and methods. Control (black) mice (Jackson Laboratory strain C57BL/6J) and CHS (beige) mice (Jackson Laboratory strain C57BL/6J-bg/bg) were used. Pooled platelet-rich plasma was prepared from 20 mice, incubated

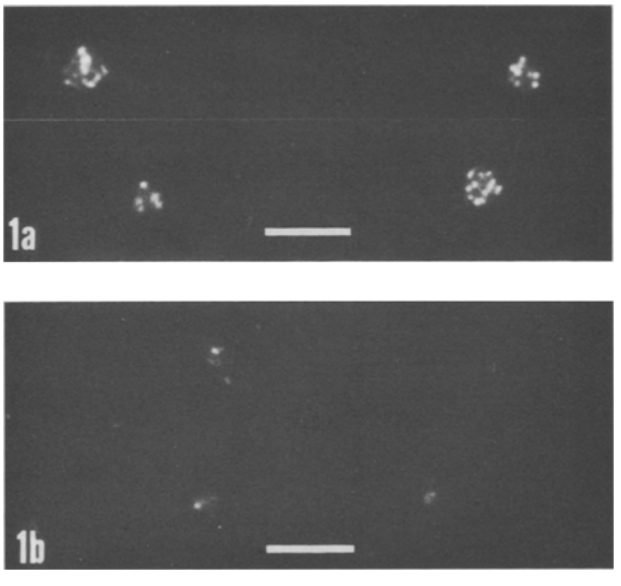

Fig. 1. Mice blood platelets incubated with mepacrine $5 \cdot 10^{-5}$ M, fluorescence micrographs. Scale: 5 µm (×2300). *a* shows 4 control mice platelets exhibiting green-yellow fluorescent granules on a virtually non-fluorescent background. In beige mice platelets (*b*, 3 platelets) the number and fluorescence intensity of the granules are decreased.

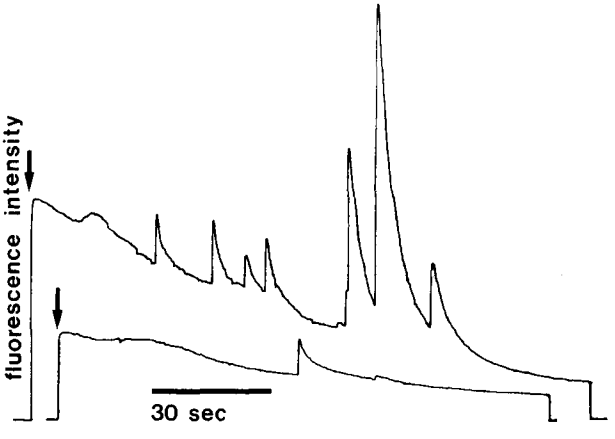

Fig. 2. Microfluorimetric measurements of the fluorescence intensity of single platelets incubated with mepacrine $5 \cdot 10^{-5}$ M. Irradiation starts at ↓. Upper curve: typical recording obtained from a normal mouse platelet (8 flashes). Lower curve: recording as obtained from a beige mouse platelet; note the lower initial fluorescence intensity and the single flash.

Number of granules and flashes and relative fluorescence intensity of single platelets from control and beige mice

	Granules	Flashes	Relative fluorescence intensity
Control mice	8.45 ± 0.32 (51)	7.54 ± 0.56 (52)	36.23 ± 2.68 (57)
Beige mice	5.49 ± 0.23 (57)	0.39 ± 0.08 (52)	17.13 ± 1.88 (53)
% of controls	65.0	5.2	47.3
p versus controls	0.001	0.001	0.001

The isolated platelets were preincubated at 37 °C for 30 min with 5×10^{-5} M mepacrine. The values are averages with SEM. The number of platelets is indicated in parentheses. The statistical significance was calculated with Student's t-test (2-tailed).

with mepacrine (10^{-4}, $5 \cdot 10^{-5}$, 10^{-5} M), and platelets submitted to fluorescence microscopy (for details see[3]). Qualitative observations, counting of fluorescent granules, microfluorimetric measurement of fluorescence intensity and microfluorimetric counting of flashes (emitted from the platelets after prolonged irradiation[2,3]) were performed as previously described[4]. Platelets were analyzed for their content of 5-HT[18], ATP[19] and proteins[20].

Results and discussion. As qualitatively compared in the fluorescence microscope, beige mice platelets differed from control platelets (figure 1) by the number of fluorescent (mepacrine-containing) granules (slight decrease), by the sharpness and fluorescence intensity of the granules (a general moderate decrease), and by the number of flashes (marked decrease). The quantitative data (table) also showed a reduced number of mepacrine accumulating granules, a deficient mepacrine uptake per granule, and a very markedly reduced number of flashes in beige mice platelets (figure 2). In controls, the numbers of fluorescent granules and flashes per platelet did not differ significantly. The 5-HT content was decreased from 21.2 ± 1.8 nmoles/mg protein in controls to unmeasurable levels (less than 1%) in beige mice platelets, and the ATP content from 21.1 ± 0.7 to 9.9 ± 1.0 nmoles/mg protein (average $\pm$ SEM; 2 determinations; $p = 0.001$ (Kolmogorov-Smirnov test)).

Evidence has been presented that fluorescent granules of platelets incubated with mepacrine represent 5-HT storage organelles[4]. Their number appeared markedly reduced (approximately 35%) in beige mice platelets, but not to the extent expected from ultrastructural studies showing a virtually complete absence of such organelles[16,17], and from the deficient 5-HT storage as shown biochemically. In addition to showing a reduced number, the fluorescence microscopical findings point to the presence of qualitatively different 5-HT organelles. In fact, ultrastructural elements possibly representing modified organelles have been described[16] in beige mice platelets. These elements may correspond to the fluorescent granules both representing storage organelles with an almost total inability to store 5-HT, but with only a reduced storage capacity for ATP and mepacrine. The reduced flashing of the platelets is probably a consequence of the deficient mepacrine storage of the 5-HT organelles, but possibly reflects additional changes. It remains to be determined if fluorescence microscopy of CHS human platelets incubated with mepacrine would reveal similar findings on 5-HT storage organelles.

1 M. Da Prada and A. Pletscher, Eur. J. Pharmac. *32*, 179 (1975).
2 H.P. Lorez, M. Da Prada, W. Boguth, H.R. Baumgartner and A. Pletscher, Experientia *31*, 742 (1975).
3 H.P. Lorez, M. Da Prada and A. Pletscher, Experientia *31*, 593 (1975).
4 H.P. Lorez, M. Da Prada, F. Rendu and A. Pletscher, J. Lab. clin. Med. *89*, 200 (1977).
5 H.P. Lorez, M. Da Prada and J.M. Launay, Experientia *33*, 823 (1977).
6 J.G. White and J.M. Gerrard, J. Path. *83*, 590 (1976).
7 R.S. Blume and S.M. Wolff, Medicine *51*, 247 (1972).
8 G.R. Buchanan and R.I. Handin, Blood *47*, 941 (1976).
9 A.R. Page, H. Berendes, J. Warner and R.A. Good, Blood *20*, 330 (1962).
10 T.G. Bell, K.M. Meyers, D.J. Prieur, A.S. Fauci, S.M. Wolff and G.A. Padgett, Blood *48*, 175 (1976).
11 J.L. Costa, A.S. Fauci and S.M. Wolff, Blood *48*, 517 (1976).
12 P.W. Lane, Mouse News Lett. *26*, 35 (1962).
13 M.A. Lutzner, C.T. Lowrie and H.W. Jordan, J. Hered. *58*, 299 (1966).
14 R.J. Elin, J. Edelin and S.M. Wolff, Fedn. Proc. *32*, 1030 Abstr. (1973).
15 J.I. Gallin, J.S. Bujak, E. Patten and S.M. Wolff, Blood *43*, 201 (1974).
16 J.M. Holland, Proc. Soc. exp. Biol. Med. *151*, 32 (1976).
17 J.G. Richards and M. Da Prada, J. Histochem. *25*, 1322 (1977).
18 D.F. Bogdanski, A. Pletscher, B.B. Brodie and S. Udenfriend, J. Pharmac. exp. Ther. *117*, 82 (1956).
19 H. Holmsen, I. Holmsen and A. Bernhardsen, Analyt. Biochem. *17*, 456 (1966).
20 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).

Effect of pretreatment with acetylsalicylate on surgical bleeding and peroperative mortality in rats undergoing kidney transplantation

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Summary. 42 rats were pretreated with L-ASA before kidney transplantation, 43 rats acted as controls. 9 rats with L-ASA, but no control rats, died with i.p. haemorrhage. However, in animals surviving the operation, the intraoperative blood loss did not differ significantly between the 2 groups.

It has been suggested that blood platelets may play an important role in rejection of transplanted organs both in laboratory animals[1-6] and in man[5,7-9]. Several drugs inhibiting platelet function have therefore been associated with conventional treatment for prevention of the rejection phenomena, especially in kidney transplantation[2,3,5,8,9].

Since rejection may start immediately after transplant[10], treatment of the recipients with antiaggregating drugs before surgery could be beneficial. These drugs, however, have been suspected of increasing surgical bleeding[11]. The clinical relevance of this haemorrhagic risk is difficult to evaluate in man since several other factors (such as the chronic uraemic condition of recipients of kidney transplants and the concomitant treatments they require) may contribute to excessive bleeding[12].

In the framework of a broader study on the role of platelets in rejection phenomena in rats, we evaluated the haemorrhagic risk connected with nephrectomy and kidney transplantation in rats pretreated with lysine acetylsalicylate.

Materials and methods. 85 out-bred Sprague Dawley male rats (250–300 g b.wt, Charles River, Italy) were randomly allocated to either control (43 animals) or tested group (42 animals). The latter received 400 mg/kg b.wt lysine acetylsalicylate (L-ASA) (Flectadol, Maggioni, Italy) i.p. 20 h