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.

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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

Table 1. Mortality of rats at operation and at different intervals after kidney transplantation

Time after transplantation	Control (n=43)	L-ASA-treated (n=42)	p
2 h	7 (16.3%)	15 (35.7%)	<0.05
2 h-7 days	16 (37.2%)	14 (33.3%)	n.s.
7-30 days	9 (20.9%)	8 (19.0%)	n.s.
>30 days	11 (25.6%)	5 (11.9%)	n.s.

Table 2. Platelet and leucocyte counts in rats following kidney transplantation

Days	Platelets $\times 10^3/\mu l$ Control	L-ASA-treated	Leucocytes/μl Control	L-ASA-treated
0	1024±43	1039± 63	15,200±1,200	18,600± 4,000
1	843±84	725± 86	16,000±1,700	21,600± 2,400
2	869±80	1053±148	22,300±2,500	25,900±16,600
3	1097±89	1049±160	24,300±1,900	27,400± 5,300

and 1 h before transplantation was performed according to the technique described by Sun Lee[13].

The dose of L-ASA used in this study was that which in preliminary experiments consistently inhibited platelet aggregation (induced by collagen) and vascular prostacyclin generation for at least 24 h[14]. L-ASA was used instead of acetylsalicylic acid because of its solubility in saline solution. Both recipients and donors (out-bred Sprague Dawley male rats) were anesthesized with ether. The surgical operation started with removal of the recipient's right kidney, followed by transplantation of donor's right kidney and subsequent removal of the recipient's left kidney. Kidneys to be transplanted were ischemic for about 45 min, during which time they were kept in and perfused with cold Ringer lactate solution containing phentolamine (0.4 mg/ml) and heparin (20 units/ml). Surgical swabs used during the operation were collected and immersed in distilled water to haemolyze red cells. Haemoglobin was measured as previously described[15] and taken as an index of surgical bleeding. Platelets and leucocytes were counted as previously described[15].

Results and discussion. 7 rats in the control group (16.3%) and 15 in the treated group (35.7%) died within 2 h of the end of operation (table 1). This difference was statistically significant ($p < 0.05$). In each group, death was ascribed to excessive anaesthesia in 5 animals; in 2 controls and 1 treated animal no apparent cause of death could be established. 9 rats pretreated with L-ASA, but no control rats, died with massive bleeding in the peritoneal cavity. No significant differences were observed in the subsequent postoperative mortality rate (table 1).

In animals surviving the operation, the average intraoperative blood loss did not differ significantly between the 2 groups (2.41±1.37 ml in controls and 2.75±1.40 ml in L-ASA group). Platelet count decreased the 1st day after transplantation, but had returned towards preoperative values within 3 days in both groups. Leucocyte counts tended to increase progressively during the first few days after surgery in both groups (table 2). A comparable slight weight loss was observed in both groups during the 1st postoperative week.

This study indicates that pretreatment with L-ASA of rats undergoing binephrectomy and renal transplantation was associated with statistically higher mortality within 2 h of surgery. The incidence of haemorrhagic complications seemed to be a contributory cause of peroperative death in 9 rats, all of which had been treated with L-ASA. These animals were indistinguishable from the others in terms of pre-operative weight, platelet and leucocyte counts, duration of extracorporeal kidney ischaemia and anaesthesia requirement. Platelet function tests were not performed. In rats surviving longer than 2 h after surgery, the intraoperative blood loss, measured by a quantitative procedure, was not significantly modified by L-ASA treatment. This indicates that, in the majority of the animals, L-ASA did not increase the haemorrhagic risk at surgery. It is worth mentioning that L-ASA was previously found not to modify the tail skin bleeding time in unanaesthesized rats[16]. It is conceivable that, in the animals which died with overt haemorrhage, L-ASA could have precipitated bleeding due to surgical problems, vasodilation or other causes. In clinical practice, it is indeed well recognized that aspirin is potentially harmful in patients with pre-existing coagulation disorders[11].

In conclusion, the present study indicates that L-ASA treatment is associated with increased haemorrhage only in some individuals undergoing major surgery. Attempts to identify them before operation seem therefore warranted. This appears of particular importance for those patients, such as the uraemics, who may have additional haemostatic defects leading to an increased haemorrhagic risk at surgery.

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