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Effect of acetylsalicylate on surgical bleeding, postoperative mortality and allograft survival in rats undergoing heart transplantation[1]

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Summary. 18 rats were treated with L-ASA before heart transplantation and daily thereafter until death or rejection. 22 animals acted as controls. A significantly higher post-operative mortality rate, without any significant modification of the transplant survival time, was found in L-ASA-treated group.

It has been suggested that blood platelets may play an important role in rejection of transplanted organs[3–11]. For this reason, several drugs inhibiting platelet function have been associated with conventional treatment in experimental and human transplantation[4,5,7,10,11]. In a previous study[12], we observed that pretreatment of rats with lysine acetylsalicylate (L-ASA) resulted in a significant increase of peroperative mortality following binephrectomy and kidney transplantation. The effect of this drug on the survival of transplanted kidneys could not be evaluated, since the animals were only treated before surgery.

The purpose of the present study was to investigate the effect of L-ASA pretreatment on peroperative mortality of rats receiving heart transplants, this surgical operation being simpler than kidney transplantation. In addition, animals were treated daily until rejection occurred, in order to assess whether L-ASA had any beneficial effect on survival of transplanted hearts.

Materials and methods. 40 outbred CD male rats (Charles River, Italy) (300–350 g b. wt) were randomly allocated to either the control (18 animals) or treated group (22 animals). The animals received 2 i.p. injections of 400 mg/kg b. wt lysine acetylsalicylate (Flectadol, Maggioni, Milan, Italy) or saline 20 h and 1 h before surgery; thereafter animals in the treated group were given 400 mg/kg L-ASA daily p.o. until death or rejection of the heart allograft. Usually 1 control and 1 treated rat were operated on the same day; a few times, an additional treated rat received the transplant to replace animals which died shortly after surgery.

Hearts from outbred CD rats were transplanted according to Van Bekkum et al.[13]. At the end of the operation, all the animals were given an intradermal injection of isotonic saline solution (0.7% of b. wt). Rejection time was taken as the day on which the palpable beat of the transplanted heart ceased; this was confirmed immediately by sight at autopsy.

Packed cell volume, total haemoglobin, leucocyte differential count and platelet counts were measured by routine techniques both before and 24 h after surgery. Leucocytes and platelets were counted every 2nd day thereafter, until death or rejection. Autopsy was carried out on animals which died or were killed shortly after rejection. Histological examination was made of recipients hearts in situ and of transplanted hearts. 3 additional control and 4 treated rats underwent sham operation and were followed the same way as the transplanted animals.

Results. 1 rat in the control group and 7 in the treated group died within 24 h of heart transplantation. Haemorrhage was found in the peritoneal cavity in the control and in 5 treated animals. 1 treated animal died of intestinal occlusion and 1 of congestive heart failure in situ.

3 control and 6 treated rats died between 2 and 7 days after surgery. The apparent causes of death in these animals were: congestive heart failure in situ (1 control and 2 treated), infectious complications (2 controls and 2 treated) and peritoneal haemorrhage (2 treated). No animals died during the 2 months after the 1st postoperative week.

The overall mortality rate was thus significantly higher ($p < 0.05$) in the treated than in the control group (table 1). No rejection episode was recorded in either group during the 1st 6 post-operative days. 11 out of the 14 surviving rats in the control group and 7 out of the 9 in the treated group rejected between 7 and 20 days after transplantation; the individual survival times were 7, 8, 8, 8, 10, 11, 13, 14, 18, 18 and 19 days (median value 11 days) for control and 7, 7, 8, 9, 16, 16, 20 days (median 9 days) for treated rats respectively. The remaining animals (3 controls and 2 treated)

Table 1. Mortality of rats following heart transplantation

Time after transplantation (days)	Control (n = 18)	L-ASA-treated (n = 22)	p*
1	1	7	0.088
2–7	3	6	0.313
Overall	4 (22.1%)	13 (59.0%)	0.041

* χ^2-test, 2-tailed.

Table 2. Some haematological parameters in rats before (B) and 24 h after (A) heart transplantation; mean ± SE

		Sham		Transplanted	
		Control (n = 3)	L-ASA (n = 4)	Control (n = 18)	L-ASA (n = 22)
Platelets ($\times 10^3/\mu l$)	B	1030 ± 32	1032 ± 35	1012 ± 35	1073 ± 33
	A	860 ± 30	933 ± 66	816 ± 19	830 ± 23
Leucocytes ($\times 10^3/\mu l$)	B	29 ± 2	30 ± 1	24 ± 2	22 ± 2
	A	34 ± 2	38 ± 2	30 ± 3	32 ± 3
Neutrophils (%)	B	39.7 ± 1.2	40.0 ± 2.7	43.5 ± 1.8	42.9 ± 2.0
	A	49.0 ± 0.6	48.5 ± 2.7	59.3 ± 1.5	55.0 ± 2.1
Lymphocytes (%)	B	57.3 ± 1.4	54.7 ± 3.5	51.9 ± 1.4	52.6 ± 2.0
	A	47.3 ± 1.4	47.5 ± 3.0	35.5 ± 1.7	39.4 ± 2.3
PCV (%)	B	41.3 ± 1.2	41.2 ± 0.2	41.9 ± 0.8	42.4 ± 0.6
	A	49.0 ± 1.2	50.0 ± 0.7	58.3 ± 2.9	59.6 ± 2.3
Haemoglobin (g%)	B	7.9 ± 0.3	8.3 ± 0.2	8.6 ± 0.2	8.3 ± 0.2
	A	7.1 ± 0.5	7.4 ± 0.2	6.8 ± 0.2	6.4 ± 0.3

were killed 60 days after transplantation when the allografts were still functioning.

Treatment with L-ASA was not associated with increased surgical bleeding, since packed cell volume (PCV) and haemoglobin modifications 24 h after transplantation did not differ significantly between control and treated groups (table 2). The same was true for the sham operated animals. 24 h after surgery, platelet counts were decreased in all groups of animals, whereas leucocyte counts were increased; differential leucocyte counts showed a marked increase of neutrophils and a corresponding decrease of lymphocytes. These modifications were more marked in transplanted than in sham operated groups, regardless of treatment (table 2). Platelet and leucocyte counts returned to normal in all groups of rats within the 1st postoperative week. No consistent modifications of either parameter were detectable either before death or at transplant rejection.

Autopsy after transplant rejection revealed no macroscopic lesions in the in situ heart of control rats; in contrast, in the hearts from the L-ASA-treated group, valvular microthrombi and haemorrhagic subendocardial purpuric spots were frequently found. The transplanted hearts from controls showed ventricular infiltration with subsequent reduction of the ventricular spaces; the transplants from treated animals showed, in contrast, diffuse microhaemorrhagic spots and a modest thickening of the ventricular walls. Histological examination of in situ hearts revealed granulocyte infiltration in both groups, more marked, however, in L-ASA-treated animals. Transplanted hearts from control rats showed mononuclear infiltration with disorganization of muscle cells; similar but less extensive mononuclear infiltration was evident in L-ASA-treated animals.

Discussion. This study indicates that daily treatment of rats with relatively high doses of L-ASA, starting 1 day before heart transplantation, was associated with a significantly higher post-operative mortality rate, without any significant modification of the transplant average survival time. A similar increase of peroperative mortality associated with L-ASA-pretreatment was previously reported by our group in rats undergoing binephrectomy and kidney transplantation[12]; in the previous study, however, rats were only treated pre-operatively with L-ASA.

6 out of 13 L-ASA-treated rats who died during the 1st post-operative week had massive i.p. haemorrhage; however, peroperative blood loss was not different in treated or control animals. This suggests that haemorrhagic complications occurring in animals treated with L-ASA cannot entirely account for the higher mortality rate observed in this group of rats. On the other hand, animals which died during the 1st post-operative week were indistinguishable from the others in terms of pre-operative platelet and leucocyte counts, PCV, total haemoglobin and body weight. The average survival time of transplanted hearts was not significantly different in control and treated rats which survived the 1st post-operative week. Histological examination showed less infiltration in transplants from treated rats. A striking finding, however, was the frequent appearance of both microthrombi and purpuric spots in in situ hearts from treated animals.

Since L-ASA inhibits both platelet aggregation and generation of vascular prostacyclin (a potent inhibitor of platelet aggregation)[14], it is tempting to speculate that the haemorrhagic and thrombotic complications observed in treated rats might be related to the opposite effects of the drug on the haemostatic balance. To our knowledge, no data are available in the literature on the effect of L-ASA on heart transplants in rats.

In conclusion, daily L-ASA-treatment of rats undergoing heart transplantation seemed not to influence the rejection time of the allografts, but post-operative mortality among treated animals was significantly increased. The possibility that dosages of L-ASA lower than that used in the present study and/or treatment starting after the 1st postoperative week might be beneficial in this experimental condition is being considered at present.

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