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Enhancement of the effect of low-dose cyclosporin A by sulphasalazine in prevention of cardiac allograft rejection in the rat

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Abstract. Sulphasalazine (SASP) is an immunomodulatory compound with disease-modifying activity in ulcerative colitis and in other autoimmune disorders. SASP was previously shown to prolong the survival of heart allografts in rats treated with cyclosporin A (CyA) for 9 days after transplantation. We have now evaluated whether SASP also exerts a beneficial effect under continuous treatment with CyA, when CyA is discontinued after 14 days, or alone if given 10 days prior to transplantation. Cardiac grafts were transplanted from PVG donors to Wistar/Kyoto recipients using an accessory cervical heart transplantation technique. Rejection was defined as the absence of palpable contractions and occurred in the control group in a very reproducible manner on day 8 or 9. SASP alone was given orally (100 mg/kg body weight) starting 10 days before transplantation and resulted in a minor prolongation of graft survival. When SASP was given in addition to oral CyA (1 mg/kg or 2 mg/kg from day 0 to rejection) there was a significant prolongation in graft survival [from medians of 8 (range 6–11) and 9 (range 8–11) days, respectively, to medians of 10 (range 8–15) and 12 (range 11–15) days, respectively]. When SASP was given from day 0 to rejection, in addition to a schedule of oral CyA (10 mg/kg) for 15 days, there was no prolongation of graft survival [median of 30 (range 26–42) days vs median of 32 (26–38) days]. The data show that SASP acts as a weak immunosuppressive agent which enhances the effect of CyA given at a low dose. This warrants further investigation as to whether SASP can be used as an additive to conventional regimes in order to allow a lowered dose of CyA for long-term treatment.

Key words: Heart allograft, rat, sulphasalazine – Sulphasalazine, heart allograft, in the rat – Rat, heart allograft, sulphasalazine

Sulphasalazine (salicylazosulphapyridine; SASP) has been used for several decades in the treatment of ulcerative colitis. Recently its use has been expanded and it has been confirmed to be of value in the treatment of various autoimmune diseases (for review see e.g. [18]). Its mode of action is not clear, although it has been found to have pleiotropic effects in vitro (for reviews see [4, 9]. It has been considered to have weak immunomodulatory properties, possibly by effects on the degradation of prostaglandin E_2 [10] or by being an antagonist to folic acid [2]. The effect of treatment with SASP on various cell populations involved in the inflammatory process is not clear, although it has been shown to inhibit cell-mediated cytotoxicity [13], NK-cell activity [1], the generation of an IL-1-like factor [19] and superoxide production by polymorphonuclear leucocytes [3].

In a previous study we observed that oral treatment with SASP starting at transplantation did not in itself prolong the survival of allogenic heart transplants in the rat, while when given in addition to a regime of cyclosporin A (CyA) administered for 10 days there was a distinct potentiation of the effect of CyA [24]. The data indicated that SASP could be used to potentiate the effect of CyA in anti-rejection programmes. However, a number of questions appeared during that study that required answers.

First, the inability of SASP alone to improve graft survival might possibly be due to the fact that a therapeutic effect of SASP requires an induction period [18]. If so, a better effect on the graft survival would be expected if treatment of the recipient started some time before transplantation.

Second, during the first weeks of treatment with CyA a state of tolerance may develop, and discontinuation of CyA after a few weeks can actually result in permanent graft survival in some rat strain combinations [12]. The beneficial effect of SASP could possibly be due to an enhancement of this type of process. Available results obtained by us actually support this contention [24]. In that study treatment with CyA until day 9 led to a postponed graft rejection of 9–10 days, i.e. there was no increase in graft survival beyond the expected time. In later pilot studies animals were given CyA until day 14, which lead

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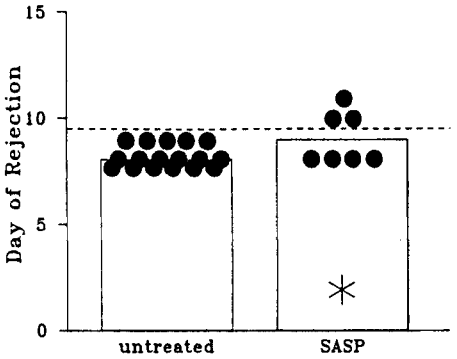

Fig. 1. Day of rejection (individual values and means) in the control and the SASP treated group. SASP was given from 10 days prior to transplantation until the day of rejection at a dose of 100 mg/kg per day. The *asterisk* indicates a statistically significant difference between the two groups

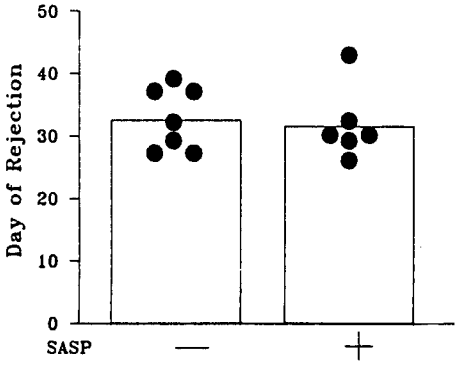

Fig. 2. Day of rejection (individual values and means) in CyA-treated groups with and without SASP. CyA (10 mg/kg per day) was administerd from the day of transplantation until day 14. SASP was given from 1 day prior to transplantation until the day of rejection at a dose of 100 mg/kg per day

to graft rejection about 8 days beyond the expected time. This indicated that a retarded rejection process developed between days 9 and 14 during CyA cover. If SASP causes more rapid development of the mechanism leading to retarded rejection, it is possible that there is a beneficial effect when it is given together with CyA for 9 days but not when given together with CyA for 14 days.

Third, in the previous study the effect of SASP on graft survival was evaluated after cessation of treatment with CyA. An evaluation of the possible effect of SASP when given together with continuous treatment with CyA is therefore necessary before a potential clinical use of SASP can be fully evaluated.

Material and methods

Animals

Inbred male PVG rats ($RT1^c$) weighing 100–150 g served as donors and male Wistar/Kyoto rats ($RT1^l$) weighing 180–220 g as recipients. The animals were obtained from Møllegard Farm, Skensved, Denmark, and were allowed to adapt to their environment for at least 1 week prior to transplantation, with food and water given ad libitum.

Heterotopic heart transplantation

The recipient rats were anaesthetized with 3.5 mg/kg body weight chloral hydrate given intraperitoneally and the donor rats with 120 mg/kg body weight thiobarbital (Inactin) also given intraperitoneally. Accessory cervical heart transplantation was performed by the technique described by Heron [8]. After removing the heart of the donor rat, the graft was perfused with a cold histidine-buffered perfusion solution [11]. The aorta and pulmonary artery of the donor heart were anastomosed to the common carotid artery and the jugular vein, respectively, of the recipient using an external cuff technique [16]. The cold ischaemia time did not exceed 10 min. The transplants were palpated daily and rejection was defined as the complete absence of palpable contraction [14].

Drug treatment

CyA (Sandoz, Basel, Switzerland) was dissolved in Intralipid (Kabi-Pharmacia, Stockholm, Sweden) to a final concentration of 0.5 or 4 mg/ml and administered orally via a gastric feeding catheter in daily doses of 1, 2 or 10 mg/kg. SASP (Kabi-Pharmacia, Stockholm, Sweden) was dissolved in drinking water at a concentration of 0.8 mg/ml. As the animals were found to drink close to 25 ml/day (regularly checked), the daily dose of SASP was close to 100 mg/kg. The addition of SASP to the drinking water did not influence water consumption.

CyA trough levels

Blood was taken from the orbital plexus on day 6 after transplantation of animals receiving low CyA doses (1 or 2 mg/kg per day). The CyA trough levels were measured with a commercial kit (Sandoz) according to the kit instructions.

Statistical analysis

The χ^2 analysis for two groups was used. A P value below 0.05 was considered statistically significant.

Experiments and results

Effect of SASP treatment started 10 days prior to transplantation (Fig. 1). One group of animals was given SASP in their drinking water commencing 10 days before transplantation and the control group was given water alone. All animals in the control group rejected their grafts at day 8 or 9 (median day 8). In recipients given SASP there was no effect on the median graft survival time of 8 days (range 8–11 days).

Effect of SASP combined with CyA until day 14 (Fig. 2). One group of animals was given oral CyA (10 mg/kg body weight) from the day of transplantation until day 14 and another group was, in addition, given SASP from the day of transplantation until rejection. Animals given CyA alone for 14 days had a median graft survival of 32 days (range 26–38 days). Addition of SASP to the CyA regimen did not affect the time of graft rejection (median 30 days; range 26–42 days).

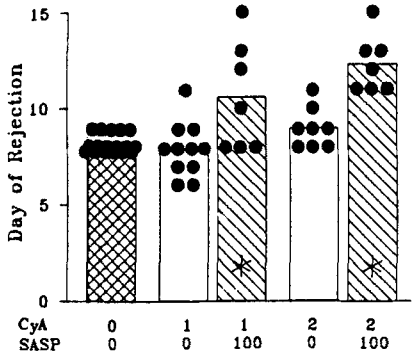

Fig. 3. Day of rejection (individual values and means) in different treatment groups. CyA was given at the indicated dose (mg/kg per day) from the day of transplantation until day 9. SASP was given from 1 day prior to transplantation until the day of rejection at a dose of 100 mg/kg per day. The *asterisks* indicate statistically significant differences between the CyA treated and the respective combined CyA and SASP treated groups

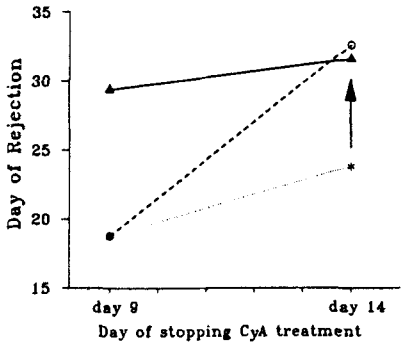

Fig. 4. The "tolerogenic" effect of CyA on graft survival. The *arrow* indicates the shift in the graft survival vs time for CyA treatment curve that is caused by the "tolerogenic" effect observed in animals given CyA for 14 days after transplantation O – – CyA; *..... CyA (estimated); ▲— CyA + SASP

Effect of SASP combined with low-dose CyA (Fig. 3). In two groups of rats CyA was administered from day 0 until rejection in daily doses of 1 or 2 mg/kg respectively. In another two groups SASP (100 mg/kg) was given in addition to CyA starting the day before grafting. While there was no significant increase in graft survival in the groups given CyA alone, isolated grafts survived for up to 11 days, which should be compared to the longest graft survival observed in untreated animals, 9 days. The addition of SASP, however, prolonged graft survival when given together both with 1 and with 2 mg/kg CyA (median 10 days, range 8–15 days, when given with CyA 1 mg/kg; median 12 days, range 11–15 days, when given with CyA 2 mg/kg). The CyA trough levels were not altered by the additional SASP treatment (data not shown).

Discussion

At least two modes of action have been claimed for CyA, an immediate graft protective effect and a process leading to permanent graft survival. The first is supposed to be due to an inhibited production of cytokines including IL-2 [15], and the other is suggested to be due to the development of active suppressor mechanisms under the protection of CyA, rather than to clonal T-cell depletion [26]. The results obtained in this and our previous report [24] can be explained by both mechanisms, which are not mutually exclusive. Thus, SASP prolongs the time to rejection from 19 to 29 days in rats given CyA for 10 days. An increase of 5 days in the treatment time with CyA, from 9 to 14 days, caused an increase in graft survival from 19 to 32 days, i.e. 13 days (Fig. 4). However, when CyA was given together with SASP there was only a prolongation from 29 to 31 days, i.e. an increase of 2 days. These data might indicate that the addition of SASP results in more rapid development of a "tolerogenic" process than that caused by CyA itself.

The mode of action SASP in systemic disorders is still unknown. SASP is hydrolysed in the colon to 5-aminosalicylic acid (5-ASA) and sulphapyridine (SP), each of which might contribute to effects observed in various disease states and experimental models (for survey see [22]). While 5-ASA has been considered the therapeutic principle in inflammatory disease, the parent molecule itself has also been shown to have effects which are compatible with the results obtained by us. The best identified of these effects are an inhibition of the metabolism of folic acid and an inhibition of the breakdown to certain prostaglandins. Thus, SASP inhibits various enzymes in the metabolism of folic acid [2, 20], effects suggested to explain antilymphocyte actions of SASP [20]. Such a mode of action might explain why SASP requires an induction time to develop its full effect, and also why a certain minor graft-protecting effect can be observed after 10 days of administration prior to transplantation. In analogy to the results obtained here, the more potent folate antagonist, methotrexate, potentiated the development of CyA-induced tolerance [5]. Furthermore, methotrexate itself has been reported to have anti-rejection properties in heart-transplanted patients treated with CyA as well [17].

The other possible mode of action of SASP is based on its ability to inhibit various enzymes of the arachidonic acid cascade. SASP is thus a potent inhibitor of 15-prostaglandin dehydrogenase (PGDH [10]) and a moderate inhibitor of 5- and 15-lipoxygenases [23]. PGDH is the major pathway for the degradation of prostaglandin E_2 (PgE_2), and inhibition of this enzyme would be expected to lead to increased tissue concentrations of locally formed PgE_2, which in itself has immunosuppressive properties with beneficial effects on graft survival time, alone or in synergy with CyA [7, 25]. The fact that SASP analogues which are more potent inhibitors of PGDH, given as a single treatment modality, cause a variable increase of graft survival time, whereas SASP itself is without any effect, suggests that this is the mode of action of SASP [25]. Furthermore, PgE_2 is suggested to be an important mediator for the development of tolerance [21]. As CyA, however, does not have any direct impact on the PgE_2 biosynthesis [6], the tolerance induced under CyA cover is caused by mechanisms other than PgE_2. A combined treatment with CyA and SASP would therefore possibly result in development of tolerance by two independent routes, allowing synergistic effects.

High concentrations of SASP are obtained in the lumen of the bowel, slightly lower concentrations in the bowel wall, while only traces of SASP and of the degradation products are absorbed to the systemic circulation [9]. It has therefore been suggested that the immunocompetent cells in the bowel wall constitute the microenvironment where SASP exerts its biological effects. Most observed effects of SASP actually require concentrations only achieved in the bowel lumen.

In the clinically relevant situation, i.e., during continuous treatment, SASP potentiated the effect of low-dose CyA. This extends previous observations where an artificial dose regimen of CyA was used [24]. Although the rejection process is one of the most vigorous immune responses, the data suggest that it can be affected by disease-modifying drugs of modest potency, like SASP. Chronic dose-dependent side effects limit the dosing of CyA compared to what would be desirable from an immunological point of view; continuous doses of one or more disease-modifying compounds like SASP might well make it possible to allow lower doses of CyA without losing immunosuppressive power.

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