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The enhancing effect of cyclosporine A and sulfasalazine on the prevention of rejection in rat cardiac allografts

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Abstract. Sulfasalazine (SASP) has been used for many years as a disease-modifying agent in inflammatory bowel disease and in rheumatoid arthritis. However, its mode of action is not entirely clear. Evidence has been accumulated which indicates that its efficacy is due to an immunomodulatory effect. In the present communication, we report that SASP has an immunomodulatory capacity in an experimental rat cardiac allograft model. A combination of 100 mg/kg per day of SASP given orally until rejection and 10 mg/kg per day of cyclosporine A (CyA) given orally for 10 days resulted in a significantly increased graft survival time as compared to that in animals given CyA alone.

Key words: Heart - Graft survival - Cyclosporine - Sulfasalazine - Rats.

Sulfasalazine (SASP) was developed in the late thirties in an attempt to obtain an efficient medication against rheumatoid arthritis. The initial studies gave "impressive results" [12]. These initial results were, however, more or less forgotten until the last decade when a number of controlled clinical studies confirmed the efficacy of SASP in rheumatoid arthritis. However, the main clinical use of this compound has been in inflammatory bowel disease. The mode of action of SASP is not well understood although the drug has many pharmacological and biochemical actions, above all on arachidonate metabolism [8]. Among the effects recently suggested to be of importance is an inhibitory effect on 15-OH-prostaglandin dehydrogenase [9], which would lead to an inhibition of prostaglandin E_2 breakdown, and an inhibition of leukotriene formation [2]. Furthermore, SASP inhibits neutrophil degranulation and superoxide production [10]. The degradation product 5-aminosalicylic acid has been reported to be a scavenger of hydroxyl radicals [3] and an inhibitor of myeloperoxidase activity [5].

In experimental models for autoimmune disease, SASP has been found to be beneficial [7], indicating that one or more of its biochemical effects might be operative in an immunological context, where the net effect would be immunomodulatory. This effect might theoretically be used in adjunctive treatment to prevent rejection in allograft recipients. We have, therefore, evaluated the extent to which SASP alone or in combination with cyclosporine A (CyA) influences the rejection time of rat cardiac allografts.

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Materials and methods

Animals

PVG (100-150 g) and male Wistar/Kyoto rats (180-220 g) were obtained from Møllegard Farm, Skensved, Denmark. They were given food and water ad libitum until experimentation. The animals were allowed to get settled for at least 1 week prior to transplantation.

Surgical technique

A non-suture technique of heart transplantation in rats was used as described in detail elsewhere [11]. The donor rats were briefly anesthetized with Inactin i.p. and, after transplant harvest, the donor heart was flushed with a cold histidine-buffered perfusion solution (Frödin et al., to be published). The caval and pulmonary veins were ligated before removal. The heart was heterotopically transplanted to the neck vessels of the recipient, where the

aortic root was anastomosed to the right common carotid artery and the pulmonary artery to the right jugular vein of the recipient. The total ischemia time was less than 10 min.

Drug treatment

CyA (100 mg/ml; Sandoz AG, Basel, Switzerland) was dissolved in Intralipid (Kabi-Vitrum, Stockholm, Sweden) to a final concentration of 4 mg/ml and administered orally via a gastric feeding catheter in a dose of 10 mg/kg body weight for 10 days, the first dose given at transplantation. Sulfasalazine (SASP, Salazopyrine, Asulfidine; Pharmacia AB, Uppsala, Sweden) was dissolved in the drinking water at a concentration of 0.8 mg/ml and given from the day before transplantation. As the daily water consumption for a rat was found to be 22-28 ml per day, the daily dose of SASP administered was very close to 100 mg/kg body weight per day. Water consumption was evaluated for 2 days prior to and after transplantation. SASP was found not to have any impact on water consumption.

Evaluation of complete rejection

The transplanted hearts were palpated daily. The day of complete rejection was defined as the day when palpable pulsations ceased.

Statistics

Between groups, significant differences were evaluated with the Wilcoxon-White rank sum test.

Results

The rejection time for pharmacologically untreated transplanted hearts in this strain combination was found to be 8-9 days (Table 1). Administration of SASP alone did not have any impact on graft survival. Administration of CyA for 10 days resulted in a completely abrogated rejection response during CyA treatment, and the hearts were rejected approximately 10 days after cessation of treatment, i.e., 17-22 days after transplantation. In the group given CyA in addition to SASP, the animals rejected their hearts 26-34 days after transplantation, i.e., 17-25 days after cessation of CyA therapy. This indicates that SASP enhanced the effect of CyA on graft survival time.

Table 1. Effect of sulfasalazine and cyclosporine A on rat cardiac allograft survival

Groups	Graft survival (days)	
	Individual values	Median value
Untreated	8, 8, 8, 8, 9, 9, 9	8
Sulfasalazine[a]	6, 7, 7, 8, 8, 9	7.5
Cyclosporine A[b]	17, 17, 18, 19, 20, 22	18.5
Sulfasalazine[a] and cyclosporine A[b]	26, 26, 28, 33, 34	28[c]

[a] 100 mg/kg per day orally day -1 to rejection
[b] 10 mg/kg per day orally day 0 to day 9
[c] $P<0.01$ vs the cyclosporine A group

Discussion

While SASP alone did not have any impact on rejection time, its combination with CyA resulted in an apparent enhancing effect of the two compounds.

SASP has been shown to downregulate the mitogenic response of isolated peripheral blood monocytic cells [1]. It has also been suggested that SASP functionally increases the suppressor cell activity in ulcerative colitis [4]. It can be speculated that increased functional suppression develops under the cover of CyA. In grafts not treated with CyA, the rejection time might be so short that a slowly maturing functional suppression would not have time to develop.

In certain rat strain combinations (e.g., PVG to DA [6]), short-term CyA treatment at transplantation is sufficient to induce tolerance and permanent graft survival. It is, therefore, possible that the addition of SASP to the conventional CyA treatment in the present strain combination results in a more modest allogeneic response, with certain similarities to the response seen in the strain combinations in which tolerance can be achieved during CyA cover only.

The results suggest that, in view of the long-term toxicity of CyA, it would be of value to investigate whether SASP, or SASP-like drugs, could be used in clinical transplantation in an attempt to lower the dose of CyA given. Furthermore, an enhancing effect might benefit those patients who reject while seemingly under an optimal immunosuppressive protocol.

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