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Pharmacokinetics of 15-deoxyspergualin studied in renal transplant patients receiving the drug during graft rejection

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Abstract The pharmacokinetics of the novel immunosuppressant 15-deoxyspergualin (DSG) were studied in five renal transplant patients who participated in a dose-finding study for the treatment of renal graft rejection. DSG, in a dose of 4 or 6 mg/kg per day, was given in a 3-h i.v. infusion for 5 days, in combination with a 4-day course of i.v. methylprednisolone. Analyses of DSG in plasma and urine were performed by high-performance liquid chromatography (HPLC). Plasma samples were taken up to 12 h following infusion on treatment day 2 and again on day 4 or 5. Urine was collected during the infusion and up to 12 h following the infusion. DSG was rapidly eliminated from the plasma in an apparently biexponential manner. The mean t1/2alpha was 0.5 h (range 0.1–1.1 h) and the mean t1/2 beta 2.4 h (range 1.0–5.9 h). The mean Cmax was 4117 ng/ml (range 1944–7166 ng/ml) and the mean AUC 12505 ng · ml^{-1} · h (range 5642–24435 ng · ml^{-1} · h). Clearance ranged from 375 to 945 ml/min (mean 653 ml/min) and volume of distribution ranged from 0,2 to 1,4 l/kg (mean 0,7 l/kg). A small fraction (mean 1.6%, range 0.1%–2.7%) of the DSG dose given was excreted unmetabolized in the urine. The amount of DSG in the urine correlated strongly to renal function ($P = 0.0019$). Pharmacokinetics were otherwise not affected by the degree of renal function. There were no significant differences in the pharmacokinetic determinants and no accumulation of the drug on study day 4 or 5, as compared to day 2. Therefore, the drug can safely be given to patients with impaired renal function. DSG did not affect cyclosporin pharmacokinetics.

Key words 15-Deoxyspergualin, renal transplantation · Renal transplantation, 15-deoxyspergualin Immunosuppression, 15-deoxyspergualin · Rejection, 15-deoxyspergualin, renal transplantation

Introduction

Spergualin is a natural substance produced by *Bacillus laterosporous* which, by synthetic dehydroxylation, is converted to ± 15-deoxyspergualin (DSG). DSG possesses weak antibiotic and antitumor effects [15]. Recently, DSG has been found to exert potent immunosuppressive effects in a variety of animal models [4, 6, 12, 19–21]. Some promising results have also been reported in clinical transplantation [1, 2, 5, 14]. The immunosuppressive mechanism of action of DSG remains unknown. The drug weakly inhibits IL-1 and IL-2 production, but it probably produces its main effects in a later stage of T- and B-cell maturation [21]. The effects of DSG are quite different from those of drugs like cyclosporin, FK 506, or rapamycin, which produce their effects by interference with IL-2 [13]. Because of poor bioavailability, DSG cannot be given orally in the present preparation, leaving intraven-

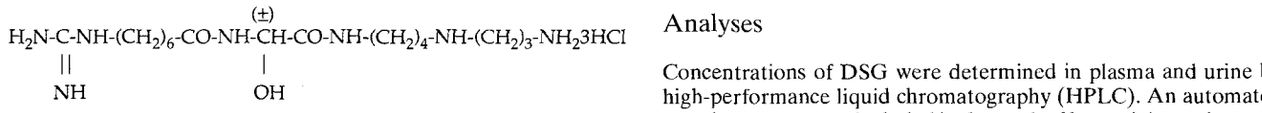

Fig. 1 Structure formula of ± 15-deoxyspergualin

ous (i.v.) administration as the only option in humans at the present time. This limits the clinical use of the drug to short-term treatment. The most common side effects observed with DSG have been hot flushes, paresthesias, nausea, leukopenia, and thrombocytopenia. The hematological side effects occur late – i.e., 7–14 days after the termination of a 5-day course of treatment [10]. To minimize the acute side effects, DSG is usually given as a 3-h continuous infusion once daily. Whether this is an accurate dosing regimen for patients remains unclear. Moreover, only limited data are available regarding the pharmacokinetics of DSG in humans. As part of a clinical dose-finding study utilizing DSG as an adjunct to treatment of renal graft rejection together with high-dose steroids, pharmacokinetic studies were performed in five patients.

Material and methods

Study drug

DSG has a molecular weight of 469.91 and the summation formula $C_{17}H_{37}N_7O_3 3HCl$ (Fig. 1) [18]. Further characterization has shown that DSG is a mixture of racemic (±) enantiomers at carbon number 11. It is not known whether the immunosuppressive and toxic effects are related to the same or to different enantiomers [17]. Unlike some other immunosuppressive agents, DSG is easily soluble in water.

Patients

Five renal transplant recipients, maintained on immunosuppression with cyclosporin A (CyA), azathioprine, and prednisolone, were part of a dose-finding study with DSG [10]. When graft rejection was suspected, a renal biopsy was performed and rejection treatment was instituted with methylprednisolone for 4 consecutive days (total dose 1.25 g). In addition, DSG was given for 5 consecutive days. The pharmacokinetics of DSG were studied in two patients on treatment days 2 and 5, after a DSG dose of 4 mg/kg per day, and in two patients on days 2 and 4, after a DSG dose of 6 mg/kg per day. An additional patient, given a DSG dose of 6 mg/kg per day, was studied only on treatment day 2. Patient characteristics are presented in Table 1.

Study procedure

The study drug was dissolved in 50 ml of 0.9% saline and administered as a 3-h continuous i.v. infusion. Plasma samples (3 ml) were obtained as follows: at the end of the infusion, at 5, 15, and 30 min, and at 1, 2, 3, 4, 6, 9, and 12 h thereafter. Urine samples were collected during the 3-h infusion and for 12 h following the infusion. All samples were kept frozen at – 20 °C until the analyses were done.

Analyses

Concentrations of DSG were determined in plasma and urine by high-performance liquid chromatography (HPLC). An automated sample clean-up was included in the method by applying an ion pair-assisted DSG enrichment procedure using a pre-column installed in the sample loop of the HPLC autoinjector. In the presence of an internal standard, DSG was separated via ion pair HPLC and detected after a post-column on-line reaction with o-phthaldialdehyde by fluorescence. The limit of quantitation of DSG in human plasma amounted to 10 ng/ml. CyA concentrations were analyzed in whole blood by specific monoclonal radioimmunoassay (RIA; CYCLO-Trac-SP, Immuno Nuclear, Stillwater, Minn., USA). The assay was performed according to the instructions supplied with the kit.

Pharmacokinetics and statistics

DSG plasma concentrations were analyzed by means of weighted, nonlinear regression, using HOEREP-PC software, version 1.05. Weighted least squares were obtained using the following formula:

$W(y) = \frac{1}{(0.0095y + 1.52)^2}$, where "y" denotes the DSG concentration measured. Thus, the weights corresponded to the inverse variance of the analytical assay, as derived from a spiking experiment at various concentrations. Half-lives ($t^1/_2$) area under the curve (AUC), clearance, and volume of distribution (V_d) were then derived from adjusted plasma curves [3]. Cmax and urinary excretion of unmetabolized DSG were obtained directly and by simple arithmetical calculations, respectively. Student's paired *t*-test was used for comparisons between different study days; a *P* value lower than 0.05 was considered a significant difference. Linear regression was performed using the method of least squares with the JMP software package (SAS Institute, Cary, N.C., USA).

Results

DSG in plasma

After termination of the 3-h infusion, DSG was eliminated from plasma in a biexponential fashion. No significant differences were observed between the pharmacokinetics determined on day 4 or 5 as compared to those on day 2 (Table 2). AUC and Cmax correlated well to the given dose ($n = 9$, $r = 0.8$, $P = 0.001$, for both determinants). Mean t1/2alpha was 0.5 h (range 0.1–1.1 h) and mean t1/2 beta was 2.4 h (range 1.0–5.9 h). Mean Cmax was 4117 ng/ml (rang 1944–7166 ng/ml) and mean AUC was 12505 ng · ml^{-1} · h (range 5642–24435 ng · ml^{-1} · h). Clearance ranged from 375 to 945 ml/min (mean 653 ml/min) and volume of distribution ranged from 0.2 to 1.4 l/kg (mean 0.7 l/kg; Table 2). All data obtained from patients studied on 2 treatment days (patients 1–4) are shown in Fig. 2.

Urinary excretion and renal function

A mean of 1.6% (range 0.1%–2.7%) of the DSG dose given was excreted in the urine collected during the infusion and up to 12 h following the infusion (total 15 h). The

Table 1 Patient characteristics

Patient number	Age/sex	Renal disease	DSG dose mg/kg (total dose mg)	Outcome of rejection crisis	Adverse effects (treatment given on days 1–5)
1	52/M	Unknown	4.1 (320)	Resolved	Paresthesias/hot flushes (day 1) Hypertension (day 2)
2	60/M	Toxic damage	4.0 (320)	Resolved	None
3	53/M	Chronic glomerular nephritis	6.0 (475)	Not resolved	Paresthesias (days 1–2)
4	58/M	Polycystic disease	6.0 (550)	Resolved	Hot flushes (days 1–2, 4) Paresthesias (day 4) Leukopenia (day 10)
5	59/M	Diabetes nephropathia	6.1 (485)	Biopsy did not confirm rejection	Nausea (day 1)

Table 2 Pharmacokinetic determinants of DSG and renal function

Patient number	Treatment day	AUC ($ng \cdot ml^{-1} \cdot h$)	Clearance (ml/min)	V_d (l/kg)	Cmax (ng/ml)	t1/2beta (h)	% DSG in urine of total dose	S-Creatinine (μmol/l)
1	2	6224	857	0.7	2342	1.1	1.8	341
	5	7517	709	1.4	2266	5.9	2.0	355
2	2	5642	945	1.4	1944	3.3	1.2	480
	5	8503	627	0.7	2788	1.4	1.4	498
3	2	17609	450	0.7	6507	5.2	0.3	468
	4	15388	514	0.6	3995	1.5	0.1	642
4	2	18232	503	0.2	6994	1.1	1.9	302
	4	24435	375	0.2	7166	1.0	2.7	331
5	2	9000	898	0.6	3048	1.0	2.6	250
Mean ± SD:								
Day 2		11341 ± 6142	730 ± 235	0.7 ± 0.4	4167 ± 2396	2.3 ± 1.9	1.6 ± 0.9	368 ± 102
Days 4/5		13961 ± 7811	556 ± 145	0.7 ± 0.5	4054 ± 2198	2.5 ± 2.3	1.5 ± 1.1	456 ± 144

amount of nonmetabolized drug excreted in the urine depended on the renal function ($n = 9$, $r = -0.88$, $P = 0.0019$; Fig. 3). However, clearance of DSG, AUC, Cmax and t1/2 did not correlate to the degree of renal function.

Cyclosporin levels

Concentrations of CyA were determined every day or every 2nd day during the study period. No major dose changes were made during this time. There was no indication of interaction of DSG on CyA pharmacokinetics since the concentrations of the latter drug remained constant (Fig. 4).

Discussion

DSG has recently been introduced as an immunosuppressant in transplanted patients, but the optimal use of this novel substance has yet to be defined. As a part of the clinical evaluation of the drug, studies of the pharmacokinetics are needed. We are aware of only one published study concerning the pharmacokinetics of DSG in renal transplant patients [14]. The study reported that DSG had a half-life of approximately 1 h, and that the total urinary excretion of the unmetabolized drug was 4 % of the given dose. The results of a similar study performed in cancer patients showed that the urinary excretion was 10 % [16]. In the cancer patients, a biexponential elimination from plasma was found with t1/2alpha and t1/2beta of 0.2 h and 1.1 h, respectively. At doses larger than 264 mg/m^2 (approximately 7 mg/kg), a second plasma peak was observed [7]. In this study also, the rapid elimination of the drug appeared to be biexponential, indicating a two-compartment model. No second peak was seen after the somewhat smaller doses given in this study. The pharmacokinetics observed in the present study are in general agreement with those of the previous studies, except for the finding of a more than twice as long mean elimination half-life than that mentioned above. Furthermore, a smaller proportion (1.6 %) of the drug was present in the urine,

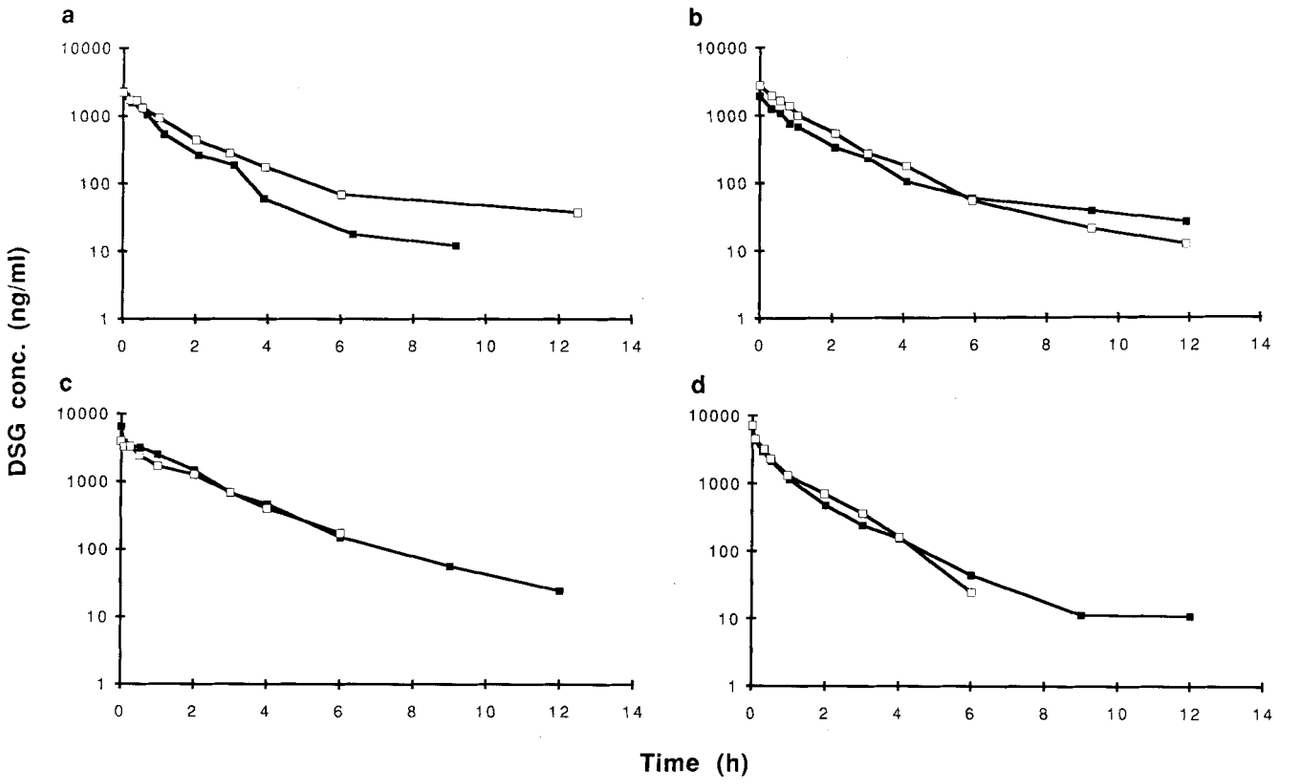

Fig. 2a–d Postinfusion plasma concentrations of DSG in patients 1–4, who were investigated on 2 treatment days. - ■ - Concentration (ng/ml) on day 2, - □ - concentration (ng/ml in day 4 or 5

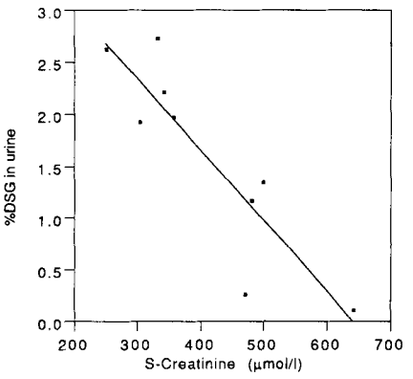

Fig. 3 Linear regression performed between s-creatinine and % DSG in urine ($n = 9, r = -0.88, P = 0.0019$)

and this proportion was strongly correlated to the degree of renal function ($P = 0.0019$; Fig. 3). The fact that all subjects in our study had impaired renal function (Table 2) probably explains the finding of a smaller excretion of the drug in the urine. However, the absence of a correlation between AUC, Cmax, clearance or t1/2 and renal function further justifies the assumption that most of the drug is metabolized. Moreover, no accumulation of unmetabolized drug occurred during the 5-day treatment, not even in patients with poor renal function. Furthermore, we found that various pharmacokinetic determinants for DSG, such as AUC and Cmax, could be well predicted by the dose given, without regard to renal function.

The clinical use of DSG has been restricted by the fact that only parenteral administration is possible with the currently available preparation. A 3-h infusion was given because toxic symptoms, such as hot flushes and paresthesias, were more severe when the drug was given at a faster rate. Animal studies show that the acute toxicity of DSG is related to the peak plasma levels [11]. The optimal mode of administration would probably be a 24-h continuous infusion. However, such a dosing regimen has practical drawbacks. The fact that hematological side effects begin 7–14 days after the termination of treatment [10] may indicate that toxic and possibly active metabolites of DSG may be present and may accumulate over a considerable period. Alternatively, this finding is simply a consequence of the turnover time for peripheral blood cells, which delays the peripheral manifestation of stem cell depression. Favoring the latter explanation is the fact that DSG is assumed to exert a cytostatic rather than a cytolytic effect [9]. We could not relate the occurrence of side effects or the clinical outcome to pharmacokinetic

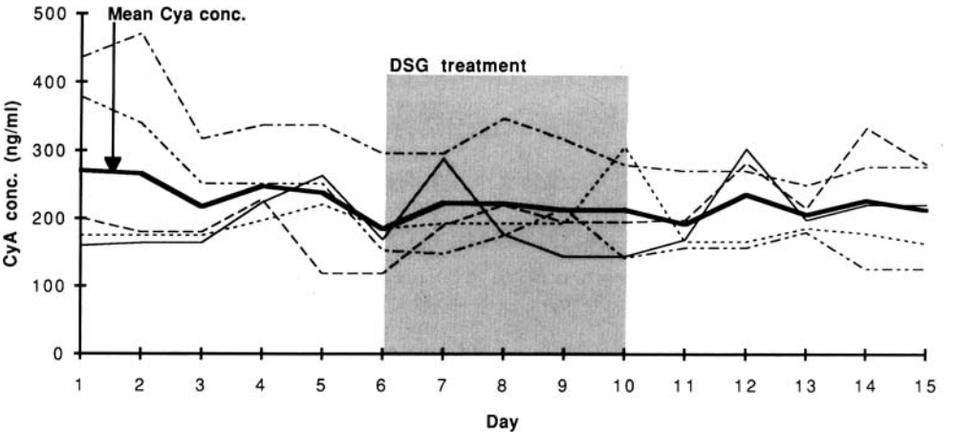

Fig. 4 Individual *(thin lines)* and mean *(bold line)* cyclosporin concentrations before, during, and after DSG treatment

determinants, such as Cmax or AUC. This would require a larger number of patients.

As regards transplant patients, it is important to know whether a new drug may interfere with other immunosuppressive drugs, such as CyA. In our study, DSG treatment caused no change in the CyA blood levels (Fig. 4). However, some studies claim that high doses of methylprednisolone increase CyA levels [8]. Since our patients received a combination of methylprednisolone and DSG, it may be that in increase caused by the former drug is counteracted by a decrease caused by DSG, which results in unaltered concentrations of CyA. The question of whether CyA has an influence on DSG pharmacokinetics cannot be answered by this study since all patients were continuously on unaltered CyA maintenance therapy.

We conclude that AUC and Cmax of DSG can be well predicted by a given dose and that DSG is rapidly eliminated from plasma by metabolization. The small proportion excreted in the urine unchanged is related to the degree of renal function. Unmetabolized DSG does not accumulate and the drug can safely be given to patients with impaired renal function. There was no evidence of a pharmacokinetic interaction between DSG and CyA.

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