CICLOSPORIN

1. Chemical and Physical Data

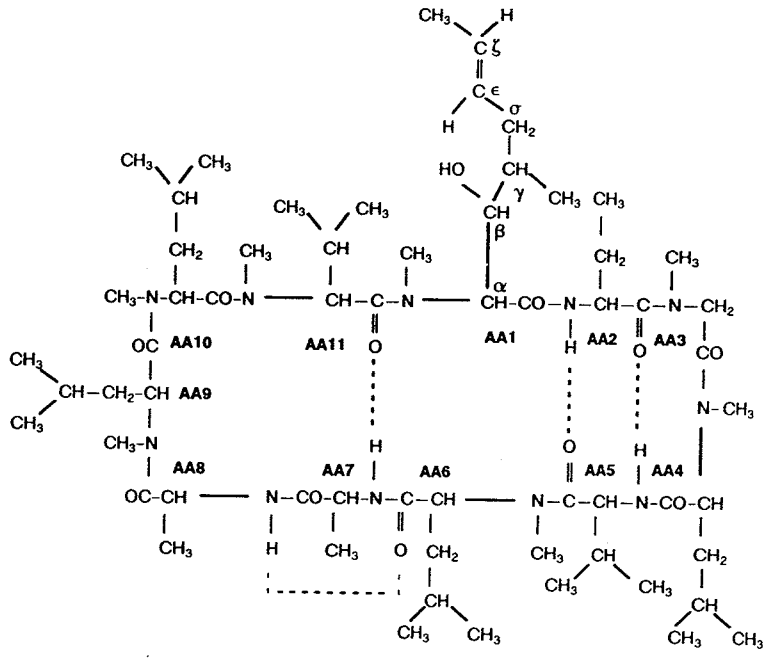
1.1 Synonyms

Chem. Abstr. Services Reg. No.: 59865-13-3 (cyclosporin A); 79217-60-0 (cyclosporine)

Chem. Abstr. Name: {R-[R*,R*-(E)]}-L-Cyclic(L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-leucyl-*N*-methyl-L-valyl-3-hydroxy-*N*,4-dimethyl-L-2-amino-6-octenoyl-L-α-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl)

Synonyms: Cyclosporin A; cyclosporine; dyclosporin; OL-27-400; cyclo{[(E)-(2S,3R,4R)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl}; cyclo{[4-(E)-but-2-enyl-*N*,4-dimethyl-L-threonyl]-L-homoalanyl(*N*-methyl-glycyl) (*N*-methyl-L-leucyl)-L-valyl(*N*-methyl-L-leucyl)-L-alanyl-D-alanyl-(*N*-methyl-L-leucyl)(*N*-methyl-L-leucyl)(*N*-methyl-L-valyl)}

1.2 Structural and molecular formulae and molecular weight



$C_{62}H_{111}N_{11}O_{12}$ Mol. wt: 1202.64

1.3 Chemical and physical properties of the pure substance

From Ruegger *et al.* (1976), Windholz (1983) and Hassan and Al Yahya (1987)

(*a*) *Description*: White prismatic crystals from acetone; neutral, hydrophobic, cyclic non-polar oligopeptide composed of 11 amino acid residues. The X-ray crystallographic structure is known.

(*b*) *Melting-point*: 148-151°C (natural); 149-150°C (synthetic)

(*c*) *Optical rotation:* $[\alpha]_D^{20} = -244°$ (c = 0.6 in chloroform); $[\alpha]_D^{20} = -189°$ (c = 0.5 in methanol)

(*d*) *Solubility*: Neutral; rich in hydrophobic amino acids; insoluble in water and *n*-hexane; very soluble in all other organic solvents

(*e*) *Spectroscopy data*: Ultraviolet, infrared, nuclear magnetic resonance and mass spectra have been reported.

(*f*) *Stability*: Stable in solution at temperatures below 30°C; sensitive to light, cold and oxidization (Reynolds, 1989)

1.4 Technical products and impurities

Trade names: Sandimmun; Sandimmune

Ciclosporin is available in bottles containing 100 mg/ml in an olive oil-based solution and 12.5% ethanol for oral administration, and in ampoules containing 50 mg/ml with 33% ethanol and 650 mg polyoxethylated castor oil for intravenous injection (Barnhart, 1989).

2. Production, Occurrence, Use and Analysis

2.1 Production and occurrence

The isolation of cyclosporins A and C from the fungus *Tolypocladium inflatum* Gams has been described (Rüegger *et al.*, 1976), and the biosynthesis of ciclosporin has been reported (Kobel & Traber, 1982; Kobel *et al.*, 1983; Billich & Zocher, 1987). It is also produced synthetically from *N*-methyl-*C*-9-amino acid with subsequent additions of appropriate peptides, followed by cyclization (Hassan & Al-Yahya, 1987).

Ciclosporin is manufactured commercially in Switzerland (Reynolds, 1989).

Cyclosporins (mostly A and C) are produced by the fungi *Tolypocladium inflatum* Gams and *T. cylindrosporum* and by other fungi isolated from soil.

2.2 Use

Ciclosporin is an immunosuppressive agent. It is used extensively in the prevention and treatment of graft-*versus*-host reactions in bone-marrow

transplantation, and for the prevention of rejection of kidney, heart and liver transplants. It has also been tested for the therapy of a large variety of other diseases in which immunological factors may have a pathogenetic role, including Graves' disease, uveitis, Crohn's disease, ulcerative colitis, chronic active hepatitis, primary biliary cirrhosis, diabetes mellitus, myasthenia gravis, sarcoidosis, dermatomyositis, systemic lupus erythematosus and psoriasis (Calne *et al.*, 1978, 1979; Powles *et al.*, 1980; Merion *et al.*, 1984; Kahan *et al.*, 1985; Reynolds, 1989).

The usual oral dose of ciclosporin is 18 mg/kg daily, beginning 12 h before transplantation and continuing for one to two weeks. Dosage may subsequently be reduced to 5-10 mg/kg or less. Ciclosporin may also be given intravenously, usually at one-third of the oral dose. This drug is often given for several months to transplant recipients (Reynolds, 1989).

2.3 Analysis

Ciclosporin has been measured in pharmaceutical preparations by high-performance liquid chromatography (HPLC; US Pharmacopeial Convention, Inc., 1989).

Ciclosporin and its metabolites have also been measured in biological fluids using HPLC (Awni & Maloney, 1988; Christians *et al.*, 1988a,b; Birckel *et al.*, 1988), and ciclosporin has been monitored in whole blood by radioimmunoassay (Donatsch *et al.*, 1981; Vine & Bowers, 1987). Vine and Bowers (1987) provided a critical summary of HPLC methods used to measure ciclosporin in biological fluids, and Hassan and Al-Yahya (1987) reviewed the methods for analysing ciclosporin. Radioimmunoassay kits for the analysis of ciclosporin in plasma are available, and their performance has been compared to that of HPLC analyses (Vernillet *et al.*, 1989; Wolf *et al.*, 1989).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Oral administration*

Mouse: Groups of 50 male and 50 female OF1 mice, weighing 26-39 and 19-28 g, respectively, were fed ciclosporin at 1, 4 or 16 mg/kg of diet for 78 weeks, at which time all survivors were killed. An untreated group of 50 males and 50 females served as controls. All mice were necropsied, and all macroscopic lesions were examined histologically. Mortality was higher in high-dose females (60%) than in

controls (40-50%) and in other treated groups (42-52%). No increase in the incidence of tumours was observed in treated mice (Ryffel *et al.*, 1983).

In a screening assay based on the accelerated induction of leukaemia in a strain highly susceptible to development of this neoplasm, 30 male AKR mice, six weeks of age, were fed ciclosporin at 150 mg/kg of diet. The first thymic lymphoma in treated mice was noted at week 17; these tumours occurred in 13/18 animals killed between 20 and 29 weeks [$p = 0.004$] and in 9/9 killed between 30 and 34 weeks [$p = 0.005$, Fisher's exact test]. In 22 mice that received the basal diet only, the first thymic lymphoma was noted at week 23, and the incidences of these tumours in animals killed between 20 and 29 weeks and 30 and 34 weeks were 2/12 and 3/9, respectively (Hattori *et al.*, 1986).

Rat: Groups of 50 male and 50 female OFA rats, weighing 242-326 and 169-244 g, respectively, were fed ciclosporin at 0.5, 2 or 8 mg/kg bw of diet for 95 weeks (males) and 105 weeks (females), at which time the experiment was terminated. An untreated group of 50 males and 50 females served as controls. All animals were necropsied, and all macroscopic lesions were examined histologically. Mortality rates were 68% in controls, 74% in low- and mid-dose groups, and 86% in the high-dose group. No increase in tumour incidence was observed in treated rats (Ryffel *et al.*, 1983). [The Working Group noted the high incidence of tumours in the controls, which may have reduced the sensitivity of the assay.]

(*b*) *Administration with other treatments*

Mouse: A group of 39 male Swiss Webster mice and 13 male C57Bl/6J mice, six to seven weeks of age, were given a single whole-body γ-irradiation of 350 rad and ten days later were fed ciclosporin [purity unspecified] at 150 mg/kg of diet for 35 weeks, at which time all survivors were killed and autopsied. A group of 26 male Swiss Webster and 14 male C57Bl/6J mice received the same irradiation and were maintained on basal diet. Two groups of 18 male Swiss Webster and 12 male C57Bl/6J mice received no irradiation and were maintained on control diet or were given ciclosporin at 150 mg/kg of diet. No tumour was observed in either of the strains of mice irradiated and maintained on basal diet alone or in either strain that received no radiation and were fed diets containing ciclosporin. Of the Swiss Webster mice that were irradiated and fed diets containing ciclosporin, 18/39 (46%) [$p < 0.001$, Fisher's exact test] developed lymphoid tumours, primarily in the spleen and mesenteric lymph nodes, within an average latent period of 24 weeks. The tumours were interpreted as B-immunoblastic lymphomas with plasmacytoid features. Four of the 39 (10%) mice developed classical thymic lymphomas within an average latent period of 23.7 weeks. Of the C57Bl/6 mice irradiated and fed diets containing ciclosporin, 7/13 (54%) [$p < 0.002$, Fisher's exact test] developed thymic

lymphomas within an average latent period of 27.4 weeks. No spleen or lymph node lymphoma developed in this strain (Hattori *et al.*, 1988).

Two groups of 13 male Swiss Webster mice, six to seven weeks old, received a single intraperitoneal injection of 1 g/kg bw urethane. One week later, ciclosporin [purity unspecified] was administered at 150 mg/kg of diet. Two groups of 15 or 14 mice not receiving injections of urethane were fed the basal diet or ciclosporin at 150 mg/kg of diet. All animals were killed 22 weeks after the beginning of treatment. No significant difference in the number of lung adenomas was found between the groups receiving urethane and ciclosporin and those receiving urethane alone (Shinozuka *et al.*, 1988). [The Working Group noted the small number of animals used and the short duration of the study.]

Groups of 28-41 male Swiss Webster mice, six to seven weeks of age, received a single intraperitoneal injection of *N*-methyl-*N*-nitrosourea (MNU) at 0, 12.5 or 25 mg/kg bw [vehicle unspecified] and one week later were fed either basal diet or ciclosporin [purity unspecified] at 150 mg/kg of diet for 35 weeks. Mice treated with MNU and ciclosporin had four- and eight-fold higher incidences of thymic lymphomas, respectively, than mice treated with either dose of MNU alone ($< 2\%$) [figures not given]. Thymic lymphomas did not develop in mice treated with ciclosporin alone or maintained on basal diet (Shinozuka *et al.*, 1988). [The Working Group noted the incomplete reporting of the study.]

Rat: Groups of 10-12 male Sprague-Dawley rats, weighing 100-120 g, received a single intraperitoneal injection of 0 or 25 mg/kg bw MNU in 10% ethanol and citrate buffer; one week later, they were fed basal diet or ciclosporin [purity unspecified] at 110 mg/kg of diet for 34 weeks, at which time the experiment was terminated. Autopsies were carried out on all rats killed during the course or at the end of the experiment, and tissues from the thymus, mesenteric lymph nodes, intestinal lymphoid plaques, spleen, lung, kidney and liver were examined histologically. Of the rats receiving MNU and ciclosporin, 6/10 developed intestinal adenocarcinomas in the region of intestinal lymphoid plaques: two in the lower portion of the ileum and four in the ascending and transverse colon; two of the latter had two tumours each in the colon. The first tumour appeared in week 23 of the study. Of the rats receiving MNU alone, 1/12 developed an intestinal adenocarcinoma in week 33 of the study ($p < 0.05$). No intestinal tumour was observed in rats receiving ciclosporin or basal diet alone, but in rats treated with ciclosporin alone, atypical epithelial proliferations of the intestinal mucosa associated with hyperplasia of gut-associated lymphoid structures was observed (Perera *et al.*, 1986). [The Working Group noted the small number of animals used.]

Rat: Young male Wistar rats, weighing 62-80 g, were divided into six groups: group 1 (five animals) received daily subcutaneous injections of ciclosporin [purity unspecified] at 10 mg/kg bw in olive oil during week 1; group 2 (15 animals) received

daily subcutaneous injections of ciclosporin at 10 mg/kg bw in olive oil during week 1, followed by administration of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) at 83 μg/ml in the drinking-water *ad libitum* from week 3 to 28; group 3 (15 animals) received MNNG in the drinking-water from week 3 to 28; groups 4 and 5 (15 animals per group) received MNNG in the drinking-water in weeks 3-28 and daily subcutaneous injections of ciclosporin at 10 mg/kg bw during week 15 or during week 30; group 6 (ten animals) served as untreated controls. All surviving animals were sacrificed in week 39. No rat in group 1 or 6 died during the experiment, and no tumour was found in any animal in these groups. In group 2, 7/9 surviving rats had a total of 14 tumours (one intestinal carcinosarcoma, 13 adenocarcinomas of the stomach and small intestine; mean number of tumours per rat, 1.56). In group 3, 8/12 survivors had a total of 12 tumours (mostly adenocarcinomas of the stomach, small intestine or both; mean number of tumours per rat, 1.00). In group 4, 10/13 survivors had a total of 19 tumours (18 adenocarcinomas of the stomach, small intestine or both, and one large-cell lymphoma involving coeliac lymph nodes, liver and spleen; mean number of tumours per rat, 1.46). In group 5, 10/12 survivors had a total of 20 tumours (one carcinosarcoma, 19 adenocarcinomas of the stomach, small intestine or both; mean number of tumours per rat, 1.67). No statistical difference in the incidence of tumours was observed among groups 2-5 (Johnson *et al.*, 1984).

Monkey: A group of 55 macaques [age and sex unspecified] that had received cardiac or heart-lung allografts and had survived the first two post-operative weeks received daily intramuscular injections of ciclosporin [purity unspecified] at 25 mg/kg bw in miglyol 812 (an oil base) for 14 days, after which they were treated either every other day or daily with intramuscular injections of 17 mg/kg bw ciclosporin continuously. Eight subgroups were formed: group 1 (16 animals) received no treatment other than ciclosporin; group 2 (nine animals) was treated concurrently with 2 mg/kg bw azathioprine; group 3 (six animals) had previously received daily injections of 10 mg/kg bw rabbit antithymocyte globulin on post-operative days 0-7; group 4 (13 animals) received concurrent weekly treatment with 14 mg/kg bw antithymocyte globulin, azathioprine and methylprednisolone; group 5 (11 animals) had received total lymphoid radiation at a dose of 100 rads per day (total dose, 600-1800 rads) prior to operation; group 6 (ten animals) received injections of azathioprine plus methylprednisolone; group 7 (23 animals) received azathioprine, methylprednisolone and antithymocyte globulin; and group 8 (nine animals) received azathioprine, antithymocyte globulin and total lymphoid irradiation. No lymphoma was observed among animals receiving treatment other than with ciclosporin (groups 6-8). Of the animals treated with ciclosporin alone or in combination with other immunosuppressive agents, B-cell lymphomas developed in 12/55 monkeys [$p < 0.001$, Fisher's exact test]: 2/16 treated with

ciclosporin alone (group 1), 4/9 with ciclosporin plus azathioprine (group 2), 1/6 with ciclosporin plus antithymocyte globulin (group 3), 2/13 with ciclosporin, antithymocyte globulin, azathioprine and methylprednisolone (group 4), and 3/11 with ciclosporin and total lymphoid radiation (group 5). Viral particles were noted within the endoplasmic reticulum of plasmacytoid cells in 6/8 tumours from animals treated with ciclosporin alone or in combination with other immunosuppressive agents. The authors noted that the incidence of spontaneous haematopoietic neoplasms in nonhuman primates is generally considered to be low, although outbreaks of lymphomas have been reported among macaques (Bieber *et al.*, 1982).

3.2 Other relevant data

(*a*) *Experimental systems*

The experimental toxicology of ciclosporin has been widely reviewed (e.g., Feutren & Bach, 1987; Aszalos, 1988; Grace, 1988; de Groen, 1988; Humes & Jackson, 1988; Kahan *et al.*, 1988a,b; Mihatsch *et al.*, 1988a,b).

(i) *Absorption, distribution, excretion and metabolism*

The toxicokinetics and toxicodynamics of ciclosporin have been reviewed (Wood *et al.*, 1983; Maurer, 1985; Wood & Lemaire, 1985; Grevel, 1986a,b; Lemaire *et al.*, 1986).

Orally administered ciclosporin (in olive oil) was rapidly absorbed in dogs and rats. About 50% of a single dose reached the circulation (plasma levels determined by radioimmunoassay) in both species; there was no tendency for accumulation in beagle dogs after repeated daily administration for a year (Ryffel *et al.*, 1983).

A single oral administration of 82 mg/kg bw to WAG/Rij rats resulted in levels of 80 µg/g in liver, kidney and brain 3 and 7 h after administration. Slow elimination occurred thereafter: even after five days, significant amounts (10 µg/g) were detected. A short time after oral administration, 3.5 µg/ml of ciclosporin were detected in blood, and the levels remained almost the same for about two days; 2% of the administered dose was eliminated unchanged in bile and 2% in urine (Nooter *et al.*, 1984a). About 2% of an oral dose of ciclosporin was absorbed into the intestinal lymphatic system in rats (Ueda *et al.*, 1983).

Pharmacokinetic studies were also performed after intravenous administration of 20, 40 or 80 mg/kg bw to WAG/Rij rats (Nooter *et al.*, 1984b). Elimination of ciclosporin at the lowest dose was best described by a two-compartment model ($t_{½}$: 6 min and 16.5 h); at the higher dose levels, a three-compartment model best described the observed data. Urine and bile excretion was 10 and 20% of the total administered dose. The bioavailability of ciclosporin in Wistar rats increased with increasing oral dose. Daily oral administration of 4 mg/kg bw was necessary to

maintain plasma levels at about 130 ng/ml in very young rats, while 7.5 mg/kg bw per day were needed in one-month-old animals (Levy-Marchal *et al.*, 1988).

Absorption of orally administered tritium-labelled ciclosporin by Sprague-Dawley and Wistar rats was slow and was not affected by the vehicle. The degree of absorption was about 30%. Labelled ciclosporin was widely distributed throughout the body. The terminal elimination half-time of the radiolabel was 46 h after dosing with 10 mg/kg bw daily in olive oil for 21 days; elimination from kidney and liver had a half-time of 70-100 h. Accumulation of the parent compound was evident after repeated treatments, with high levels in kidney, liver, blood and lymph nodes and particularly in skin and adipose tissue (Wagner *et al.*, 1987).

In male CD-COBS rats treated intravenously, blood concentrations during elimination were best described by a three-compartment model, with half-times of 0.11 h, 1.8 h and 23.8 h. The apparent distribution volume ranged from 4.88 to 6.84 l/kg. Elimination was almost entirely by hepatic metabolism (Sangalli *et al.*, 1988). Total body clearance was lower in obese rats than in lean Zucker rats (Brunner *et al.*, 1988).

A non-linear pharmacokinetic behaviour was seen in New Zealand white rabbits injected intravenously. The volume of distribution at steady state increased with increasing dose (Awni & Sawchuk, 1985). The mean half-time after intravenous administration of 15 mg/kg bw to male New Zealand rabbits was 229.7 min (D'Souza *et al.*, 1988).

In rabbits, the concentrations of ciclosporin in blood were about 100 ng/ml from day 43 to 120 after repeated subcutaneous injections; the calculated absorption half-time was 33 days following injection with 20 mg/kg twice a week during days 7-29 of the experiment (Shah *et al.*, 1988). In BALB/c mice injected subcutaneously with 12.5, 50 or 200 mg/kg bw, ciclosporin was detected (by radioimmunoassay) in every organ investigated (Boland *et al.*, 1984). The organs in mice that are susceptible to toxicity (e.g., brain, kidney, liver) retained ciclosporin after intraperitoneal injection (Belitsky *et al.*, 1986).

Following oral, intraperitoneal, subcutaneous or intravenous administration of radiolabelled ciclosporin to C57Bl mice, a high initial concentration of radiolabel was observed in liver, pancreas, salivary glands, spleen and fat tissue by whole-body autoradiography. Relatively high levels were retained in liver, bone marrow, thymus and lymph nodes. In kidney, the radiolabel was confined to the outer zone and outer medulla. No radioactivity was seen in the central nervous system or in fetuses (Bäckman *et al.*, 1987, 1988).

When ciclosporin was mixed with human or rat blood *in vitro*, 50% was found in erythrocytes, 15% in leukocytes and 30-40% in plasma. At concentrations of

25-100 ng/ml in human plasma, 65-80% of tritiated ciclosporin was associated with lipoproteins (Lemaire & Tillement, 1982; Niederberger *et al.*, 1983).

Ciclosporin is extensively metabolized by cytochrome P450-mediated oxidation, hydroxylation and *N*-demethylation (Maurer *et al.*, 1984; Maurer, 1985; Burke & Whiting, 1986; Maurer & Lemaire, 1986; Bertault-Peres *et al.*, 1987; Wagner *et al.*, 1987). Figure 1 shows some characteristics of the metabolites that have been isolated. The numbers in the following text refer to the amino acids and metabolites identified in the figure.

Fig. 1. Structures and molecular weights of metabolites of ciclosporin that have been isolated[a]

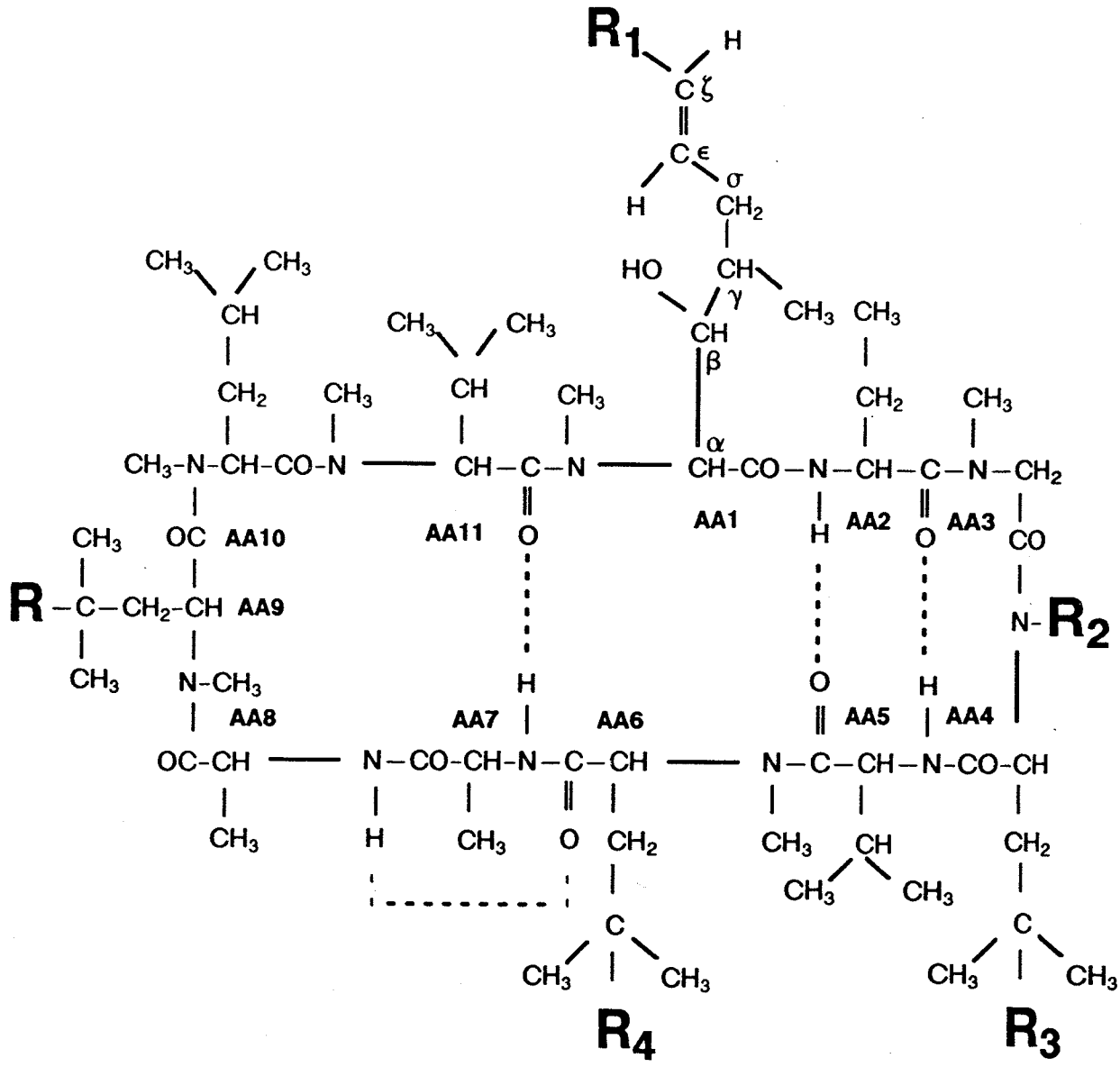


Fig. 1 (contd)

Metabolite no.	R	R_1	R_2	R_3	R_4	Other modification	Molecular weight
Ciclosporin	H	CH_3	CH_3	H	H		1202.64
1	OH	CH_3	CH_3	H	H		1218.64
8	OH	CH_2OH	CH_3	H	H		1234.64
9	OH	CH_3	H	H	OH		1220.62
10	OH	CH_3	CH_3	OH	H		1234.64
13	Hydroxylated and *N*-demethylated derivative of ciclosporin						1204.62
16	OH	CH_3	CH_3	H	OH		1234.64
17	H	CH_2OH	CH_3	H	H		1218.64
18	H	CH_2OH	CH_3	H	H	O / \ CH CH-CH_2 of AA1 β ϵ ζ	1218.64
22	H	CH_3	H	H	H		1188.62
25	H	CH_2OH	H	H	H		1202.64
26	OH	CH_2OH	CH_3	H	H	O / \ CH CH-CH_2 of AA1 β ϵ ζ	1204.62
203-218	H	COOH	CH_3	H	H		1234.64

[a]From Maurer & Lemaire (1986)

All ciclosporin metabolites from dog urine and from rat bile and faeces retained the intact cyclic oligopeptide structure of ciclosporin. Conjugations with sulfuric or glucuronic acid were not detected (Maurer *et al.*, 1984). Using perfused rabbit liver, 27 metabolites were characterized, including three dihydrodiol metabolites probably derived from epoxide intermediates (Wallemacq *et al.*, 1989a).

An α,β-unsaturated carboxylic acid metabolite of amino acid **9** (**AA9**) was isolated in rabbit urine after intravenous administration of ciclosporin (Hartman *et al.*, 1985). In a study on ciclosporin metabolism in rats, parent ciclosporin predominated over metabolites in blood. Metabolite **1** was found to be the major one in this species. Intraperitoneal injections of phenobarbital and methyl prednisolone to Wistar rats receiving daily subcutaneous treatments with ciclosporin decreased ciclosporin levels in blood (Pell *et al.*, 1988). In rats injected intravenously, covalently bound ciclosporin was detected in protein fractions of liver and kidney homogenates, and phenobarbital treatment enhanced adduct formation.

Covalent binding to protein was found *in vitro* after incubation of labelled ciclosporin with a rat liver microsomal fraction in the presence of NADPH. Binding also occurred in isolated hepatocytes. SKF-525A inhibited the covalent binding, and glutathione depletion increased ciclosporin binding to protein (Nagelkerke *et al.*, 1987).

No association of radioactivity was observed with cellular proteins or with DNA in liver homogenates from mice administered the drug parenterally (Bäckman *et al.*, 1987, 1988).

(ii) *Toxic effects*

The LD_{50}s for ciclosporin after a single oral administration to mice, rats and rabbits were 2.3, 1.5 and > 1.0 g/kg bw, respectively. The corresponding figures after a single intravenous administration were 107, 25 and > 10 mg/kg bw. Toxic signs were hyperventilation, drowsiness and muscular spasms. After oral administration, weight loss and diarrhoea were noted (Ryffel *et al.*, 1983, 1986).

Daily subcutaneous injections of ciclosporin into BALB/c mice at a dose of 200 mg/kg bw per day resulted in a median survival time of about 13 days. Nephrotoxicity, hypocellularity of the thymus, lymph nodes and spleen and fatty changes in the liver were observed; no abnormality of femoral bone marrow was found (Boland *et al.*, 1984).

Histological findings in OFA rats fed a diet containing ciclosporin for 13 weeks included leukocytosis, lymphopenia, hypochromic anaemia, monocytosis and eosinopenia without myelotoxic effects. Lymphoid tissues were atrophied. Doses of 45 mg/kg bw per day and more produced nephrotoxicity and hepatotoxicity. A chronic nonspecific gingivitis with atrophy of periodontal tissue was observed in treated rats. Nephrotoxicity and hepatotoxicity were also observed among rats administered ciclosporin orally for 104 weeks (Ryffel *et al.*, 1983).

OF1 mice were given ciclosporin in the diet at 1.4 and 16 mg/kg per day for 78 weeks. Females given the high dose had higher mortality rates than other mice and had haematological changes without myelotoxic signs (Ryffel *et al.*, 1983).

NZW and RB rabbits treated subcutaneously with ciclosporin at 15 mg/kg bw daily had weight loss and reduced food and water intake. High mortality was observed within 60 days of treatment, and animals had distended stomachs and intestines (Gratwohl *et al.*, 1986).

After intravenous treatment at 45 mg/kg bw day for four weeks, cynomolgus monkeys showed blood chemistry changes, marked neurological side-effects, and degenerative changes in kidney and liver. Rhesus monkeys tolerated high oral doses of ciclosporin (200-300 mg/kg bw) for 13 weeks, with small functional and histopathological changes (Ryffel *et al.*, 1983).

The renal effects of ciclosporin in experimental systems have been studied extensively and reviewed (Sullivan *et al.*, 1985; Ryffel & Mihatsch, 1986; Humes & Jackson, 1988).

The severity of histological changes in the kidneys of rats receiving subcutaneous injections daily for up to 30 days were directly correlated with tissue levels of ciclosporin (Kumar *et al.*, 1988).

Ciclosporin induced marked renal vasoconstriction in rats (Kaskel *et al.*, 1988; Monaco *et al.*, 1988; Stanley Nahman *et al.*, 1988) and sheep (Friedman *et al.*, 1988). Various defects in renal function accompanied the vasoconstriction, including decreased glomerular filtration rate (Whiting *et al.*, 1982; Sabbatini *et al.*, 1988; Tejani *et al.*, 1988), decreased sodium reabsorption (Whiting & Simpson, 1988), impairment of the diluting capacity of the thick ascending limb of the loop of Henle (Gnutzmann *et al.*, 1986) and release of cellular enzymes into the urine (Whiting *et al.*, 1986).

Sprague-Dawley rats given ciclosporin at 50 or 100 mg/kg bw per 48 h over 21 days by gastric intubation had elevated serum urea and creatinine levels, and urinary *N*-acetyl-β-D-glucosaminidase activity was increased (Thomson *et al.*, 1981; Whiting *et al.*, 1982). The renal and hepatic functional disturbances were reversible (Thomson *et al.*, 1981). There was cytoplasmic vacuolization of the proximal tubule, swollen cells and cell necrosis—the latter at the higher dose. Vacuolization was due to dilatation of smooth and rough endoplasmic reticulum. The number of lysosomes was increased, and myeloid bodies were present (Whiting *et al.*, 1982).

Rats given ciclosporin at 20 or 40 mg/kg bw in the diet showed augmentation of autoplagic vacuoles, lipid drops and loss of microvilli in the proximal nephron as well as prenecrotic damage of proximal tubular S2 and S3 cells (Pfaller *et al.*, 1986). Similar observations were made by Verani (1986), Jackson *et al.* (1987), Dieperink *et al.* (1988), Gillum *et al.* (1988), Jackson *et al.* (1988) and Starklint *et al.* (1988a,b), although strain differences have been reported (Duncan *et al.*, 1986).

When ciclosporin was given by gavage at 30 mg/kg bw per day to Sprague-Dawley rats for four weeks, serum testosterone levels were decreased by 50%; this change was reversible (Sikka *et al.*, 1988).

Rats injected intraperitoneally with ciclosporin at 5, 10 or 15 mg/kg bw for one or three weeks had significantly raised levels of serum bile acids. Both bile salt-dependent and independent-flow were decreased (Stone *et al.*, 1988).

Ciclosporin markedly decreased pancreatic insulin content and insulin release in rats administered the drug by intramuscular injection for two weeks (Hahn *et al.*, 1986). Electron microscopy demonstrated cytoplasmic degranulation, nuclear

inclusions and cisternal dilatation of endoplasmic reticulum and of the Golgi apparatus in pancreatic β cells (Hamaguchi *et al.*, 1988).

When Sprague-Dawley rats were fed ciclosporin at 150 mg/kg of diet, their thymuses and lymph nodes were smaller after eight weeks. Proliferative changes were observed in gut-associated lymphoid tissue, with mitotically active lymphocytes that displayed local tissue invasion and destruction (Demetris *et al.*, 1984).

Orál administration of immunosuppressive doses of ciclosporin reduced the trabecular bone volume of Sprague-Dawley rats. Osteoclast number and bone resorption were significantly increased at low (7.5 mg/kg bw per day) and high (15 mg/kg bw per day) doses of ciclosporin (Movsowitz *et al.*, 1988).

Thromboxane synthesis in rats and its excretion in urine were increased by ciclosporin treatment (Perico *et al.*, 1986a,b; Coffman *et al.*, 1987; Benigni *et al.*, 1988; Rogers *et al.*, 1988). Prostaglandin production was stimulated by ciclosporin (Coffman *et al.*, 1987), and administration of prostaglandin E_2 (Ryffel *et al.*, 1986) or its analogues (Paller, 1988a,b) reduced the nephrotoxicity of ciclosporin. A thromboxane synthetase inhibitor (CGS 12970) also prevented nephrotoxicity in rats (Smeesters *et al.*, 1988a,b).

Ciclosporin affected protein synthesis *in vivo* and *in vitro* (Bäckman *et al.*, 1988; Buss *et al.*, 1988), altered hepatic glycogen metabolism (Betschart *et al.*, 1988) and inhibited P450-dependent metabolism *in vivo* (Augustine & Zemaitis, 1986; Moochhala & Renton, 1986).

It induced dose-dependent malonaldehyde production in rat renal microsomes (Inselmann *et al.*, 1988). It bound with high affinity to cyclophilin, a low-molecular-weight cytosolic protein that occurs ubiquitously in eukaryotic cells and is thought to be a regulator of T- and B-cell activation (Harding & Handschumacher, 1988; Quesniaux *et al.*, 1988).

Ciclosporin inhibited T-lymphocyte proliferation (Borel *et al.*, 1977) but did not affect protein kinase C. It inhibited the augmentation of ornithine decarboxylase levels in mouse skin induced by phorbol ester (Elder *et al.*, 1988) and interfered with intracellular calcium metabolism (for reviews, see Aszalos, 1988; Bijsterbosch *et al.*, 1988).

(iii) *Effects on reproduction and prenatal toxicity*

In routine studies to evaluate the safety of ciclosporin, oral administration at 1.5, 5 or 15 mg/kg bw to male and female rats daily from before mating (males, 12 weeks; females, two weeks) until weaning had no adverse effect on reproduction. In rats administered ciclosporin at 10-300 mg/kg bw orally from day 6 to 15 of gestation, there was no embryotoxic effect at doses up to 17 mg/kg bw. At 30 mg/kg bw, which was clearly toxic to the mother, high rates of embryolethality (90%)

occurred, average fetal weights were lower than those of controls and skeletal retardations were seen frequently, but there was no increase in the frequency of minor or major anomalies. At higher doses, embryolethality was 100%. In a similarly designed study in rabbits, using doses of 10-300 mg/kg bw, no adverse effect was observed up to 30 mg/kg. At 100 mg/kg and above, maternal toxicity was seen, with an increased frequency of resorptions; however, no major or minor anomaly was found. In a peri-/postnatal study in rats at three dose levels (5, 15, and 45 mg/kg bw), a distinct increase in pre-/perinatal and early postnatal mortality of offspring was observed at the highest dose level (Ryffel *et al.*, 1983).

Two further studies confirm the toxic effects of ciclosporin on rat fetuses after daily exposure during late gestational stages at a maternally toxic dose (25 mg/kg). Fetal kidneys that could be examined showed evidence of ciclosporin-induced proximal tubular-cell damage (Brown *et al.*, 1985; Mason *et al.*, 1985).

When ciclosporin was administered subcutaneously for 14 days at daily doses of 10, 20 and 40 mg/kg bw to sexually mature male rats, dose-dependent changes in body and reproductive organ weights were noted. Histological examination of the testis showed degenerative changes, and sperm counts and motility were decreased in all three treated groups. Rats treated with the two highest doses were infertile (Seethalakshmi *et al.*, 1987). This effect was reversible after withdrawal of the drug (Seethalakshmi *et al.*, 1988).

(iv) *Genetic and related effects*

Ciclosporin did not induce mutation in *Salmonella typhimurium* in either the presence or absence of an exogenous metabolic system (Matter *et al.*, 1982).

It did not induce mutations at the *hprt* locus of Chinese hamster V79 cells in the presence or absence of an exogenous metabolic system (Zwanenburg *et al.*, 1988). It induced sister chromatid exchange in human peripheral lymphocytes *in vitro* (Yuzawa *et al.*, 1986, 1987).

At doses up to 1000-3000 mg/kg, ciclosporin did not induce chromosomal aberrations or micronuclei in bone-marrow cells of CD-1 mice or Chinese hamsters *in vivo*, or unscheduled DNA synthesis [dose unspecified] or dominant lethal mutations in CD-1 mice (Matter *et al.*, 1982).

(*b*) *Humans*

(i) *Pharmacokinetics*

The kinetics of ciclosporin has been reviewed (Bowers *et al.*, 1986; Grevel, 1986a,b; Lemaire *et al.*, 1986; Vine & Bowers, 1987; Grevel, 1988; McMillan, 1989). In studies on the kinetics of ciclosporin, radioimmunoassay and liquid chromatography have generally been used. If not indicated otherwise, the data

given below are from studies in which high-performance liquid chromatography analysis was used, which is the most specific for ciclosporin.

Absorption of orally administered ciclosporin is variable and low: the oral bioavailability was 35 ± 11% in heart transplant patients (Venkataramanan *et al.*, 1986), 36 ± 17% in adult uraemic patients (Grevel *et al.*, 1989) and 27 ± 20% in 41 renal transplant recipients; it was < 10% in 17% of these subjects (Ptachcinski *et al.*, 1985). Peak blood ciclosporin concentrations were reached between 1 and 8 h after oral dosing (Beveridge *et al.*, 1981; Ptachcinski *et al.*, 1985; Venkataramanan *et al.*, 1986).

Ciclosporin is rapidly and widely distributed; distribution half-times after intravenous administration have been reported to be 0.1 ± 0.03 h (Follath *et al.*, 1983) and 0.3-0.5 h (Yee *et al.*, 1984). The steady-state apparent volume of distribution is large, and means of 2.7-5.1 l/kg have been calculated (Follath *et al.*, 1983; Yee *et al.*, 1984; Ptachcinski *et al.*, 1985; Venkataramanan *et al.*, 1986; Clardy *et al.*, 1988). Concentrations of ciclosporin in rejected kidney were higher than preoperative values in the blood of three patients (Kahn *et al.*, 1986; Rosano *et al.*, 1986). High concentrations of ciclosporin and its metabolites are found in, e.g., fat, gall-bladder, liver, gastrointestinal tract and pancreas (Atkinson *et al.*, 1983a; Kahan *et al.*, 1983a; Ried *et al.*, 1983).

After the distribution phase, two further first-order disappearance phases may be discerned, with half-times of approximately 1 and 16 h, respectively (Follath *et al.*, 1983). Even in a case of acute overdose of ciclosporin (5000 mg), saturation of clearance was not observed (Schroeder *et al.*, 1986). Clearance of ciclosporin from the blood is rapid: in bone-marrow transplant recipients with normal liver and kidney function, clearance of 12.8 ± 1.6 ml/min per kg was reported; in those with elevated serum bilirubin but normal renal function, it was 9.8 ± 2.1 ml/min per kg. In another study, however, no relationship was noted between the disappearance of ciclosporin from the blood and the degree of impairment of hepatic function in patients with primary biliary cirrhosis (Robson *et al.*, 1984). In renal and heart transplant recipients, average clearance values of 6.5 and 5.7 ml/min per kg were reported (Ptachcinski *et al.*, 1985; Venkataramanan *et al.*, 1986), while in patients with renal failure clearance was 369 ml/kg per h [6.15 ml/min per kg] (Follath *et al.*, 1983). In healthy subjects, a value of 51 ml/h per kg [8.5 ml/min per kg] was reported (Grevel *et al.*, 1986); in this study, however, the radioimmunological assay method was used, which provides an underestimate of clearance (Grevel *et al.*, 1989).

After administration of tritiated ciclosporin to two patients, 6% of the dose was recovered in the urine (Maurer *et al.*, 1984; Maurer, 1985; Lemaire *et al.*, 1986). In healthy volunteers, approximately 0.1-0.2% of a dose was excreted in the urine as unchanged ciclosporin (Beveridge *et al.*, 1981; Maurer & Lemaire, 1986).

More ciclosporin and ciclosporin metabolites were detected in the bile than in urine after intravenous and oral administrations (Kahan *et al.*, 1983b; Venkataramanan *et al.*, 1985). Unchanged ciclosporin is a minor component in the bile (mean, 0.29% of an oral dose) (Venkataramanan *et al.*, 1985).

The concentration of ciclosporin in blood cells is approximately double that in the plasma (Follath *et al.*, 1983). The majority of ciclosporin and/or its metabolites in serum is bound to different lipoprotein fractions (Mraz *et al.*, 1983; Gurecki *et al.*, 1985). After treatment of pregnant women with ciclosporin, it was detected in cord blood at concentrations somewhat lower than those in maternal blood (Lewis *et al.*, 1983; Venkataramanan *et al.*, 1988; Rose *et al.*, 1989). Ciclosporin has also been detected in breast milk (Lewis *et al.*, 1983).

The first study of the metabolism of ciclosporin in humans was performed by Maurer *et al.* (1984), who isolated and identified nine ether-extractable metabolites from the urine of two patients who had received a single oral dose of 300 mg ^{3}H-ciclosporin. All identified metabolites retained the intact cyclic peptide structure; the sites on the molecule that are changed by metabolism are indicated in Figure 1. The primary metabolites were products of hydroxylation; the secondary metabolites identified were products of oxidation or demethylation of oxidized primary metabolites or of a cyclization reaction. Similar oxidized ciclosporin metabolites have been identified in the blood and bile of patients treated with ciclosporin (Hartman *et al.*, 1985; Rosano *et al.*, 1986; Lensmayer *et al.*, 1987a,b; Wallemacq *et al.*, 1989a,b; Wang *et al.*, 1989). Twenty-seven ciclosporin metabolites were identified in human bile; these included a vicinal dihydrodiol and a demethylated vicinal dihydrodiol, suggesting that an epoxide is the intermediate (Wallemacq *et al.*, 1989a).

In addition to metabolites generated by oxidation, demethylation and cyclization reactions, three further metabolites have been isolated in which the double bond in amino acid **1** (**AA1** in Fig. 1) is probably saturated (Wang *et al.*, 1989). This metabolite and metabolites **1**, **8**, **17** and **203-218** (Fig. 1) were reported to be the major metabolites of ciclosporin in human bile (Hartman *et al.*, 1985; Maurer, 1985; Wang *et al.*, 1989; Maurer & Lemaire, 1986). A sulfate conjugate of ciclosporin was also identified in human bile and plasma (Henricsson *et al.*, 1989). Metabolite **17** was the main metabolite in human blood, and metabolites **1**, **8** and **21** were the other major ones (Maurer, 1985; Maurer & Lemaire, 1986; Rosano *et al.*, 1986). Metabolite **17** was the main metabolite detected in kidney (Rosano *et al.*, 1986).

A cytochrome P450 isolated from human liver catalysed the formation of mono- and dihydroxylated and demethylated metabolites from ciclosporin (Combalbert *et al.*, 1989). This cytochrome is encoded by the gene P450IIIA3, as is

nifedipine oxidase; it is induced by treatment with rifampicin (Kronbach *et al.*, 1988; Combalbert *et al.*, 1989).

(ii) *Immunosuppressive action*

The pharmacological effects of ciclosporin on the human immune system have been reviewed (Thomson, 1983; Shevach, 1985; Drugge & Handschumacher, 1988; Kerman, 1988; Kahan, 1989; Lorber, 1989). The ratio of T-helper cells to T-suppressor cells was decreased in renal transplant recipients during treatment with ciclosporin and prednisolone (Kerman *et al.*, 1987). Production of α-interferon, γ-interferon and interleukin-2 by isolated leukocytes was decreased in renal and heart transplant patients receiving ciclosporin and prednisolone, as compared to healthy volunteers (Dupont *et al.*, 1985).

Many studies have been published on the immunosuppressive effects of ciclosporin since the detection (Borel *et al.*, 1977) of its biological and clinical significance in the early 1970s (for review, see Feutren & Bach, 1987). Its immunosuppressive effects have been demonstrated experimentally to lead to tolerance of tissue grafts (Morris *et al.*, 1980; Pennock *et al.*, 1981; Bain *et al.*, 1988; Chisholm & Bevan, 1988; Finsen *et al.*, 1988; Kimura *et al.*, 1988; Lear *et al.*, 1988; White & Lim, 1988; for reviews, see Lorber, 1986; Tutschka, 1986; Hopt *et al.*, 1988; Kahan *et al.*, 1988a,b) and to affect a variety of experimental autoimmune diseases, such as uveitis (Nordmann *et al.*, 1986; Dinning *et al.*, 1987; Mahlberg *et al.*, 1987; Caspi *et al*, 1988a,b; Kaswan *et al.*, 1988), myasthenia gravis (for review see Feutren & Bach, 1987; for a tabular summary, see Gunn *et al.*, 1988), mercuric chloride-induced glomerulonephritis (Aten *et al.*, 1988), allergic encephalomyelitis (Polman *et al.*, 1988) and serum sickness nephritis (Shigematsu & Koyama, 1988).

Ciclosporin is preferentially active on proliferating T cells (White *et al.*, 1979) and selectively inhibits T-helper cell function (Caspi *et al.*, 1988a,b) while sparing T-suppressor cell activities (Kupiec-Weglinski *et al.*, 1984; Bucy, 1988). It inhibits the production of interleukin-2 (Larsson, 1980; Bunjes *et al.*, 1981; Caspi *et al.*, 1988b; Tracey *et al.*, 1988) from T-helper cells and of interleukin-1 from splenic adherent cells (Bunjes *et al.*, 1981). Ciclosporin metabolites also suppressed concanavalin A-stimulated human peripheral blood mononuclear cell proliferation (Cheung *et al.*, 1988).

Ciclosporin was bound to a low-affinity site (K_D = 3-6 × 10^{-7} M) on human splenic T-lymphocytes *in vitro*, while B-lymphocytes showed both a high-affinity ($K_D = 2 \times 10^{-9}$ M) and a low-affinity binding site (LeGrue *et al.*, 1983).

Ciclosporin depressed the synthesis of γ-interferon by human thymocytes and T-lymphocytes *in vitro* (Reem *et al.*, 1983; McKenna *et al.*, 1989), as well as the synthesis of lymphotoxin and tumour necrosis factor by lymphocytes activated in mixed-lymphocyte culture or by concanavalin A (McKenna *et al.*, 1989; Szturm *et*

al., 1989). Ciclosporin reduced T-cell growth factor (interleukin-2) gene transcription in a cloned human leukaemic T-cell line (Krönke *et al.*, 1984) and binding of radiolabelled human recombinant interleukin-2 to high-affinity receptors in human T-lymphocytes (Povlsen *et al.*, 1989). Ciclosporin also inhibited the release of γ-interferon from alloactivated human peripheral blood mononuclear cells (Bishop & Hall, 1988).

(iii) *Adverse effects*

The adverse effects of ciclosporin therapy have been reviewed (Kahan *et al.*, 1985; Bennett & Norman, 1986; Myers, 1986; Keown *et al.*, 1987; Mihatsch *et al.*, 1988a,b; Racusen & Solez, 1988; Schachter, 1988; Weidle & Vlasses, 1988; Dieperink, 1989; Mihatsch *et al.*, 1989; Reynolds, 1989; Steinmuller, 1989).

The first report on the use of ciclosporin in the treatment of renal allograft rejection (Calne *et al.*, 1978) documented nephrotoxicity, hepatotoxicity and hirsutism as side-effects of the therapy. Nephrotoxicity has since been amply documented as the most prevalent and serious complication of ciclosporin therapy, in recipients of kidney transplants (Calne *et al.*, 1979; Klintmalm *et al.*, 1981a,b; Merion *et al.*, 1984) and in other transplant recipients (Powles *et al.*, 1980; Klintmalm *et al.*, 1981b; Shulman *et al.*, 1981; Atkinson *et al.*, 1983b; Hows *et al.*, 1983; Myers *et al.*, 1984). Morphological changes related to ciclosporin administration include diffuse interstitial fibrosis (associated with oligo- or anuria), tubular toxicity, peritubular capillary congestion and a combination of the last two. These two changes have been associated with acute renal damage; acutely impaired renal function was not, however, necessarily accompanied by microscopic changes. Arteriolopathy and interstitial fibrosis with tubular atropy, or a combination of the two, have been attributed to chronic ciclosporin toxicity (Mihatsch *et al.*, 1988a,b, 1989). Mechanisms of the renal toxicity of ciclosporin have been reviewed (Bennett *et al.*, 1988; Dieperink *et al.*, 1988; Grace, 1988; Neild, 1988; Benigni *et al.*, 1989).

Mild functional disturbances of the liver have been reported in 20-40% of treated patients (Klintmalm *et al.*, 1981a; Kahan *et al.*, 1985).

Other side-effects reported include gastrointestinal disturbances, hirsutism, acne, gingival hyperplasia, neurotoxicity, altered blood coagulability, hypertension, electrolyte changes and gout. Anaphylactoid reactions have occurred following intravenous administration of preparations containing ciclosporin (Kahan *et al.*, 1985; Bennett & Norman, 1986; Weidle & Vlasses, 1988; Lin *et al.*, 1989; Reynolds, 1989).

(iii) *Effects on reproduction and prenatal toxicity*

In two of three published reports of babies born to mothers treated throughout pregnancy with ciclosporin (Lewis *et al.*, 1983; Klintmalm *et al.*, 1984; Endler *et al.*,

1987), growth was retarded. However, whether this effect was due to the drug or to the general condition of the mother is uncertain.

(iv) *Genetic and related effects*

A group of 25 kidney transplant patients received daily oral treatment with ciclosporin at 12-14 mg/kg bw (reduced to 4 mg/kg) combined with variable doses of prednisolone for over one year (Fukuda *et al.*, 1987). In an extension of this study (Fukuda *et al.*, 1988), the number of patients was increased to 40. More patients receiving ciclosporin had chromosomal aberrations in their peripheral lymphocytes (68% and 48% in the two studies, respectively) than did 50 healthy individuals (0%) or 50 haemodialysis patients (2%). [The Working Group noted the poor reporting of the studies and that cells were cultured for 72 h.]

Unscheduled DNA synthesis was reported to be elevated in the lymphocytes of kidney transplant patients treated with ciclosporin [dose and length of treatment unspecified] in comparison with those from healthy individuals (Petitjean *et al.*, 1986). [The Working Group noted the incomplete reporting of the study.]

3.3 Case reports and epidemiological studies of carcinogenicity to humans

(*a*) *Case reports*

Numerous case reports have been published of neoplasms occurring in organ transplant recipients who received only ciclosporin, without azathioprine or cytotoxic agents. The majority of these neoplasms were lymphomas, commonly of the gastrointestinal tract (Thiru *et al.*, 1981; Beveridge *et al.*, 1984; Bencini *et al.*, 1985; Bloom *et al.*, 1985; Castro *et al.*, 1985; Thompson *et al.*, 1985; Walker *et al.*, 1989), but Kaposi's sarcoma and skin cancers have also been reported (Thompson *et al.*, 1985; Gorg *et al.*, 1986; Arico *et al.*, 1987; Cockburn, 1987; Bencini *et al.*, 1988; Civati *et al.*, 1988). Malignancies at other sites have also been seen (Maung *et al.*, 1985; Thompson *et al.*, 1985). Regression of lymphomas when the drug was discontinued has sometimes been reported (Bencini *et al.*, 1988).

In the most recent report from a registry of organ transplant recipients who developed tumours (Penn & Brunson, 1988), 412 tumours had been recorded in ciclosporin-treated patients. Of these, the most frequently reported were lymphoma (29%), skin cancer (22%) and Kaposi's sarcoma (11%). [The Working Group noted that the size of the underlying population was unknown; but, given the low incidence of Kaposi's sarcoma in the general population, the number of cases in this registry is strikingly large.]

Cockburn and Krupp (1989) described the occurrence of 186 neoplasms in organ transplant recipients treated with ciclosporin and reported to the drug manufacturer. The most frequent malignancies were lymphomas and leukaemias

(55 cases) and Kaposi's sarcoma (26 cases). The lymphomas were found predominantly in the gastrointestinal tract.

(*b*) *Cohort studies*

Anderson *et al.* (1978) reported that among 143 cardiac transplant recipients treated with ciclosporin and other immunosuppressive agents, six developed lymphomas.

Calne *et al.* (1979) followed up 34 organ transplant recipients treated with ciclosporin, six of whom had also received a cyclophosphamide derivative; three lymphomas developed—two in patients treated with ciclosporin only and one in a patient treated with both drugs.

Starzl *et al.* (1984) reported lymphoproliferative lesions (15 lymphomas, two other lesions) that occurred during follow-up in eight of 315 renal transplant, four of 129 heart transplant, three of 48 liver transplant and two of six heart-lung transplant patients treated, in general, with ciclosporin alone. In seven renal transplant patients with these lesions who were operated on for bowel perforation, discontinuation of ciclosporin treatment resulted in tumour regression, as determined by a second laparotomy.

Bencini *et al.* (1986) followed 67 renal transplant recipients treated with ciclosporin for 1-17 months (mean, 3.2 months); one developed a squamous epithelioma and one, skin nodules thought to be a lymphoma.

Sheil *et al.* (1987) reported three-year results of a trial of ciclosporin in renal transplant patients. One malignant melanoma and one adenocarcinoma of the remaining kidney were observed among 140 renal transplant patients receiving long-term treatment with ciclosporin alone, while no tumour was reported among a further 140 patients who received treatment with ciclosporin alone for three months followed by treatment with azathioprine.

Smith *et al.* (1989) reported that lymphomas developed in two of 712 organ transplant patients who received azathioprine, none of 160 treated with ciclosporin and seven of 132 who received both.

Cockburn and Krupp (1989) followed up 4040 organ transplant recipients treated with ciclosporin and compared observed with expected numbers based on population rates. Increased risks were noted for lymphoma (relative risk, 27.5; 11 cases observed), skin cancers (6.8; 11) and urinary-tract cancers (5.9; 11). [The Working Group noted that it was not clear that the only immunosuppressive treatment received was ciclosporin.]

Table 1 summarizes the studies in which lymphomas were reported in transplant patients who had received ciclosporin but not azathioprine or cytotoxic drugs. The Working Group estimated upper limits for the expected values (not

Table 1. Non-Hodgkin's lymphomas in organ transplant patients treated with ciclosporin (without azathioprine or cytotoxic drugs)

No. of patients	Maximal follow-up (years)	Non-Hodgkin's lymphomas		Reference
		Expected[a]	Observed	
28	1.5	0.02	2	Calne *et al.* (1979)
498	4	1.0	11	Starzl *et al.* (1984)
67	1.5	0.05	0	Bencini *et al.* (1986)
120	5[b]	0.3	0	Sheil *et al.* (1987)
160	5	0.4	0	Smith *et al.* (1989)
873 (total)		1.8	13	

[a]As estimated by the Working Group
[b]Mean, as given in paper

provided in the original papers), on the basis of assumptions adverse to a causal relationship, as follows:

(i) When the total period of follow-up was not given, the time of observation of every patient was equivalent to the maximal observation time of the relevant study.

(ii) The incidence rate for any age group below 70 years was the highest published in the Connecticut Tumor Registry (higher than in any UK or Australian registry), i.e., 50/100 000 per year (Muir *et al.*, 1987).

(iii) All patients followed up received only ciclosporin. In fact, it is known that some had received other agents, but only patients with lymphomas who had not received other agents were included in the count of observed cases.

Even with the above assumptions, the occurrence of lymphomas was remarkably high.

[The Working Group noted that in many studies no information on dose, survival or follow-up time was given for any group, and it was difficult to compare rates. As is clear from estimates of expected numbers made by the Working Group, however, the incidence of lymphoma in the cohort studies is remarkably high. In addition, Kaposi's sarcoma has figured prominently in case reports. It is also noteworthy that lymphomas regressed following discontinuation of ciclosporin in two studies. A higher incidence of lymphomas was noted when ciclosporin was

used in combination with other imunosuppressive agents, as was a frequent practice soon after its introduction (Anderson *et al.*, 1978; Calne *et al.*, 1979; Kinlen, 1982; Beveridge *et al.*, 1984). This is consistent with other evidence that the intensity of immunosuppression has an important influence on lymphoma incidence.]

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Ciclosporin has been used as an immunosuppressive agent since the mid-1980s.

4.2 Experimental carcinogenicity data

Ciclosporin was tested for carcinogenicity by oral administration in two studies in mice and in one study in rats. In one study in mice, it accelerated the development of leukaemias; tumours were not induced in a chronic bioassay. In rats, negative results were obtained in a study with limited sensitivity.

Ciclosporin enhanced the development of lymphomas induced in two strains of male mice by single whole-body irradiation or *N*-methyl-*N*-nitrosourea. In grafted macaques, ciclosporin increased the incidence of lymphomas, a neoplasm that occurs extremely infrequently in this species of monkeys. When given in combination with various other immunosuppressive regimens, ciclosporin induced a substantial increase in the incidence of lymphomas when compared to immunosuppressive regimens excluding ciclosporin. This drug also enhanced the incidence of intestinal adenocarcinomas induced in male rats by *N*-methyl-*N*-nitrosourea.

4.3 Human carcinogenicity data

In case reports, both lymphomas and Kaposi's sarcoma have been associated frequently with exposure to ciclosporin. Four cohort studies recorded a high incidence of lymphoma in organ transplant recipients; in two of these, ciclosporin was given without azathioprine or cytotoxic drugs. In several cases, there has been well-documented regression of lymphoma following withdrawal of the drug.

4.4 Other relevant data

Ciclosporin induced dose-dependent changes in reproductive organ weights in male rats and caused sterility at high doses. Fetal mortality was observed in rats and rabbits when the drug was administered during the second half of gestation at maternally toxic doses. No other sign of embryo- or fetotoxicity was noted.

Ciclosporin is rapidly absorbed and widely distributed in humans and in experimental animals. It is extensively metabolized by the cytochrome P450 system. Adverse effects include nephro- and hepatotoxicity. The compound is immunosuppressive, resulting in tolerance to tissue grafts; its main effect is on the early proliferation of T-cells.

In a single study, ciclosporin was reported to increase the incidence of chromosomal aberrations in the lymphocytes of kidney transplant patients.

Ciclosporin did not induce dominant lethal mutations in mice, chromosomal aberrations in the bone marrow of Chinese hamsters or micronuclei in the bone marrow of Chinese hamsters or mice *in vivo*. It induced sister chromatid exchange in human peripheral lymphocytes *in vitro* but did not induce gene mutations in Chinese hamster cells. Ciclosporin did not induce mutations in *Salmonella typhimurium*. (See Appendix 1.)

4.5 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity of ciclosporin in humans.

There is *limited evidence* for the carcinogenicity of ciclosporin in experimental animals.

Overall evaluation

Ciclosporin *is carcinogenic to humans (Group 1).*

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[1]For description of the italicized terms, see Preamble, pp. 26–29.

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