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Cyclosporin Increases the CNS Sensitivity to the Hypnotic Effect of Phenobarbitone but not Ethanol in Rats

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Abstract—The purpose of this investigation was to determine whether repetitive administration of cyclosporin affects the pharmacodynamics of phenobarbitone- and ethanol-induced anaesthesia. Sabra male rats received either cyclosporin (50 mg kg^{-1} day^{-1}, i.m.) for four days, or the same volume of the vehicle. Two hours after the last cyclosporin dose, phenobarbitone or ethanol solutions were infused intravenously at a constant rate until the onset of anaesthesia. Repetitive treatment with cyclosporin increased the CNS sensitivity to the hypnotic action of phenobarbitone. This was evidenced by the lower CSF phenobarbitone concentration, at the onset of the hypnotic effect, in the cyclosporin-treated group vs control values (115 ± 4 vs 93 ± 7 mg L^{-1}, $P = 0{\cdot}01$). However, the same pretreatment had no apparent effect on the pharmacodynamics of ethanol-induced sleep. It is suggested that anaesthesiologists must be alert to the possible increase in brain sensitivity when placing cyclosporin patients under anaesthesia with barbiturates.

Treatment with cyclosporin, a widely used immunosuppressive agent, is frequently associated with neurotoxic episodes including headaches, confusion, tremor, ataxia, seizure and coma (De Groen et al 1987; McEvoy 1991). The impact of this immunosuppressive agent on the CNS could theoretically alter the pharmacodynamics of neuroactive drugs.

There exists a clinical impression that pretreatment of patients with cyclosporin for transplantation prolongs general anaesthesia (Cirella et al 1987). This concept has been verified in mice, where a single cyclosporin dose of 60 mg kg^{-1} significantly lengthened pentobarbitone sleeping time and increased fentanyl analgesia. This intensified activity could result from a pharmacokinetic mechanism or by increased CNS sensitivity to the anaesthetic agent.

Previously, we had investigated the mechanism of this drug-drug interaction in rats, using heptabarbitone as an anaesthetic drug model (Hoffman & Levy 1990). While no significant effect on the pharmacokinetics of the barbiturate was observed, cyclosporin prolonged barbiturate-induced general anaesthesia by a pharmacodynamic mechanism. This effect was more pronounced following three days pretreatment with cyclosporin (50 mg kg^{-1} day^{-1}) than following a single dose of cyclosporin; 22 and 16% lower CSF heptabarbitone concentrations were required to induce onset of sleep. However, the effect of the same acute cyclosporin pretreatment failed to demonstrate any alteration in the concentrations of phenobarbitone at the onset of loss of righting reflex (LRR) (Ramzan 1988). Several differences exist between the two barbiturates, phenobarbitone and heptabarbitone, phenobarbitone is less lipophilic, has a very long biological half-life, has a slower penetration to the brain, and is less potent in producing hypnotic-anaesthetic action (Rall & Schleifer 1990). Harris (1990) concluded that all barbiturates do not share the same site of action on the GABA-activated chloride channels; thus, it is not appropriate to regard the barbiturates as a homogeneous class of durgs, and it is pertinent to investigate the effect of cyclosporin on anaesthetic action and to examine whether repetitive administration of this immunosuppressive agent can affect the pharmacodynamics of phenobarbitone.

We have extended this investigation to compare the effects of the same pretreatment on the pharmacodynamics of ethanol-induced anaesthesia since its depressant activity is also mediated by the GABA-chloride channel complex (Harris 1990). Unlike phenobarbitone, pentobarbitone, thiopentone and methohexital (the most clinically used anaesthetic barbiturates) are racemic mixtures and therefore they are not suitable as model drugs for the investigation of pharmacodynamic drug-drug interaction.

The experimental strategy used in this study permits determination of the relationship between drug concentration (rather than dose) and pharmacologic effect, without interference of pharmacokinetic variables (Danhof & Levy 1984). It has been established that phenobarbitone concentrations in the CSF (but not in the serum or the whole brain) at the onset of LRR are independent of pharmacokinetic variables and reflect the drug concentrations at the site of action (biophase). On the other hand, ethanol distributes very rapidly from the blood to the receptor sites in the brain. Consequently, ethanol concentrations in the serum, brain, or CSF are equally suitable as sampling sites for pharmacodynamic evaluation. It is important to use this experimental model in view of the many reported cyclosporin-drug interactions attributed to pharmacokinetic mechanisms (Carstensen et al 1986; McEvoy 1991).

Materials and Methods

Male Sabra rats, 200–250 g, were acquired from the Animal Breeding Unit of the Hebrew University-Hadassah Medical School. During the experimental period, all animals were

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housed in individual metal cages in a light controlled room (lights on from 0700 to 1900 h), and were maintained on freely available laboratory chow and water. Rats designated for treatment with cyclosporin received an intramuscular (i.m.) injection of cyclosporin (Sandimmune I.V.; Sandoz Pharmaceuticals, East Hanover, NJ) (50 mg kg^{-1} day^{-1}) for four days, whereas control animals received injections of the same volume of the vehicle (65% Cremophor EL (polyoxyethylated castor oil) in ethanol), both under light ether anaesthesia.

One day before the pharmacodynamic experiment, a blood sample (0·3 mL) was withdrawn for haematological evaluation by Coulter-Counter Model S-plus (Counter Electronics, Luton, UK) and an indwelling cannula was implanted in the right jugular vein (Weeks & Davis 1964), under light ether anaesthesia. The cannulae were filled with heparin-free saline.

To determine the effect of pretreatment with multiple doses of cyclosporin on the pharmacodynamics of the anaesthetic action of phenobarbitone, 2 h after the last dose of cyclosporin, sodium phenobarbitone (40 mg mL^{-1} in water) was administered intravenously to rats at a constant rate of 1·2 mL h^{-1} (48 mg h^{-1}) until the predefined pharmacological endpoint, onset of LRR. The LRR was determined without nociceptive stimulus (i.e. pressure on the tail). Concurrently, samples of CSF from the cisterna magna, blood (for serum) from the abdominal aorta and brain (which was stripped of its external vasculature and meninges), were taken in this order and frozen pending analysis.

To investigate the effect of pretreatment with cyclosporin on the concentration-hypnotic effect of ethanol, 2 h after the last cyclosporin injection, a solution of 20% v/v ethanol in water was infused at a constant rate of 0·106 mL min^{-1} (equivalent to 16·3 mg min^{-1}) until onset of LRR, at which point a large blood sample (for serum) was withdrawn through the jugular vein cannula. Throughout the experiments the rats were placed on thermal pads to maintain normal body temperature. The rectal temperature was measured just before the pharmacodynamic experiments.

Phenobarbitone concentrations in serum, brain and CSF were assayed by a high-performance liquid chromatography (HPLC) (HPLC system and Data System 450, Kontron Instruments, Switzerland), using a modified method of Danhof & Levy (1984). The standard curve was linear over the range 0–400 mg L^{-1} ($r > 0·996$).

Ethanol concentrations in the serum were determined by a commercially available kit (no. 332-uv, Sigma Chemical Co., St Louis, MO). The standard curve was linear over the range 0–0·16% w/v ($r = 0·992$).

Serum urea nitrogen, total serum protein and transaminase enzymes were determined using commercially available kits (no. 535, 540, 505, respectively, Sigma Chemical Co., St Louis, MO).

Since the test for homogeneity of variances (Bartlett's test) revealed some cases of heteroscedasticity, and because of the relatively low number of samples, the non-parametric Mann-Whitney test was used in all the statistical analyses; a P value of $< 0·05$ was considered statistically significant. Results were reported as mean ± s.e.m.

Results

A description of the animals used in the phenobarbitone investigation is summarized in Table 1. Pretreatment with cyclosporin had no effect on serum alanine or aspartate aminotransferase activities. It did cause a minor, but statistically significant ($P < 0·01$), elevation in serum urea nitrogen (within the normal range) and a decrease in rectal temperature. The effect of cyclosporin treatment on certain haematologic indices of the animals used in both pharmacodynamic experiments is summarized in Table 2. Cyclosporin did not alter the total white blood cell count, or the lymphocyte or neutrophil count, but it did significantly elevate ($P > 0·04$) the haematocrit and the red blood cell count, and markedly reduced the platelet count ($P < 0·01$), as expected (McEvoy 1991).

The results of the experiment examining the effect of pretreatment with cyclosporin (50 mg kg^{-1} day^{-1} for 4 days) on the pharmacodynamics of phenobarbitone are summarized in Table 3. The phenobarbitone infusion time and the dose required to induce onset of LRR were significantly ($P < 0·05$) lower in cyclosporin-treated animals. Phenobarbitone concentration in the CSF and serum at the onset of LRR were significantly ($P < 0·05$) lower in cyclosporin-treated animals. There was also a tendency toward lower phenobarbitone concentrations in the brain of animals treated with cyclosporin compared with control animals. It is interesting to note that the differences between the brain phenobarbitone concentrations of the two groups were not statistically significant. The same phenomenon occurred in the cyclosporin-heptabarbitone pharma-

Table 1. Description of male Sabra rats used in the phenobarbitone investigation.

	Control	Treated group
No. of animals	10	10
Body weight (g)	231 ± 13	238 ± 16
Rectal temperature (°C)	37·8 ± 0·3	36·8 ± 0·3*
Serum urea nitrogen (mg dL^{-1})	12·1 ± 0·8	33·7 ± 2·5*
Serum alanine aminotransferase (units L^{-1})	45·7 ± 1·1	45·1 ± 0·9
Serum aspartate aminotransferase (units L^{-1})	96·1 ± 2·1	94·3 ± 2·4

Results are reported as mean ± s.e. *$P < 0·05$ compared with control group.

Table 2. Effect of pretreatment with cyclosporin for four days on haematologic indices.

	Control	Treated group
No. of animals†	24	23
Total white blood cells (10^3 mL^{-1})	3·5 ± 0·6	3·2 ± 0·3
Lymphocytes (%)	75·6 ± 4·7	80·6 ± 3·5
Neutrophils (%)	14·1 ± 3·1	10·1 ± 2·1
Red blood cell	7·4 ± 0·1	8·0 ± 0·2*
Haemoglobin	14·2 ± 0·2	14·6 ± 0·4
Haematocrit (%)	45·1 ± 0·6	48·1 ± 1·3*
Platelets	467 ± 55	144 ± 24*

†Combined data of the phenobarbitone and ethanol studies. Results are reported as mean ± s.e. *$P < 0·05$ compared with control group.

Table 3. Effect of four days of pretreatment with cyclosporin on phenobarbitone concentrations at onset of LRR in rats receiving an intravenous infusion of phenobarbitone.

	Control	Treated group
Infusion time (min)	39·9 ± 2·9	34·4 ± 1·3*
Total dose (mg kg^{-1})	136 ± 6	117 ± 6*
CSF concn (mg L^{-1})	115 ± 4	93 ± 7*
Brain concn (mg kg^{-1})	174 ± 7	158 ± 11
Serum concn (mg L^{-1})	227 ± 10	176 ± 13*

Phenobarbitone was infused at a rate of 0·8 mg min^{-1}. Results are reported as mean ± s.e. *$P < 0.05$ compared with control group.

Table 4. Description of male Sabra rats used in the ethanol investigation.

	Control	Treated group
No. of animals	14	13
Body weight (g)	227 ± 9	235 ± 13
Rectal temperature (°C)	37·5 ± 0·2	36·6 ± 0·1*
Serum urea nitrogen (mg dL^{-1})	15·6 ± 0·3	23·7 ± 0·6*
Serum alanine aminotransferase (units L^{-1})	56 ± 1	60 ± 1*
Serum aspartate aminotransferase (units L^{-1})	80·6 ± 1·9	83·4 ± 2·1

Results are reported as mean ± s.e. *$P < 0.01$ compared with control group.

codynamic investigation (Hoffman & Levy 1990), and probably resulted from the effect of the cyclosporin treatment on binding of the barbiturate to tissues in the brain which are not involved with the anaesthetic effect. Descriptions of the animals used to investigate the effect of cyclosporin on the pharmacodynamics of ethanol-induced anaesthesia are presented in Table 4. These indices were virtually the same as those used for the rats in the phenobarbitone investigation (see Table 1). Treatment with cyclosporin did not alter the infusion time and dose required to induce sleep, nor the ethanol serum concentration at this pharmacologic endpoint (Table 5).

Table 5. Effect of four days of pretreatment with cyclosporin on ethanol concentrations at onset of LRR in rats receiving an intravenous infusion of ethanol.

	Control	Treated group
Infusion time (min)	37·2 ± 1·1	36·8 ± 0·8
Total dose (mg kg^{-1})	2705 ± 100	2634 ± 135
Serum concn (mg mL^{-1})	3·4 ± 0·2	3·87 ± 0·31

Ethanol (20% v/v in water) was infused at a rate of 0·106 mL min^{-1} until onset of LRR. Results are reported as mean ± s.e.

Discussion

It is essential to identify the effect of concomitant drug therapy and pathophysiologic conditions on the pharmacodynamics of drugs in order to enable the rational design of individualized drug dosage regimens. In anaesthetic practice, it is of utmost importance to identify conditions which affect the brain sensitivity to anaesthetics, in particular, concomitant pharmacotherapy which is not generally known to exhibit CNS activity.

Based on the CSF phenobarbitone concentrations at the onset of LRR (Table 3), it could be concluded that following four days of daily treatment with cyclosporin the sensitivity of the CNS to the general anaesthetic action of phenobarbitone had increased. The mechanism responsible for this increased sensitivity could not be clearly deduced from these results; however, it could be related to one or more mechanisms which have been suggested to elucidate the effect of cyclosporin on the CNS. These mechanisms could be divided into two major categories: direct effect of cyclosporin and indirect effects due to changes induced by cyclosporin treatment.

Dougherty & Dafny (1988) demonstrated that direct cyclosporin administration to the brain reduced the severity of opiate withdrawal syndrome. The dose dependency of this effect had a U shape (i.e. increasing doses of cyclosporin lessen the severity of the withdrawal syndrome up to a certain dose after which higher doses produced a smaller magnitude of effect) which may indicate two opposite direct effects of cyclosporin. This direct effect was consistent with the differences found between the unaffected CNS sensitivity to phenobarbitone following acute administration of a single dose of cyclosporin, as compared with increased CNS sensitivity following repetitive dosing for four days. This could result because of the slow rate of cyclosporin penetration through the blood-brain barrier (BBB). Repetitive administration of the immunosuppressant drug was required in order to accumulate a sufficient brain concentration to affect the CNS sensitivity to the barbiturate. This proposed mechanism corroborated the increased neurotoxicity found in cyclosporin-treated patients with an impaired BBB due to severe systemic diseases (Gottrand et al 1991). Taking into consideration this theoretical mechanism, the magnitude of this cyclosporin-barbiturate interaction would be increased in patients with impaired BBB, who are actually the major group of patients treated by this drug combination. Another explanation links cyclosporin neurotoxicity with its metabolites rather than the parent drug (Kunzendorf et al 1988), since brain metabolite concentration following repetitive administration is likely to be higher than after a single dose administered 2 h before the pharmacodynamic experiment.

The proposed indirect mechanism to explain the modulation of the severity of the opiate withdrawal syndrome is based on the immunosuppressive activity of cyclosporin. Systemic administration of cyclosporin has a more pronounced effect on the severity of characteristic opiate withdrawal behaviour than direct administration to the brain (Dougherty & Dafny 1988), a result further substantiated by the fact that the transfer of spleen cells from cyclosporin-treated rats to morphine-dependent rats 24 h earlier, attenuated the severity of opiate-withdrawal behaviour and was equivalent to direct administration of cyclosporin (Dougherty et al 1987). According to McEvoy (1991), the white cell count (which reflects the cellular component of the immune system) is not affected by cyclosporin treatment (see Table 2). This element together with the fact that the immunosuppressive activity of cyclosporin is recognized primarily after exposure to an antigen, restricted elucida-

tion of the role of immunosuppression in the current pharmacodynamic effect.

Four days of treatment with relatively large doses of cyclosporin did not cause any apparent nephrotoxicity or liver dysfunction and induced only a slight reduction in the rectal temperature (Tables 1, 4). Therefore, potential changes in the sensitivity of the CNS due to pathophysiological states could be excluded in this case.

The commercial vehicle of cyclosporin, consisting of 65% Cremophor EL (polyoxyethylated caster oil) in ethanol, which is known to cause various adverse effects (Lorenz et al 1982; Besarab et al 1987), had no apparent effect on the pharmacodynamics of heptabarbitone anaesthetic action (Hoffman & Levy 1990). To exclude the potential effect of the vehicle in the present experiment, the control group received the vehicle alone.

Treatment with cyclosporin for four days did not affect the sensitivity of the CNS to the anaesthetic action of ethanol, as evidenced by the equivalent serum ethanol concentrations in both control and cyclosporin-treated animals at onset of LRR.

The difference between the effects of the cyclosporin treatment on these two depressant agents is itself of interest because of the potential clinical implications. Both phenobarbitone (unlike pentobarbitone) and ethanol seem to share the same binding site on the GABA-activated chloride channels (Olsen 1987; Harris 1990). Therefore, the effect of cyclosporin on brain sensitivity is probably via a mechanism other than the GABA-receptor complex. Differences between the magnitude and direction of the effect of several pathophysiological states on the pharmacodynamics of phenobarbitone- and ethanol-induced anaesthesia have been previously reported. Acute renal failure markedly increased the CNS sensitivity to phenobarbitone but only slightly that to ethanol (Hisaoka et al 1985); liver dysfunction moderately affected the pharmacodynamics of ethanol but did not alter the sensitivity to phenobarbitone (Danhof et al 1985). In addition, acute starvation decreased the sensitivity to phenobarbitone anaesthetic action but did not affect the pharmacodynamics of ethanol (Wanwimolruk & Levy 1987).

In essence, the results demonstrate that CNS sensitivity to the depressant action of ethanol in alcohol-consuming patients maintained on cyclosporin is unaffected. However, the concentration-anaesthetic effect relationship of the three barbiturates, heptabarbitone, phenobarbitone and pentobarbitone, is altered by this immunosuppressive treatment. Thus, it can be deduced that the sensitivity of the brain to the anaesthetic effect of barbiturates is enhanced by cyclosporin treatment.

Thus, extrapolation of these results to other clinically used barbiturates is valid since we rely upon results of two different model drugs of the barbiturate category (heptabarbitone and phenobarbitone), which provide under the conditions of the present experimental design, the same pharmacodynamic information. Although these drugs are not used in anaesthetic practice, they provide unambiguous information concerning pharmacodynamic interactions. In addition to being optically pure, these drugs have no active metabolites interfering with the concentration-response relationship, no pharmacokinetic perturbations (such as slow equilibration between the sampling site and the site of action) affecting the results of the pharmacodynamic examination and no acute tolerance developing during the pharmacodynamic study.

The results also stress that the magnitude of this interaction is more pronounced in patients treated with cyclosporin for long periods rather than in acute cases. This pharmacodynamic interaction is of utmost importance to anaesthesiologists who must take this information into consideration when placing patients who have cyclosporin treatment for long periods under anaesthesia.

These conclusions are subject to the usual reservations in extrapolating animal data to man. It is interesting to note that FK-506 (a recent clinically introduced immunosuppressive agent with an analogous mechanism of action) exhibited similar neurotoxicity (Freise et al 1991), and may also alter the pharmacodynamics of anaesthetic preparations. Further studies are warranted to evaluate the full clinical significance of this interaction.

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