Pilot Study of PK 11195, a Selective Ligand for the Peripheral-Type Benzodiazepine Binding Sites, in Inpatients with Anxious or Depressive Symptomatology

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Summary

PK 11195 is a selective ligand for the peripheral-type benzodiazepine binding sites which exhibits anticonflict activity in animals. In a pilot open study, PK 11195 was administered to 10 psychiatric inpatients characterized by a rating of at least "moderate" for the item "felt loss of vitality" and a rating of at least "moderate" for the items "anxiety" and/or "inhibition of drive" from the psychopathological scale of the system developed by the Association for Methodology and Documentation in Psychiatry (AMDP). The duration of the study was two weeks, with an initial daily dose of 200 mg of PK 11195 which could be increased up to 400 mg. Patients were assessed weekly using the psychopathological and somatic AMDP scales and at days 0, 4, 7, and 14 using the Hamilton anxiety scale and a checklist of symptoms and side-effects.

The results showed significant improvement in the AMDP factor scores related to somatic complaints, depression, anxiety, apathy-retardation, and psycho-organic symptoms. However, anxiolytic activity, confirmed on the Hamilton anxiety scale, remained moderate and reached maximum effect after one week. No side-effects, drowsiness in particular, were reported.

This study therefore suggests a potential beneficial activity of PK 11195 on anxiety and inhibition, which merits further investigation in controlled studies.

Etude pilote du PK 11195, un ligand spécifique des sites de liaison benzodiazépinique de type périphérique, chez des patients hospitalisés avec une symptomatologie anxieuse ou dépressive

Le PK 11195 (Fig. 1) est un ligand specifique des sites de liaison benzodiazépiniques de type périphérique qui possède une activité anxiolytique chez l'animal dans les tests de conflit. Dans une étude pilote ouverte, le PK 11195 a été administré à 10 patients psychiatriques hospitalisés caractérisés par une évaluation de degré au moins «modéré» à l'item «trouble de l'éprouvé vital» associé une évaluation de degré au moins «modéré» aux items «anxiété psychique éprouvée» et/ou «inhibition de l'énergie» de l'échelle psychopathologique du système développé par l'Association pour la Méthodologie et la Documentation en Psychiatrie (AMDP). Les caractéristiques individuelles des patients sont reprises dans le tableau 1. La durée de l'étude était de 2 semaines, avec une dose journalière initiale de 200 mg de PK 11195 qui pouvait être augmentée jusqu'à un maximum de 400 mg. Les patients étaient évalués chaque semaine par les échelles psychopathologique et somatique de l'AMDP et aux jours 0, 4, 7 et 14 par l'échelle d'anxiété de Hamilton et une liste de symptômes et effets secondaires.

Les résultats ont mis en évidence une amélioration significative des notes des facteurs AMDP suivants: plaintes somatiques, dépression, anxiété, apathie-ralentissement et symptômes psychoorganiques (Fig. 2). Cependant, l'action anxiolytique, confirmée sur l'échelle d'anxiété de Hamilton, est restée modérée et déjà maximum après une semaine. Aucun effet secondaire, somnolence en particulier, n'a été rapporté. Dès lors, cette étude suggère une activité thérapeutique potentielle du PK 11195 sur l'anxiété et l'inhibition qui devra cependant être confirmée dans des études ultérieures.

Introduction

In 1977, using tritiated diazepam, *Braestrup* and *Squires* and *Möhler* and *Okada* demonstrated two different types of benzodiazepine binding sites. The first type is only present in the central nervous system and mediates the "classic" anxiolytic, anticonvulsant, hypnotic, and muscle-relaxant properties of the benzodiazepines. The second type of binding site was demonstrated in various peripheral organs such as kidney, heart, spleen, lung, liver, salivary gland, tongue, nasal

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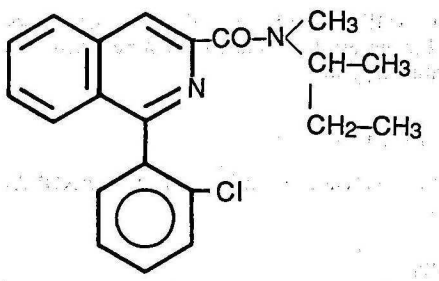

Fig. 1 Structural formula of PK 11195 (52028 RP), a selective antagonist of the benzodiazepine binding sites of peripheral type.

epithelium, pituitary, testis, adrenal gland, as well as on mast cells, platelets, and cells of the pineal gland (*Anholt*, 1986). These peripheral-type binding sites differ from the central type by virtue of their dissimilar affinities for pharmacological compounds such as clonazepam, specific for the central type, and Ro 5-4864 or 4'-chlorodiazepam, specific for the peripheral type (review in *Weiss* et al., 1986). In addition to differences in pharmacological properties, these two types are distinct in the time course of their phylogenetic and ontogenetic manifestation. The peripheral-type benzodiazepine binding sites appear late in evolution, but early in ontogeny; while central-type benzodiazepine receptors are demonstrable in avian, reptilian, and amphibian species as well as in fish, peripheral-type benzodiazepine binding sites are readily detectable only in mammals (*Anholt*, 1986). Recent evidence shows that these peripheral-type benzodiazepine binding sites are associated with the mitochondrial outer membrane and exhibit a range of effects on calcium mobilisation, growth, and differentiation of cells in culture, suggesting that these receptors may be important control sites for the modulation of intermediary metabolism (*Anholt*, 1986). Porphyrins have been suggested as endogenous ligands (*Verma* et al., 1987) and species differences exist (*Cymerman* et al., 1986).

Interestingly, peripheral-type benzodiazepine binding sites have also been found in mammalian central nervous system (*Schoemaker* et al., 1981). While they exhibit the same biocheimcal characteristics as peripheral-type sites in other organs, they differ completely from the central-type receptors both in their localization and their biochemical properties. They are GABA-independent (*Taniguchi* et al., 1982) and insensitive to photolabelling by flunitrazepam (*Richards* and *Möhler*, 1984). In contrast to central-type binding sites which are rather regularly distributed throughout the brain as a whole, peripheral-type binding sites are mainly concentrated in the olfactory bulb, ependyma, and choroid plexus, at least in the rat (*Richards* et al., 1982).

Until now, these binding sites have not been considered as receptors, but only as acceptors, as their physiological role remains unknown (*Richards* et al., 1982). However, a slight increase of their density has been found in Huntington chorea and in Alzheimer disease (*Schoemaker* et al., 1982; *Owen* et al., 1983).

Structurally completely different from benzodiazepines, PK 11195 (also 52028 RP) or 1-(2-chloro-phenyl)-N-methyl-N-(1-methyl-propyl)-3-isoquinolinecarboxamide (Fig. 1) exhibits a very high affinity for the peripheral-type benzodiazepine binding site and is thought to act as an antagonist there (*Le Fur* et al., 1983 a, b, c; *Benavides* et al., 1984). While low doses of Ro 5-4864 increase the "anxious" behavior of the rat placed in a conflict situation with a dose-related effect, low doses of PK 11195 are capable of inhibiting this proconflict effect. Moreover, higher doses of PK 11195 alone exhibit anticonflict activity (*Mizoule* et al., 1985). The fact that the central benzodiazepine receptor antagonist flumazenil potentiates the proconflict effect of Ro 5-4864 and the anticonflict effect of PK 11195 led to the hypothesis that peripheral and central type benzodiazepine binding sites could act in a contrary way (*Mizoule* at al., 1985). This hypothesis is supported by the fact that bulbectomy, which destroys a brain area particularly rich in peripheral-type binding sites in the rat, suppresses the deterrent effect of punishment (*Mizoule* et al., 1985).

At low doses, PK 11195 facilitates audiogenic seizures and blocks the anticonvulsant effects of carbamazepine, but not of diazepam, on amygdala-kindled seizures and prevents or reverses the contrary actions of a low dose of Ro 5-4864 (*Benavides* et al., 1984). However, PK 11195 is inactive against normal experimental convulsions elicited by electric shock, pentylenetetrazole, and picrotoxin and, unlike classic benzodiazepines, does not antagonize the convulsant effect of high doses of Ro 5-4864 in mice, most probably because peripheral benzodiazepine binding sites do not play a role in these effects (*Weissman* et al., 1985).

PK 11195 exhibits no other action on the central nervous system, whether sedative, psychoanaleptic, nooanaleptic, muscle-relaxant, or analgesic. PK 11195 is also devoid of effect on other body systems and is characterized by excellent cardiovascular tolerance and very low toxicity in animals. In humans, single doses of 100, 200, and 400 mg of PK 11195 (which has an elimination half-life of 10–15 h) as well as repeated daily doses of 200 mg over two weeks were devoid of any clinical, laboratory, cardiological, or electro-encephalographic side-effects. The purpose of the present pilot study was therefore to determine the potential beneficial activity of PK 11195 on neurotic symptomatology, including anxious, asthenic, and depressive symptoms. As will be discussed, it is felt that a pilot study of this type may benefit from a lack of rigorous selection criteria and from the use of comprehensive rating instruments.

Subjects and Methods

Subjects

Ten inpatients from the Psychiatric Unit of the University of Liège, Belgium, were included in the study. Their clinical characteristics are given in Table 1. All patients exhibited a psychiatric condition, psychosis excluded, characterized by a rating of a least 2 (moderate) for the item "felt loss of vitality" on the psychopathological scale of the system developed by the Association for Methodology and Documentation in Psychiatry (AMDP) (*Guy* and *Ban*, 1982) as well as a rating of at least 2 (moderate) for either "anxiety" or "inhibition of drive" on the same comprehensive scale. Patients presenting any evidence of somatic illness, alcoholism, or drug addiction were excluded from the study. Finally, the protocol was approved by the Ethical Committee of the University of Liège Medical School and all patients gave their informed consent.

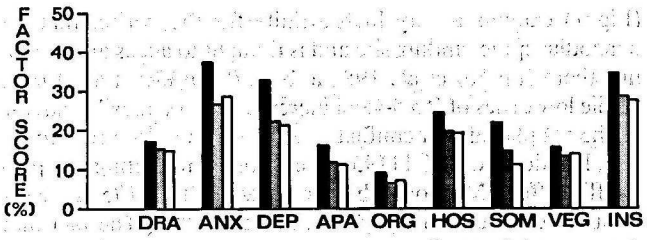

Fig. 2 Factor scores on the AMDP psychopathological and somatic scales in 10 patients before treatment (black columns), after one week of PK 11195 (hatched columns), and after two weeks of PK 11195 (white columns). DRA, dramatisation; ANX, anxiety ($p = 0.02$ after 2 wks); DEP, depression ($p = 0.01$ after 1 wk and 0.0006 after 2 wks); APA, apathy-retardation ($p = 0.04$ after 2 wks); ORG, psycho-organic symptoms ($p = 0.02$ after 1 wk and 0.03 after 2 wks); HOS, hostility; SOM, somatic complaints ($p = 0.006$ after 1 wk and 0.0009 after 2 wks); NEU, neurovegetative symptoms; INS, insomnia.

Table 1 Characteristics of the sample.

Patient #	sex	age	weight	DSM-III Diagnosis
1	F	49	61	Dysthymic disorder (300.40)
2	M	18	63	Dysthymic disorder (300.40)
3	F	39	52	Dysthymic disorder (300.40)
4	M	24	68	Dysthymic disorder (300.40)
5	M	38	78	Adjustment disorder with depressed mood (309.00)
6	F	55	64	Major depression without melancholia, bipolar (296.52)
7	F	31	47	Major depression without melancholia, single episode (296.22)
8	M	32	80	Dysthymic disorder (300.40)
9	M	26	70	Adjustment disorder with depressed mood (309.00)
10	M	50	68	Major depression without melancholia, single episode (296.22)
Mean	6M, 4F	36.2	65.1	
SD		12.2	10.3	

Design of the study

After a drug-free period of at least seven days, PK 11195 was given at a daily dose of 200 mg in two intakes (morning and evening). From day 4, the dose could be individually adapted according to the patient's clinical condition, with a maximum daily dose of 400 mg. The duration of the study was two weeks. No associated drugs were permitted.

Assessments

All assessments, performed on days 0, 4, 7, and 14, included the Hamilton anxiety scale (*Hamilton, 1959*), a checklist of symptoms and side-effects previously described (*Ansseau* et al., *1989*), and a general physical examination, including pulse, standing and supine blood pressure, and weight. Moreover, on days 0, 7, and 14, the patients' psychological and somatic symptoms were comprehensively assessed by means of the AMDP system (*Guy* and *Ban*, 1982). Factor analysis of the 115 items of the psychological AMDP scale and of the 50 items of the somatic AMDP scale has revealed 14 main factors (*Bobon*, et al., 1982). Laboratory tests and ECGs were performed before and after the treatment period.

Data analysis

Statistical analysis used variance analysis (ANOVA) with repeated measures.

Results

Subjects and doses

Nine subjects completed the entire two-week test; patient # 7 left the hospital on day 10 for administrative reasons. The 200 mg daily dose of PK 11195 remained stable in one case, was increased to 300 mg from day 4 in three cases and from day 7 in four cases, and increased to 400 mg from day 7 one case. Patient # 7 was still at the 200 mg dose when she left the study at day 10.

AMDP scales

The changes in the main factor scores of the AMDP psychological and somatic scales are shown in Fig. 2. Baseline scores for five factors (obsessions, dissociation-depersonalization, delusions, mania-agitation, and neurological symptoms) which are not displayed in Fig. 2 were less than 5 % and did not exhibit any significant change during the treatment. Of the other factors, five exhibited significant improvement over the study period: somatic complaints ($F(2,16) = 11.2$, $p = 0.0009$), depression ($F(2,16) = 12.1$, $p = 0.0006$), anxiety ($F(2,16) = 5.0$, $p = 0.02$), apathy-retardation ($F(2,16) = 4.3$, $p = 0.04$), and psycho-organic symptoms ($F(2,16) = 4.6$, $p = 0.03$). Significant decrease were already present after one week for somatic complaints ($p = 0.006$), depression ($p = 0.01$), and psycho-organic symptoms ($p = 0.02$).

Hamilton anxiety scale

Mean Hamilton anxiety scores exhibited a significant decrease over the two-week study period: from 26.8 ± 5.5 to 19.4 ± 11.6, $F(3,24) = 5.66$, $p = 0.005$. This was already noted after one week ($p = 0.02$).

Side-effects and physical examinations

No side-effects appeared during the treatment with PK 11195. On the contrary, the factors on the checklist of symptoms and side-effects related to behavior, central nervous system, and autonomous nervous system exhibited a significant decrease over time ($F = 3.6$, $p = 0.03$, $F = 4.6$, $p = 0.01$, and $F = 4.6$, $p = 0.01$ respectively).

Finally, physical examinations and laboratory tests did not show pathological changes.

Discussion

The results of this pilot open study of PK 11195, a selective ligand for the benzodiazepine binding sites of peripheral type, suggest a beneficial action on psychopathologic symptomatology. In this group of 10 psychiatric inpatients characterized by anxiety and/or inhibition, the com-

prehensive assessment by the AMDP system shows significant improvement of somatic complaints, depression, anxiety, apathy-retardation, and psycho-organic symptoms. The compound's rather large range of action does not appear to correspond to other typical psychotropic drugs, such as classic antidepressants or anxiolytic benzodiazepines. PK 11195 seems to exhibit both anxiolytic and stimulating properties. However, its anxiolytic effect seems to be less clear than that of benzodiazepine anxiolytics, and was most pronounced after seven days of treatment, without further improvement during the remaining seven days of the present study. This might be due to the doses of PK 11195 selected for this study being too low, and higher doses should be tested in further trials. At any rate, the possibility of a placebo effect can never be excluded in such an open pilot study.

The relatively high doses of PK 11195 used in this study (200–400 mg/d) may appear excessive in view of the compound's affinity at the receptor level which, in membranes from rat and cat brain cortex, is in the nanomolar range (*Le Fur* et al., 1983a). However, oral doses of 10 mg/kg are necessary to inhibit the binding of radiolabelled Ro 5-4864 (a selective marker of peripheral-type benzodiazepine binding sites) in the mouse brain, and this suggests that high doses are needed to reach nanomolar concentrations at the receptor level (*Le Fur* et al., 1983 b). Moreover, the dose of PK 11195 which evokes anticonflict activity in the rat is the same as that needed to displace Ro 5-4864 (12.5 mg/kg) (*Mizoule* et al., 1985).

No patient reported sedative side-effects; similarly, no increase in insomniac complaints was noted, despite an evening administration of the drug. Indeed, there was a trend toward significant improvement of sleep disturbances. Not side-effects of any other type were mentioned during treatment with PK 11195. On the contrary, the treatment induced a clear improvement in somatic complaints, which can be attributed to the anxiolytic activity of the drug.

The results of this pilot study suggest that peripheral-type benzodiazepine binding sites play a role in the modulation of psychopathologic symptoms such as anxiety and inhibition, and that a selective ligand for this binding site, such as PK 11195, may find clinical applications. Although the physiological functions of the peripheral-type benzodiazepine binding sites remain unknown, intriguing patterns of tissue distribution, an unusual association with the mitochondrial outer membrane, and their possible role in cell growth and differentiation are relevant for the clinical application of substances acting at the binding site. Particularly, the possibility of unwanted side-effects, such as mutagenesis during long-term administration, should be kept in mind. PK 11195, however, appears to be extremely safe after acute administration in animals. In mice and rats, oral doses of as much as 5000 mg/kg are completely atoxic. Thirteen weeks of chronic administration of doses up to 450 mg/kg in the rat and up to 300 mg/kg in the monkey were perfectly well tolerated. Moreover, in the classic tests, PK 11195 is devoid of any mutagenic effect of genic or chromosomic type. One should also remember that classic benzodiazepines, such as diazepam, also bind, even if they do not do so selectively, to the peripheral-type benzodiazepine binding sites and are devoid of mutagenic effect after long-term use. In any case, special caution is advisable during administration of a substance that interacts with such an ubiquitous binding site, whose function is hardly understood. The potential risk should be weighed against the potential therapeutic benefits.

The use of inclusion criteria based on the presence of symptoms rather than on specific diagnosis may seem somewhat questionable. In fact, the authors feel that a pilot study with a drug unrelated to known psychotropic drugs may benefit from such an approach (*Ansseau* et al., 1987; *Digonnet* et al., 1987). Such a methodology may enable us better to define the profile of activity of the compound, without an *a priori* hypothesis. Most new families of psychotropic drugs were originally tested for illness unrelated to their final application. The use of comprehensive scales, such as those derived from the AMDP system, may record any changes in psychopathology and then help to define better the type of patients needed for further studies.

In conclusion, the results of this pilot study suggest that peripheral-type benzodiazepine binding sites play a role in the modulation of psychopathologic symptoms such as anxiety and inhibition, and that a selective ligand for these binding sites, such as PK 11195, may find clinical applications. Obviously, these preliminary findings need to be confirmed in large controlled trials including specific diagnostic categories of anxiety or depressive disorders.

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