

IV. Pharmacology and Toxicology

A. General Description

Fallypride is a selective, high affinity dopamine D₂ receptor antagonist with K_D of 30 pM (Christian et al 2000; Kessler et al 1993). Pharmacological doses of this agent would be expected to show typical D₂ receptor antagonist activities of extrapyramidal side effects, sedation, and catalepsy. However, the proposed dose is well below that of pharmacological effects. Thus, the current study is not expected to cause any effects or side effects.

Robert Kessler, MD has an active IND for [¹⁸F]fallypride (also called *N*-allyl-5-fluoropropylepride). Dr. Kessler has authorized the FDA to review his IND on behalf of the current proposal for all information regarding pharmacology of this tracer, including toxicity, pharmacokinetics, safety, and efficacy. Please note that Dr. Kessler's IND was approved for the use of a mass dose no greater than 0.9 µg, calculated as follows:

$$5 \text{ mCi} * 1 \text{ } \mu\text{mol}/2,000 \text{ mCi} * 364 \text{ } \mu\text{g}/\mu\text{mol} = 0.9 \text{ } \mu\text{g}$$

Since the time of original submission, Dr. Kessler has studied many human subjects and found no pharmacological effects or side effects. See attached letter in Appendix E. With this information, we request a maximal mass dose of 2 µg, which corresponds to ~0.04 µg/kg in a 70 kg adult. Dr. Kessler's original IND submission has information on Pharmacology and Toxicology, beginning on page 411. His data support a 100-1,000 fold safety factor for this proposed dose (0.04 µg/kg) relative to the minimal effective dose in animals.

The Pharmacology and Toxicology Data in Dr. Kessler's IND application can be summarized as follows:

1. Acute Toxicity in Rats. The maximal dose in rats (600 µg/kg) caused mild sedation, no deaths, and no abnormalities on postmortem examination. This dose is approximately 20,000 fold higher than the proposed human dose (0.03 µg/kg).
2. Subacute Toxicity in Rats. Ten consecutive daily doses of fallypride (1.5 and 6 µg/kg I.V.) showed no pathological effects on postmortem examination. The higher dose (6 µg/kg) is ~200 fold higher than the proposed human dose.
3. Subacute Toxicity in Rhesus Monkeys. Ten consecutive daily doses of fallypride (3 µg/kg I.V.) showed no significant effect on blood chemistries, CBC, and chest X-ray. This dose (3 µg/kg) is 100 fold higher than the proposed human dose.
4. Pharmacological Effects. The lowest dose in monkeys to produce the earliest observable pharmacological effects was 2.2 nmol/kg, which corresponds to ~0.8 µg/kg I.V. The behavioral effect was decreased pacing and decreased reactivity to eye contact. The HED (Human Equivalent Dose) would be 0.26 µg/kg (= 0.8 * 0.324). The maximal proposed human dose is 0.029 µg/kg (= 2 µg / 70 kg subject). Thus, the proposed human dose is 9 times lower than the minimally effective behavioral dose in monkeys. For this reason and the fact that the proposed dose is only two fold higher than that used by Dr. Kessler in humans with no effects, we expect that our studies will show no pharmacological effects. Dr. Kessler also calculated that a fallypride dose of ~0.015 µg/kg would occupy ~0.7% of striatal D₂ receptors. Since this low dose is in the linear range of

receptor occupancy, our proposed dose (0.03 µg/kg) would occupy 1.4% of receptors, at least 30 fold lower than any side effects.

5. Radiation Dosimetry. Rat biodistribution data were used to calculate organ dosimetry. A dose of 5 mCi was estimated to cause the following exposures (rem): 0.8 to red marrow; 0.25 to spleen; 0.1 to testes, 0.19 to whole body, and 1.05 to small intestine. See Table IV of Kessler's IND for other organs. These exposures are within guidelines for research studies. After the original IND application, Dr. Kessler submitted additional data on dosimetry based upon human whole body imaging. See Section VI "Human Experience."



APPENDIX

F. Pharmacology & toxicology tl!tta from Dr. Kessler's IND

8. PHARMACOLOGY AND TOXICOLOGY DATA

Animal Toxicity

1) Acute Toxicity - Rather than determine the acute LD₅₀, we have elected to demonstrate that the acute LD₅₀ is greater than 40,000 fold higher than the maximal scan dose given. The scan dose will be less than 2.5 nanomoles (5 mCi at 2,000 Ci/mmol), i.e. 2.50 nanomoles given to a 70 kg man which corresponds to a dose of 14.3 nanograms/kg. We have administered doses of 611g/kg, 6011g and 60011g/kg to groups of 5 male Sprague Dawley rats with no deaths and no apparent effects other than sedation. All animals were observed for 48 hours following dosing. The rats receiving the 60011g/kg dose were sacrificed at 48 hours and the brain, lungs, heart, liver, stomach, intestines, spleen, kidneys, adrenals pancreas and striated muscle were removed and grossly examined. No gross abnormality was noted. The peak dose of 60011g/kg corresponds to a dose more than 40,000 times the scan dose in man.

2) Subacute Toxicity of N-allyl-5 fluoropropylepidepride in Rats

The goal of this study was to study possible organ toxicity due to N-allyl-5 fluoropropylepidepride, a potential PET radioligand for the dopamine D₂ and D₃ receptor. The anticipated maximal scan dose is less than 2.5 nanomoles which corresponds to a mass dose of 15 µg for a 70 kg man or about 14.3 nanograms/kg. To demonstrate a lack of toxicological effects, a dose 400 times higher than that to be given to man, i.e. 6111g/kg IV as given daily for 10 days to male 250 gram Sprague Dawley rats. A total of twenty-one ~~250~~ gram, male Sprague Dawley rats were studied. Eleven received 1.5 µg (6111g/kg) dissolved in 0.2 ml 99% sterile saline and 1% ethanol (95%) via tail vein daily for 10 days. A second group of ten received 0.2 ml of a 99% sterile saline/1% ethanol (95%) solution via tail vein daily for 10 days. All doses were filtered through a Millipore filter. At 24 hours following the final dose, the animals were sacrificed. The brain, lungs, heart, liver, spleen, kidneys, intestines, red marrow and gonads were dissected and

tissues fixed in formalin except for the red marrow and spleen which were fixed in B5. All tissues were stained with hematoxylin and eosin for studying of pathological changes. The histological sectioning and microscopic examination were performed under the supervision of Dr. Fred Gorstein, Chairman, Department of Pathology, Vanderbilt University School of Medicine. All slides were read with the reader blinded as to whether saline or N-allyl-5-fluoropropylepidepride had been given. No pathological changes were seen suggesting toxicity to any of the organs studied. Dr. Gorstein's report is appended.

3) Subacute Toxicity of N-allyl-5 fluoropropylepidepride in Rhesus Monkeys

The goal of this study was to examine the potential toxic effects of N-allyl-5 fluoropropylepidepride, a new PET dopamine D2 receptor ligand, in a species which is from an evolutionary perspective close to man. This is particularly important in evaluating the substituted benzamides as they have different patterns of metabolism in rodents and dogs than occurs in man and primates. 1-3 The maximum scan dose to be given in man is 2.5 nanomoles or 1 μ g for a 70 kg man corresponding to a dose of about 14.3 nanograms per kilogram. For the 9, 11, and 12 kg rhesus monkeys that are available, this corresponds to a dose of 0.13 μ g, 0.16 μ g, and 0.17 μ g. As many over the counter medications, e.g. aspirin, benadryl and phenylephrine, have LD₅₀: clinical dose ratios of 50-80, a 200 fold ratio of subacute dose: scan dose appears to be a conservative ratio for subacute testing. Allowing for a 200 fold margin of safety, this corresponds to doses of 27, 33 and 36 μ g respectively administered in 2 ml of 99% sterile saline/1% ethanol. One rhesus monkey (animal 4) was studied with just the injection vehicle i.e. 2 ml of 99% sterile saline/1% ethanol. These doses were given intravenously daily for 10 consecutive days. Prior to the start of the 10 consecutive doses, each monkey had clinical chemistries equivalent to an SMA6 and SMA12 and a CBC with differential including platelets drawn. These were obtained twice and the results verified prior to starting the dosing schedule. When all laboratory results were obtained, the 10 consecutive daily doses of N-allyl-5 fluoropropylepidepride or vehicle were given intravenously. At 24 hours and six days following

the last dose, all laboratory investigations, including the clinical chemistries, CBC with platelets and differential were repeated. A chest film was obtained at 24 hours following the last dose. Two sets of clinical chemistries and CBC's The results of this study shows no changes in clinical chemistries, CBC and no abnormalities seen on chest x-ray. Animal I had a mildly elevated SGPT on the determination performed 24 hours following 10 days dosing. Neither of the other two treated animals had significant changes in SGPT making the apparent mild elevation of SGPT in animal I of questionable significance. All animals, including the control had high LDH and alkaline phosphatase values (compared to canine control values) prior to dosing which did not change following dosing. The CBC for the control animal at 24 hours following the 10 day dosing and the CBC for six days following dosing for animal I were lost or not performed. No changes were seen in the CBC's for all treated animals at 24 hours following 10 days dosing. These results demonstrate no significant abnormalities in the CBC's or clinical chemistries following 200 times the scan dose daily for 10 days. No pulmonary or cardiac abnormalities were seen on chest X-ray. Following this study the primates have been observed for over 3 months and show no deleterious effects.

4. Pharmacological Effects

N-allyl-5-fluoropropylepidepride is a substituted benzamide and a close structural analogue of epidepride 4-6 and raclopride. 7,8 Similar to these compounds, it has high affinity at the dopamine D_2 ($K_o = 30 \text{ pM}$) and dopamine D_1 ($K_o = 31 \text{ pM}$) receptors with little or very low affinity ($K_i > 100 \text{ nM}$ to over $10 \text{ } \mu\text{M}$) for a variety of other neurotransmitter receptors including the dopamine D_4 , D_5 , serotonin 5_2 and 5_3 , noradrenergic α_1 and α_2 , opiate, GABAergic, histaminergic, and sigma receptors (see Table 1). In primates its Uptake in striatum was about 0.1-0.12% administered dose per cc. Doses of unlabelled N-allyl-5-fluoropropylepidepride were administered intravenously to each of three rhesus monkeys weighing 12.1, and 11.4 and 9.2 kg while under ketamine sedation. The monkeys were then rated following the anesthesia at 4, 6, 8, 24 and 48 hours following each dose. The amount given was 10, 20, or 30 nanomoles or vehicle

on four separate occasions. At the 0 and 10 nanomole dose no pharmacological effects were seen in any animal. Some decreased pacing and decreased reactivity to eye contact was noted in the 9.2 kg animal at the 20 nanomole dose and similar findings were noted in the 11.4 kg and 12.1 kg. animals at the 30 nanomole dose. These effects were apparent at 4, 6 and 8 hours post administration but had resolved or were minimally present by 24 hours and were inapparent at 48 hours following these doses. Thus a dose of 2.2 to 2.6 nanomoles/kg appears to produce the earliest observable pharmacological effects in rhesus monkeys. The dose to be given to man will not exceed 0.0357 nanomoles/kg; this is a safety margin of seventy to eighty fold and so at the 2.5 nM (1 Jig) maximum dose, no pharmacological effect should be apparent. This is consistent with our previous results with [¹H]epidepride where 2 Jig (5 nanomoles) doses have produced no pharmacological effects in man. Epidepride⁴⁻⁶ is a very close chemical analogue having slightly higher affinity for the dopamine D₂ receptor ($K_o = 24$ pM), a considerably slower dissociation rate in vivo (55 minutes versus 12 minutes), somewhat lower lipophilicity (log K_w pH 7.5 of 2.05 versus 2.48), and an almost identical pattern of affinities for dopamine D₂, D₃, and other neurotransmitter receptors.

A second way of estimating the level at which pharmacological effects occur would be to compute the fraction of occupied dopamine D₂ receptors following a 1 Jig dose. Farde⁹ has demonstrated that the earliest pharmacological effects for dopamine antagonists are seen at or greater than 55% receptor blockade. As raclopride has similar lipophilicity and brain uptake is most closely related to lipophilicity for substituted benzamides⁴, peak striatal uptake in man should be similar for [¹⁸F]N-allyl-5-fluoropropylepidepride and [¹¹C]raclopride. Wang reports that [¹¹C]raclopride has a peak uptake in human striatum of 0.005% injected dose/cc 10. For a 2.5 nM dose of N-allyl-5-fluoropropylepidepride, this corresponds to a receptor saturation of 0.7% - or at least 75 fold below the minimal pharmacological dose. This degree of receptor occupancy would indicate that the administered dose of [¹⁸F]N-allyl-5-fluoropropylepidepride is a true tracer dose.

A third way of estimating the minimal pharmacological dose is to compare N-allyl-5-fluoropropylepidepride to studies with IV raclopride. Farde⁹ reports that no pharmacological effects were seen with high specific activity doses- i.e. under 9 Jlg. With low specific activity doses, 200-500 Jlg of raclopride IV, 19 of 33 subjects had akathisia- an inner restlessness and desire to move. It would appear that a 1 Jlg dose of N-allyl-5-fluoropropylepidepride is highly unlikely to produce any pharmacological effects. While it may be argued that N-allyl-5-fluoropropylepidepride is more potent than raclopride, i.e. in vitro KD of 30 pM versus 1.1-2 nM, 7,8 both have a very high bound fraction in vivo because of the high affinities of both compounds. Assuming a tracer dose, equilibrium kinetics, a KD in vivo similar to that in vitro, and little nonspecific binding and a Bmax of 17-18 picomoles/gram tissue in the striatum, then the bound fraction would be 89-95% for raclopride versus just over 98% for N-allyl-5-fluoropropylepidepride. In both cases nearly all ligand in tissue is bound. Receptor occupancy is more a function of delivery to tissue than affinity. We have previously shown that striatal uptake is a function of lipophilicity which determines the ability of these compounds to cross the blood brain barrier. As N-allyl-5-fluoropropylepidepride and raclopride have similar lipophilicity, i.e. $\log k_{ow}$'s, pH 7.5 of 2.48 and 2.66 respectively, their striatal uptake should be very similar. Thus, experience with raclopride, a chemically and pharmacologically similar substituted benzamide, would indicate that a 1 Jlg dose of N-allyl-5-fluoropropylepidepride is below a level expected to produce effects in man.

In conclusion, N-allyl-5-fluoropropylepidepride is a specific dopamine D₂ and D₃ receptor antagonist. Behavioral studies in rhesus monkeys, estimations of receptor occupancy in man, and behavioral pharmacological-PET studies in man all indicate that a 1 Jlg dose is at least 75 fold and perhaps as much as 200 fold lower than the minimal pharmacological dose.

5. Radiation Dosimetry

To calculate radiation dosimetry, three groups of five male, 250 gram Sprague Dawley rats received 120 JICi of [ISF]N-allyl-5-fluoropropylepidepride via tail vein injection in 0.2 ml of

90% normal saline/10% ethanol solution. At 5 minutes, 1 hour, and 3 hours following injection, groups of 5 animals were euthanized and rapidly dissected; the brain, lung, heart, stomach, stomach contents, small bowel, small bowel contents, upper and lower large intestines, upper and lower large intestinal contents, kidney, liver, adrenals, spleen, testes, blood, muscle and bone (femur) were removed, weighed, and gamma spectrometry performed. In calculating radiation dosimetry, the reference mean for each organ was taken from ICRP23 and all activity for three hours onward was assumed to leave the body only by decay. Radiation dosimetry was calculated using MJRDOSE (IBM PC Version- January, 1988). The results of the rat uptake study are shown in Table III and dose estimates for various organs and whole body are shown in Table IV. The doses for a 5 mCi injection in man appear to be 0.8R to the red marrow, 0.246R to the spleen, 0.095R to the testes, 0.189R to the whole body and a dose of 1.045R to the small intestine. Given limits of 3 rads per quarter and 5 rads per year, it would appear that up to two studies per quarter and four studies per year are within permissible limits. The Phase I study calls for one 5 mCi dose while the two Phase II study calls for a single dose. The radiation dose resulting from these studies is within permissible limits. Following the results of Phase I studies of biodistribution, radiation dosimetry will be recalculated. All doses will be adjusted to stay within the 3 rad/quarter, 5 rad/year limit.

6) Summary of Toxicological, Pharmacological and Radiation Dosimetry Data

Pharmacological testing in vitro indicates that N-allyl-5-fluoropropylepidepride is a specific and reversible dopamine D_2 and D_3 receptor ligand. A variety of studies (see Pharmacological Effects) indicate that the minimal pharmacological dose is at least 75 times higher than the scan dose of 1 μ g and the expected peak receptor occupancy from the scan dose will be under 1% of available receptors. The uptake in brain is reversible as demonstrated both by imaging and behavioral studies in primates. These data are consistent with a 1 μ g dose of N-

allyl-5-fluoropropylepidepride being a true tracer dose and being a dose that will produce no pharmacological effects in man.

Subacute testing in rats reveals that 400 times the scan dose daily IV for ten days produces no histologically observable changes in a variety of organs including brain, heart, lungs, liver, kidneys, spleen and bone marrow. No significant abnormalities in blood count, clinical chemistries or chest film were seen in rhesus monkeys following a 10 day course of a daily dose 200 times that of the scan dose. At an acute dose of 40,000 times the scan dose, no deaths were seen in rats. The scan dose of 1 g for an average 70 kg subjects is an extremely low dose. These findings indicate that no toxicological effects should be seen in man from a 111g dose. It may be instructive to compare these findings and this dose to known toxins. Botulinus toxin, perhaps the most potent known neurotoxin on a weight basis, has a minimal lethal dose 0.03 g/g IP in mice. Scaling up to a 70 kg human, the estimated minimal lethal dose would be 210 tg. I 1 Thus the scan dose for N-allyl-5-fluoropropylepidepride is over 200 fold lower than the estimated minimal lethal dose of the most potent known neurotoxin. Given the pharmacological specificity, lack of toxicity in rats and monkeys, and extremely low dose of N-allyl-5-fluoropropylepidepride, we believe that the scan dose represents a safe dose which should produce no pharmacological or toxicological effects in man. The radiation dosimetry is within FDA guidelines.

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